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Short Communication

Minor compounds of olive oil have postprandial anti-inflammatory effects

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High postprandial levels of TAG may further induce endothelial dysfunction and inflammation in subjects with high fasting levels of TAG, an effect that seems to be related to oxidative stress. The present study investigated whether minor compounds of olive oil with antioxidant activity decrease postprandial levels of soluble isoforms of intercellular adhesion molecule 1 (sICAM-1) and vascular cell adhesion molecule 1 (sVCAM-1), as surrogate markers of vascular inflammation, after a high-fat meal. A randomized crossover and blind trial on fourteen healthy and fourteen hypertriacylglycerolaemic subjects was performed. The study involved a 1-week adaptation lead-in period on a National Cholesterol Education Program Step I diet supplemented with extra-virgin olive oil (EVOO) containing 1125 mg polyphenols/kg and 350 mg tocopherols/kg, or refined olive oil (ROO) with no polyphenols or tocopherols. After a 12 h fast, the participants ate a high-fat meal enriched in EVOO or ROO (50 g/m^2 body surface area), which on average provided 3700 kJ energy with a macronutrient profile of 72 % fat, 22 % carbohydrate and 6 % protein. Blood samples drawn hourly over the following 8 h demonstrated a similar postprandial TAG response for both EVOO and ROO meals. However, in both healthy and hypertriacylglycerolaemic subjects the net incremental area under the curve for sICAM-1 and sVCAM-1 were significantly lower after the EVOO meal. In conclusion, the consumption of EVOO with a high content of minor antioxidant compounds may have postprandial anti-inflammatory protective effects.

Postprandial metabolism: Adhesion molecules: Hypertriacylglycerolaemia: Olive oil

The arrest of white blood cells at the surface of the activated endothelium is a prominent feature of several inflammatory and immunologic disorders regulated by several endothelial adhesion molecules, such as intercellular adhesion molecule 1 (ICAM-1, CD54) and vascular cell adhesion molecule 1 (VCAM-1, CD106) of the Ig superfamily members (McEver, 2001). It has been suggested that postprandial hypertriacylglycerolaemia induces endothelial dysfunction that is accompanied by inflammation of the vessel wall, in part through mechanisms involving oxidative stress and increased levels of soluble forms of ICAM-1 (sICAM-1) and VCAM-1 (sVCAM-1) (Tsai *et al.* 2004; Burdge & Calder, 2005). Antioxidant therapy with vitamins E and C may inhibit postprandial oxidative damage and restore endothelial function (Neri *et al.* 2005). Further, antioxidants from red wine are thought to achieve such effects by decreasing the postprandial activity of the redox-sensitive transcription factor NF-κB (Blanco-Colio *et al.* 2000).

Extra-virgin olive oil (EVOO) is a key component of the Mediterranean diet that has been attributed preventive properties with regard to CVD. These beneficial properties are thought to be due to the high oleic acid content of EVOO and the minor compounds with high antioxidant activity, mainly phenolics, that are present (Giugliano & Esposito, 2005). Potency of fatty acids to inhibit endothelial activation does not depend on chain length but on the number of double bonds; the monounsaturated oleic acid is indeed able to produce all the effects obtainable with PUFA, albeit at higher concentrations (De Caterina & Massaro, 2005). It is noteworthy that inhibition of NF-κB activation was reproduced upon incubation of endothelial cells with oleic acid (Carluccio *et al.* 1999). Alternatively, transfection studies using different VCAM-1 promoter constructs showed that antioxidants from EVOO could repress VCAM-1 gene transcription in human endothelial cells (Carluccio *et al.* 2003). Moreover, phenolic minor compounds of EVOO have recently been reported to down-regulate endothelial cell surface expression of ICAM-1 and VCAM-1 (Dell'Agli *et al.* 2006). These observations raise the question of whether minor compounds of EVOO could selectively reduce the postprandial levels of sICAM-1 and sVCAM-1 after the ingestion of a high-fat meal.

The aim of the present study was to compare the effects of two diets enriched in olive oils, having the same fatty acid composition but with (EVOO) and without (refined olive oil,

Abbreviations: EVOO, extra-virgin olive oil; ICAM-1, intercellular adhesion molecule 1; netAUC, net increment in the area under the curve; ROO, refined olive oil; sICAM-1, soluble isoform of intercellular adhesion molecule 1; sVCAM-1, soluble isoform of vascular cell adhesion molecule 1; VCAM-1, vascular cell adhesion molecule 1.

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ROO) minor compounds, on postprandial levels of TAG and on the accumulation of sICAM-1 and sVCAM-1 in healthy and hypertriacylglycerolaemic subjects with a permanently activated endothelium (Ooi & Ooi, 1998). The levels of these soluble cell adhesion molecules were measured as we consider them to be surrogate markers of vascular inflammation.

Methods

The present study was conducted according to the guidelines of good clinical practice. Prior to the beginning of the study, all subjects provided their informed consent using protocols approved by the Human Clinical Commission and Ethics Committee of Hospitales Universitarios Virgen del Rocio (SAS), Seville. The investigation conforms with the principles outlined in the Helsinki Declaration of the World Medical Association.

Experimental design

By advertising, we recruited fourteen healthy and fourteen hypertriacylglycerolaemic male subjects, aged between 21 and 38 years. None of them displayed evidence of an established CHD and they were excluded if they showed any evidence of kidney or liver dysfunction, or hypothyroidism, based on clinical biochemistry tests. Hypertriacylglycerolaemic subjects, with type IIb or IV hyperlipoproteinaemia and without clinical evidence of target organ damage, had a BMI lower than 27. For BMI calculation, weight (kg; measured to the nearest 100 g) was divided by height (measured to the nearest 0·1 cm) squared (in m); the latter was measured only at baseline. None of the subjects consumed tobacco, special diets, vitamins or antioxidants.

The study was designed as a randomized crossover study that was blind to the investigators (for more details, see Pacheco *et al.* 2006). ROO was obtained by physically refining EVOO in a discontinuous deodorizer and as a result, it contained no minor compounds with antioxidant activity (Leon-Camacho *et al.* 2004). In contrast, EVOO contained 1125 ppm polyphenols and 350 ppm tocopherols (Cert *et al.* 2000), both of which act as antioxidants. The fatty acid composition and TAG molecular species were identical in EVOO and ROO (Mateos *et al.* 2005). After a washout period of 1 week, the subjects were submitted to a period of adaptation in which they were submitted to a National Cholesterol Education Program Step I diet supplemented with the corresponding fat (EVOO or ROO) for a further week. The diets were prepared by the subjects themselves under the supervision of a registered dietitian, and they consisted of whole foods according to calculated menus and standardized recipes. Participants were instructed to avoid consuming foods rich in polyphenols and tocopherols, and to refrain from intense physical exercise during the study. The subjects were then sampled after a 12 h overnight fast (baseline values), and they were immediately administered a fat-rich meal containing the corresponding dietary fat (EVOO or ROO, 50 g/m^2 body surface area) mixed with a portion of plain pasta (50 g), one slice of brown bread (28 g) and one skimmed yogurt. The average total energy provided by the meals was 3700 kJ (885 kcal) with a macronutrient profile of 72 % fat, 22 % carbohydrate and 6 % protein. Subsequently, blood samples were drawn every 1 h over a period of 8 h (postprandial values) into precooled tubes containing sodium citrate (final concentration 0·129 mmol/l). The plasma was separated immediately by centrifugation (2000 ***g***, 4°C, 20 min), and the aliquots were transferred into sterile cryovials of 1 ml and stored at −70°C until further analysis. TAG and other plasma lipids were quantified by autoanalyser using commercially available reagents (Roche Diagnostics GmbH, Mannheim, Germany). Plasma levels of sICAM-1 and sVCAM-1 were determined in duplicate with commercially available immunosorbent kits (ICAM-1 and VCAM-1 Eli-pair; Diaclone, Besançon, France). The intra- and inter-assay CV were below 5 %. All the assays were standardized according to the Standardization Program of the Spanish Society of Chemical Chemistry and the International Federation of Chemical Chemistry. Measurements were taken from each individual following both the EVOO and the ROO meals.

Statistics

Statistical analyses were carried out to compare the effects of each fat on the fasting and postprandial values, and to analyse the values from each fat at different time intervals. The net increment in the area under the curve (netAUC), including the entire incremental area below the curve and the area below the fasting concentration, was analysed by a one-factor repeated-measures ANOVA. A Bonferroni correction was used for the *post hoc* detection of significant pairwise differences. The netAUC was calculated by the trapezoidal method using Microsoft Excel 2000 version 9 (Microsoft Corp., Redmond, WA, USA). Univariate correlation analysis between variables was performed with Pearson's product moment correlations. The data were analysed by using Statview version 5 for Windows (SAS Institute, Cary, NC, USA). The designated level of significance was $P<0·05$.

Results

Fourteen healthy and fourteen hypertriacylglycerolaemic men aged 27 (SD 7) and 33 (SD 7) years, weight 75 (SD 6) and 79 (SD 8) kg, and BMI 24 (SD 2) and 24 (SD 5), respectively, participated in the study. There was no significant order or period effect of any of the measured parameters. Dietary intake of macro- and micronutrients was similar between diets and meals supplemented with EVOO and ROO, with the exception that EVOO and not ROO provided polyphenols and tocopherols. Table 1 shows the comparisons for plasma TAG, sICAM-1 and sVCAM-1 at the end of the adaptation period on the EVOO and ROO diets. The fasting values of TAG, sICAM-1 and sVCAM-1 were all higher in hypertriacylglycerolaemic subjects when compared to the healthy subjects ($P<0·001$). Fig. 1 presents the postprandial responses for plasma TAG, sICAM-1 and sVCAM-1 after the intake of the EVOO and ROO meal. There was no difference in the netAUC for TAG between the subject groups, or between the meal. However, compared with the ROO meal, healthy and hypertriacylglycerolaemic subjects had a lower netAUC for sICAM-1 and sVCAM-1 ($P<0·001$) on the EVOO meal.

Table 1. Fasting values of TAG, soluble isoform of intercellular adhesion molecule 1 (sICAM-1) and soluble isoform of vascular cell adhesion molecule 1 (sVCAM-1) after the lead-in washout and adaptation period on extra-virgin olive oil-enriched (EVOO) and refined olive oil-enriched (ROO) diets in healthy (*n* 14) and hypertriacylglycerolaemic (*n* 14) subjects

(Mean values and standard deviations)

	TAG (mg/l)		sICAM-1 (ng/ml)		sVCAM-1 (ng/ml)	
	Mean	SD	Mean	SD	Mean	SD
Healthy subjects						
EVOO	636	203	559·1	75·0	796·0	102·1
ROO	601	168	535·4	90·9	758·3	110·6
Hypertriacylglycerolaemic subjects*						
EVOO	3754	928	1209·1	196·5	1371·5	210·2
ROO	3622	514	1105·0	201·7	1342·8	238·7

Mean values were significantly different from those of the healthy subjects: *$P<0·001$.

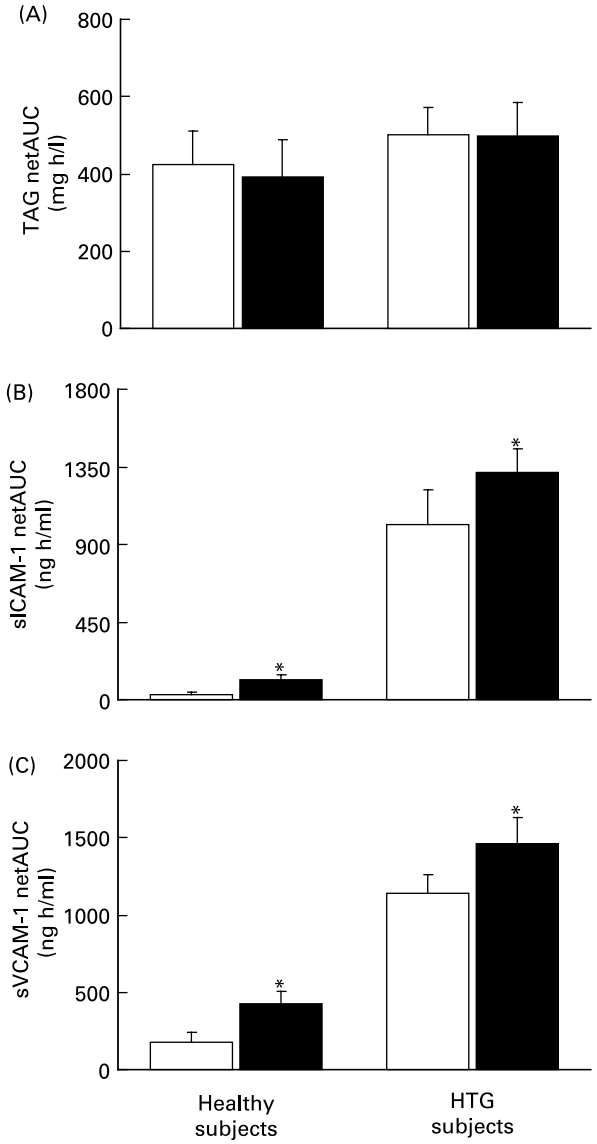

Fig. 1. TAG (A), soluble isoform of intercellular adhesion molecule 1 (sICAM-1; B) and soluble isoform of vascular cell adhesion molecule 1 (sVCAM-1; C) postprandial responses (net increment in the area under the curve (netAUC)) after the ingestion of extra-virgin olive oil-enriched (□) and refined olive oil-enriched (■) meals in healthy (*n* 14) and hypertriacylglycerolaemic (HTG; *n* 14) subjects. Values are means with their standard deviations depicted by vertical bars. Mean values were significantly different from those of the refined olive oil meal group: *$P<0·001$.

Discussion

The present study was designed to determine whether the acute intake of two olive oils with (EVOO) and without (ROO) minor compounds could have a selective influence on the postprandial levels of sICAM-1 and sVCAM-1. The primary novel finding is that the minor compounds of EVOO may postprandially reduce the release of sICAM-1 and sVCAM-1 after the acute intake of a high-fat meal in healthy and hypertriacylglycerolaemic subjects. These data are in line with both long- and short-term, as well as postprandial studies, demonstrating the cardiovascular benefits and protection induced by EVOO through endogenous antioxidant defences (Weinbrenner *et al.* 2004; Giugliano & Esposito, 2005; Ruano *et al.* 2005). Oxidative stress appears to be common during postprandial lipaemia (Tsai *et al.* 2004) and it is a result of the production of reactive oxygen species that activate several intracellular targets, including NFκB. The subsequent up-regulation of adhesion molecules may also be promoted by the impaired bioavailability of NO (Laroux *et al.* 2000). It is worth noting that minor compounds of EVOO, mainly phenolics, improve postprandial ischaemic reactive hyperaemia, as well as reduce oxidative stress and increase the accumulation of metabolites of NO (Ruano *et al.* 2005). Accordingly, unlike other fats EVOO does not elicit postprandial activation of NFκB in human monocytes (Bellido *et al.* 2004).

These novel effects of EVOO, shown here to be specifically mediated by its minor antioxidant compounds, complement the cardioprotective effects of oleic acid (Massaro *et al.* 1999; De Caterina & Massaro, 2005) and are consistent with a recent study showing that the chronic consumption of a Mediterranean diet enriched in EVOO decreased the activation of NF-κB and levels of sVCAM-1 compared with a typical Western diet in healthy men (Perez-Martinez *et al.* In the press). It is unlikely a specific effect of the fatty acids present in the meals, because EVOO and ROO had the same fatty acid composition and TAG molecular species. A recent study on the effect of simvastatin in reducing the postprandial levels of sICAM-1 and sVCAM-1 stressed the relevance of oxidative damage to the vascular endothelium rather than plasma levels of TAG following a meal (Ceriello *et al.* 2004). Therefore, the present study agrees with the notion that additional mechanisms which are dependent upon the antioxidant content of the meal are involved in sICAM-1 and sVCAM-1 postprandial response (Burdge & Calder, 2005).

In conclusion, the results of the study indicate that the consumption of EVOO with a high content in minor antioxidant compounds may help in reducing postprandial levels of adhesion molecules of the Ig superfamily, which suggests a protective postprandial anti-inflammatory effect in healthy and hypertriacylglycerolaemic subjects.

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