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Minor components of olive oil facilitate the triglyceride clearance from postprandial lipoproteins in a polarity-dependent manner in healthy men

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ABSTRACT

Postprandial triglyceride-rich lipoproteins (TRLs) are recognized as atherogenic particles whose lipid composition and function can be modified by the composition of dietary oils. This study was designed to test the hypothesis that minor components of pomace olive oil (POMACE) can not only change the composition of postprandial TRL but also affect the clearance of triglyceride (TG) molecular species of postprandial TRL. Meals enriched in either POMACE or refined olive oil (OLIVE) were administered to 10 healthy young men. TRL were isolated from serum at 2, 4, and 6 hours postprandially, and their fatty acid and TG molecular species compositions were analyzed by gas chromatography. The apolipoprotein B concentration was determined by immunoturbidimetry. POMACE and OLIVE, differing mainly in their unsaponifiable fraction, led to similar fatty acid and TG molecular species profiles in postprandial TRL. However, POMACE-TRL presented a higher particle size, estimated as TG to apolipoprotein B ratio, which was also found for the main TG molecular species (trioleoyl-glycerol, palmitoyl-dioleoyl-glycerol, palmitoyl-oeloyl-linoleoyl-glycerol, and dioleoyl-linoleoyl-glycerol). TG from POMACE-TRL also showed higher clearance rates. In this regard, apolar TG (with a higher equivalent carbon number) disappeared more rapidly from TRL particles obtained after the ingestion of either POMACE or OLIVE. In conclusion, minor components of POMACE facilitated TG clearance from TRL by modifying their particle size and the hydrolysis of the most apolar species.

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1. Introduction

There is a growing consensus that postprandial hypertriglyceridemia is a potential independent cardiovascular risk factor [1]. Triglyceride-rich lipoproteins (TRL) can cross the endothelial barrier and enter into the vascular wall [2], where they can enhance lipid accumulation into macrophages, leading to foam cell formation [3]. TRL consist of

Abbreviations: ANOVA, analysis of variance; apo, apolipoprotein; ECN, equivalent carbon number; GC, gas chromatography; LDL, low-density lipoprotein; LPL, lipoprotein lipase; MUFA, monounsaturated fatty acids; OLIVE, refined olive oil; OLL, oleoyl-dilinoleoyl-glycerol; OOL, dioleoyl-linoleoyl-glycerol; OOO, trioleoyl-glycerol; POMACE, pomace olive oil; POL, palmitoyl-oleoyl-linoleoyl-glycerol; POO, palmitoyl-dioleoyl-glycerol; SOL, stearoyl-oleoyl-linoleoyl-glycerol; SOO, stearoyl-dioleoyl-glycerol; TG, triglyceride; TRL, triglyceride-rich lipoprotein; VLDL, very low-density lipoprotein.

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chylomicrons, which are secreted by the small intestine and contain apolipoprotein (apo) B-48 as the structural protein, and very low-density lipoproteins (VLDL), which originate in the liver and contain apo B-100. In addition, TRLs also include chylomicron and VLDL remnant particles, partially depleted of triglycerides (TG) and enriched with cholesteryl esters. The transformation of TRL into remnant particles is dependent upon TG hydrolysis by lipoprotein lipase (LPL), which is attached to the surface of the vascular endothelium [4]. The enzyme can differentiate between substrates and exhibits specificity with respect to fatty acid length chain and unsaturation [4,5]. Therefore, the composition of TRL-TG is decisive for the activity of LPL and the formation of TRL remnants.

The Mediterranean diet, characterized by a high consumption of monounsaturated fatty acids (MUFA), has been proposed as a healthy dietary standard because it is associated with a low rate of cardiovascular mortality [6]. However, we have demonstrated that not all MUFA-rich oils exert the same effects on the magnitude and duration of postprandial triglyceridemia [7]. Other factors, such as minor nonfatty acid constituents (unsaponifiable fraction), rather than the content of oleic acid, may be responsible for the postprandial responses to virgin olive oil and for the effects of TRL and their remnants. In this regard, we have recently reported that the unsaponifiable fraction of virgin olive oil, contained in circulating TRL, improves the balance between vasoprotective and prothrombotic factors released by endothelial cells [8].

Pomace olive oil (POMACE) is obtained by chemical processes from residues of the extraction of virgin olive oil. The new improved procedures for POMACE extraction allow the presence of a number of unsaponifiable components from the skin of the olive, including elevated amounts of sterols, tocopherols, waxes, and triterpenic acids and alcohols, such as oleanolic acid and erythrodiol [9]. To our knowledge, there is no study assessing the effects of POMACE on the composition and clearance of TG molecular species contained in postprandial lipoproteins. Therefore, the hypothesis of the present work was that, in addition to modification of postprandial TRL-TG composition, minor components of POMACE can affect the clearance of their TG molecular species in men. To test this hypothesis, we aimed to determine the TG composition of postprandial TRL after the intake of POMACE and to compare this effect with that of a refined olive oil (OLIVE), with a low unsaponifiable content, to evaluate its potential impact on TRL metabolism and their metabolic consequences. Because postprandial studies are only slightly invasive, they allow the use of human beings for experimentation, provided all ethical issues are considered.

2. Methods and materials

2.1. Subjects and study design

Ten healthy men, aged 26.2 ± 4.3 years and with body mass indexes of 23.7 ± 2.0 kg/m^2, participated in the study. Subjects were excluded if they had any digestive or metabolic disorder, were taking dietary supplements, or were under medication of any kind. The number of participants was chosen in accordance to similar studies [7,8,10-12]. A fasting blood sample was collected to ensure that recruited subjects had plasma TG and glucose concentrations within normal limits (Table 1). These parameters were checked at the beginning of the 2 phases of the study, that is, at baseline and before administration of either experimental meal. Participants gave written informed consent to a protocol approved by the Institutional Committee on Human Research (Hospital Universitario Virgen del Rocio, Seville, Spain). All procedures were in accordance with the institutional and national ethical standards for human experimentation and the Helsinki Declaration of 1964 and its later amendments.

The study was designed as a randomized cross-over trial. On the day of the experiment, the subjects consumed 2 different meals that were enriched with one of the test oils, either POMACE or OLIVE. Meals consisted of 1 slice of brown bread (28 g), 1 skimmed yogurt (125 g), and plain pasta (100 g, cooked with 200 mL of water) with fresh tomato (130 g) that was previously mixed with the corresponding oil (70 g). A washout period of 2 weeks was established between experiments. The oils contributed 2587 kJ of energy, whereas the whole meal provided 4523 kJ, distributed as follows: 32.5% carbohydrate, 7.6% protein, and 59.9% fat.

Participants were asked to have a low-fat dinner the prior evening and to abstain from alcohol drinking and smoking for 24 hours before the postprandial study. On arrival, after an overnight fast (12 hours), a cubital vein was catheterized, and a baseline blood sample was taken immediately before consumption of the test meal. Following the intake, blood samples were collected at 2, 4, and 6 hours postprandially. During the course of the experiment, subjects were allowed to drink water and undertake only light activities.

Serum was recovered by centrifugation (1620*g*, 30 minutes, 4°C), and sodium azide, phenymethylsulfonyl fluoride, and aprotinin (Sigma-Aldrich, Poole, UK) were added to a final concentration of 1 mmol/L, 10 μmol/L, and 0.5 mg/L, respectively.

2.2. Olive oil composition

Refined olive oil and POMACE were kindly supplied by Oleicola El Tejar, S.A. (El Tejar, Cordoba, Spain). To determine the fatty acid composition of the oils, TG were transmethylated using a solution of KOH (2 N) in methanol, following the procedure

Table 1 – Baseline lipid and apo serum concentrations in normolipidemic men participating in the study

	Concentration (mg/dL)
Total cholesterol	149 ± 28
LDL cholesterol	75 ± 12
HDL cholesterol	70 ± 18
Triglyceride	60 ± 17
apo A	149 ± 23
apo B	59 ± 17
LDL/HDL	1.6 ± 0.4

Data are expressed as means ± SD; n = 10. Abbreviation: *HDL*, high-density lipoprotein.

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