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

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24 **ABBREVIATIONS**

25 Apo; apolipoprotein

26 CN; carbon number

27 DB; number of double bonds

28 ECN; equivalent carbon number

29 LRP; LDL receptor-related protein

30 LPL; lipoprotein lipase

31 MPP; myristoyl-dipalmitoyl-glycerol

32 MTP; microsomal triacylglycerol transfer protein

33 MUFA; monounsaturated fatty acids

34 NUFA; number of unsaturated fatty acids

35 OLIVE, refined olive oil

36 OLL; oleoyl-dilinoleoyl-glycerol

37 OOL; dioleoyl-linoleoyl-glycerol

38 OOO; trioleoyl-glycerol

39 PLL; palmitoyl-dilinoleoyl-glycerol

40 POMACE; pomace olive oil

41 POL; palmitoyl-oleoyl-linoleoyl-glycerol

42 POO; palmitoyl- dioleoyl-glycerol;

43 PN; partition number

44 SLL; stearoyl-dilinoleoyl-glycerol

45 SOL; stearoyl- oleoyl-linoleoyl-glycerol;

46 SOO; stearoyl-dioleoyl-glycerol

47 SPE; solid-phase extraction

48 TG; triglyceride

49 **ABSTRACT**

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

68

69 **KEY-WORDS**: olive oil; human; triglyceride; lipoprotein; triolein; apolipoprotein B; fatty
70 acid.

71 **1. INTRODUCTION**

72 There is growing agreement that postprandial hypertriglyceridemia is a potential independent
73 cardiovascular risk factor [1]. Triglyceride-rich lipoproteins (TRL) can cross the endothelial
74 barrier and enter into the vascular wall [2], where they can enhance lipid accumulation into
75 macrophages, leading to foam cell formation [3]. TRL consist of chylomicrons, which are
76 secreted by the small intestine and contain apolipoprotein (apo) B-48 as the structural protein,
77 and VLDL, originated in the liver and containing apo B-100. In addition, TRL also include
78 chylomicron and VLDL remnant particles, partially depleted of triglycerides (TG) and
79 enriched with cholesteryl esters. The transformation of TRL into remnant particles is
80 dependent upon TG hydrolysis by lipoprotein lipase (LPL), which is attached to the surface of
81 the vascular endothelium [4]. The enzyme can differentiate between substrates and exhibits
82 specificity with respect to fatty acid length chain and unsaturation [4,5]. Therefore, the
83 composition of TRL-TG is decisive for the activity of LPL and the formation of TRL
84 remnants.

85

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

93 the unsaponifiable fraction of virgin olive oil, contained in circulating TRL, improves the
94 balance between vasoprotective and pro-thrombotic factors released by endothelial cells [8].
95
96 Pomace olive oil (POMACE) is obtained by chemical processes from residues of the
97 extraction of virgin olive oil. The new improved procedures for POMACE extraction allow
98 the presence of a number of unsaponifiable components from the skin of the olive, including
99 elevated amounts of sterols, tocopherols, waxes and triterpenic acids and alcohols, such as
100 oleanolic acid and erythrodiol [9]. To our knowledge there is no study assessing the effects of
101 POMACE on the composition and clearance of TG molecular species contained in
102 postprandial lipoproteins. Therefore, the hypothesis of the present work was that, in addition
103 to modification of postprandial TRL-TG composition, minor components of POMACE can
104 affect the clearance of their TG molecular species in men. To test that hypothesis, we aimed to
105 determine the TG composition of postprandial TRL after the intake of POMACE and to
106 compare this effect with that of a refined olive oil (OLIVE), with a low unsaponifiable
107 content, in order to evaluate its potential impact on TRL metabolism and their metabolic
108 consequences. Since postprandial studies are only slightly invasive, they allow they use of
109 human beings for experimentation, provided all ethical issues are considered.

110 **2. METHODS AND MATERIALS**

111

112 **2.1. Subjects and Study Design**

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

124

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

132 Participants were asked to have a low-fat dinner the prior evening and to abstain from alcohol
133 drinking and smoking for 24 h before the postprandial study. On arrival, after an overnight
134 fast (12 h), a cubital vein was catheterized and a baseline blood sample was taken
135 immediately before consumption of the test meal. Following the intake, blood samples were
136 collected at 2, 4 and 6h postprandially. During the course of the experiment, subjects were
137 allowed to drink water and undertake only light activities.
138
139 Serum was recovered by centrifugation (1620 x g, 30 min, 4ºC) and sodium azide,
140 phenymethylsulfonyl fluoride and aprotinin (Sigma-Aldrich, Poole, UK) were added to a final
141 concentration of 1 mmol/L, 10 μmol/L, and 0.5 mg/L, respectively.
142
143 **2.2. Olive oil composition**
144 OLIVE and POMACE were kindly supplied by Oleicola El Tejar, S.A. (El Tejar, Cordoba,
145 Spain). To determine the fatty acid composition of the oils, TG were transmethylated using a
146 solution of KOH (2N) in methanol, following the procedure described on the EU Regulations
147 (CE Nº2568/91). Resultant fatty acid methyl esters were analyzed by gas chromatography
148 (GC), using a model 5890 series II gas chromatograph (Hewlett-Packard Co, Avondale, USA)
149 equipped with a flame ionization detector and a capillary silica column Supelcowax 10
150 (Sulpelco Co, Bellefonte, USA) of 60 m length and 0.25 mm internal diameter with hydrogen
151 as a carrier gas. The injector and detector were set at a constant temperature of 250 ºC during
152 the analysis. The oven temperature was programmed to start at 180 ºC during 10 min and then
153 to increase until 250 ºC at 2 ºC/min rate. External standards used for identification and

154 quantification of the resulting chromatographic peaks were purchased from Sigma-Aldrich
155 (Poole, UK). Fatty acid methyl esters were quantified as weight percentages.
156
157 TG molecular species contained in the oils were also determined by GC after dissolving oil
158 samples in hexane. The gas chromatograph (model 5890 series II, Hewlett-Packard Co,
159 Avondale, USA) was equipped with a Quadrex Aluminium-Clad 400-65HT column (Quadrex,
160 Woodbridge, USA) with 30 m length and 0.25 mm internal diameter, using a linear gas rate of
161 50 cm/s and a split ratio 1:80. The injector and detector temperatures were both 380 ºC, the
162 oven temperature was 345 ºC and a head pressure gradient from 70 to 120 kPa was applied.
163
164 The composition of the unsaponifiable fraction of the oils, including total unsaponifiable
165 matter, tocopherols, sterols, squalene, waxes and erythrodiol+uvaol, was provided by the
166 manufacturer (Oleicola El Tejar, S.A., El Tejar, Cordoba, Spain).
167
168 **2.3. Postprandial TRL isolation**
169 Postprandial TRL were isolated from 4.5 mL of serum collected at 2, 4, and 6 h after the
170 intake of the test meals enriched with OLIVE or POMACE. Serum was layered under 6 mL of
171 NaCl solution (d = 1.006 kg/L) and TRL were obtained by a single ultracentrifugation spin
172 (39,000 rpm, 18 h, 12ºC). Ultracentrifugation was performed using a SW 41Ti swinging
173 bucket rotor in a Beckman L8-70M preparative ultracentrifuge (Beckman Instruments, Palo
174 Alto, USA). Different postprandial time points for TRL isolation were chosen according to the
175 hours at which the maximum and minimum serum TG concentration values had been
176 previously found [10].

177 **2.4. Triglyceride-rich lipoprotein composition**

178 Total lipids in TRL were extracted following the method of Folch et al. [13]. To determine the
179 fatty acid composition of TG in TRL, this lipid class was separated from a 150 μL total lipid
180 aliquot by solid-phase extraction, using diol bonded-phase columns (Supelclean LC-Diol,
181 Supelco, Bellefonte, USA). TG were transmethylated using sodium methoxide in methanol
182 (0.5 %, m/v) and the resulting fatty acid methyl esters were analyzed by GC, using the same
183 equipment and conditions described above. The analysis of TG molecular species in TRL was
184 carried out by GC using the equipment described above for the TG analysis of the oils, and
185 following the same experimental conditions. The equivalent carbon number (ECN) of TRL-
186 TG was calculated as described elsewhere [14] using the following equation: ECN=CN-2·DB-
187 0.2·NUFA, where CN is the number of carbon atoms in the TG molecule corresponding to
188 fatty acids, DB is the number of double bonds of the fatty acids and NUFA is the number of
189 unsaturated fatty acids in the molecule.

190

191 The apo B composition was determined by immunoturbidimetry (Tina-quant; Roche
192 Diagnostics, Mannheim, Germany), following the manufacturer's instructions.

193

194 **2.5. Statistical analyses**

195 Results were expressed as means ± SD (n=10). Data analyses and graphs were performed
196 using the GraphPad Prism® 5 statistical package (GraphPad Software Inc., San Diego, USA).
197 Statistical significance of differences between the effects of both experimental oils was
198 evaluated by paired t-test analyses, while postprandial changes were analyzed by one-way
199 ANOVA followed by Bonferroni’s multiple comparison test. Correlations between variables

200 were assessed using Pearson's correlation coefficients. Differences were considered

201 statistically significant at $P<0.05$.

202

203 **3. RESULTS**

204 **3.1. Fatty acid and molecular species composition of OLIVE and POMACE**

205 The fatty acid composition of OLIVE and POMACE was similar (Table 2) in terms of MUFA
206 (nearly 80%) and saturated fatty acids (SFA) concentrations, although the amount of stearic
207 acid (18:0) was slightly higher in OLIVE and that of linoleic acid (18:2, n-6) higher in
208 POMACE. The main TG molecular species were composed of the main fatty acids (Table 2),
209 with trioleoyl-glycerol (OOO) accounting for almost half of the TG determined in both oils.
210 Only slight differences were found in the TG molecular species composition of OLIVE and
211 POMACE. Whereas OLIVE was richer in palmitoyl-dioleoyl-glycerol (POO) and stearoyl-
212 oleoyl-linoleoyl-glycerol (SOL), a higher concentration of dioleoyl-linoleoyl-glycerol (OOL)
213 and oleoyl-dilinoleoyl-glycerol (OLL) was observed in POMACE. In any case, although
214 significant, differences were lower than 4%.

215

216 **3.2. Unsaponifiable fraction composition of POMACE and OLIVE.**

217 More important differences were found among the components of the unsaponifiable fraction
218 (Table 3). The concentration of sterols in POMACE doubled that of OLIVE and the
219 concentration of tocopherols was nearly 5 times higher in POMACE than in OLIVE, with α-
220 tocopherol as the main species. The concentration of the triterpenic alcohols erythrodiol and
221 uvaol was nearly 30 times higher in POMACE. Likewise, a high difference was found in the
222 content of waxes (fatty alcohols) between the two oils. In contrast, the squalene content was
223 similar in both oils.

224

225

226 **3.3. Fatty acid composition of postprandial triglyceride-rich lipoproteins**

227 Table 4 shows the fatty acid composition of postprandial TRL at 2, 4 and 6h after the intake of
228 the experimental meals. The intake of POMACE-rich meal caused a higher presence of oleic
229 acid in the particles at 2h compared to the intake of OLIVE. While the content of this fatty
230 acid in POMACE-TRL was not modified throughout the postprandial period, in TRL obtained
231 after the intake of OLIVE its concentration was higher at 4 and 6h compared to 2h. The
232 linoleic acid content was also higher at 2h after the intake of POMACE and, alike oleic acid
233 but not, it did not change at 4 and 6h after the intake of this oil. No modifications in the
234 linoleic acid concentrations of OLIVE-TRL were observed at any time point. The palmitic
235 and stearic acid content was reduced in TRL during the postprandial period after the OLIVE-
236 rich meal was administrated but not after the intake of POMACE. It is noteworthy that the
237 stearic acid content in postprandial TRL at 2h after POMACE was approximately half of that
238 observed in the particles after OLIVE was ingested.

239

240 **3.4. Triglyceride molecular species composition of postprandial triglyceride-rich**
241 **lipoproteins**

242 Table 5 shows the TG molecular species composition of postprandial TRL at 2, 4 and 6h after
243 the intake of the experimental meals. TG molecular species concentrations did not vary
244 significantly during the postprandial period and differences were only significant for minor
245 TG (<1 mg/100mg). In contrast, significant differences were found when the concentrations
246 of TG molecular species analyzed from TRL obtained after administration of POMACE or
247 OLIVE were compared at each experimental time point. The intake of POMACE resulted in
248 higher concentrations of linoleic acid-containing TG, such as OLL, and OOL at 2h, and at 4

249 and 6h a higher presence of in palmitoyl-dilinoleoyl-glycerol (PLL), stearoyl-dilinoleoyl-
250 glycerol (SLL) and palmitoyl-oleoyl-linoleoyl-glycerol (POL) was observed. Conversely,
251 consumption of OLIVE resulted in higher concentrations of TG rich in oleic acid and SFA,
252 such as OOO, POO, myristoyl-dipalmitoyl-glycerol (MPP) and stearoyl-dioleoyl-glycerol
253 (SOO). All these TG were present in higher concentrations at 2h after the intake of OLIVE,
254 but the oleic acid-rich TG (OOO, POO and SOO) were found in higher concentrations also at
255 4 and 6h (except for OOO).
256

257 **3.5. Triglyceride/apolipoprotein B ratios**

258 Apo B concentration in TRL was significantly lower at 2h and 6h after the intake of
259 POMACE compared to OLIVE (Fig. 1). These concentrations were used to calculate the TG
260 to apo B ratios (Fig. 2A, 2B, 2C and 2D) to estimate the TG molecular species clearance from
261 the particle during the postprandial period. For the four main TG (OOO, POO, POL, OOL),
262 the TG/apo B ratio was significantly higher at 2h after the intake of POMACE compared to
263 OLIVE. However, at 4 and 6h only OOL/apo B and POL/apo B ratios remained higher in
264 POMACE-TRL. In any case, for the four main TG, the TG/Apo B ratio was more drastically
265 reduced if they formed part of TRL obtained after the intake of POMACE compared to
266 OLIVE. This effect was highlighted when the variations of TG concentrations (Fig. 3A) and
267 TG/Apo B ratios (Fig. 3B) between 2h and 4h were plotted against the equivalent carbon
268 number (ECN) of TG, taken as an estimation of their polarity. Both TG concentrations and
269 TG/Apo B ratios correlated negatively with ECN regardless of the TRL origin, POMACE or
270 OLIVE.

271 **4. DISCUSSION**

272 POMACE, which is obtained from the residues of virgin olive oil extraction, is specially rich
273 in lipophilic minor components that may have important roles in the composition of
274 postprandial and fasting TRL [10]. In the present study, we report the fatty acid and TG
275 molecular species composition of postprandial TRL obtained from healthy males at 2, 4 and
276 6h after the intake of meals rich in POMACE or OLIVE. These dietary oils have similar fatty
277 acid and TG profiles but considerable differences in the content of minor components from
278 the unsaponifiable fraction.

279 The fatty acid composition of postprandial TRL obtained after the intake of POMACE was
280 similar to that of particles obtained after administration of OLIVE. Significant differences
281 were found only for stearic, oleic and linoleic acids and mainly at 2h after the intake of the
282 oils. This weak effect was also reflected in the TG molecular species composition of
283 postprandial TRL. Out of 26 TG species quantified, only 9 were different between POMACE-
284 TRL and OLIVE-TRL and among these, only 3 were significantly different at the three time
285 points studied (2, 4 and 6 h). Consequently, differences were very modest and related to the
286 slight differences in TG molecular species composition of the experimental oils. This is
287 consistent with previous observations using virgin olive oil [15,16]. In these studies, the
288 effects on TRL composition of virgin olive oil supplemented in minor components was
289 compared to the effect of non-supplemented virgin olive oil; hence, with exactly the same TG
290 molecular species composition. From these findings, we suggested that postprandial TRL-TG
291 profile was mainly dependent upon the TG molecular species composition and not the
292 unsaponifiable fraction of virgin olive oil.

293 Nevertheless, minor components of virgin olive oil have been related to modifications of the
294 TG molecular species composition of TRL. In a randomized cross-over controlled trial,
295 consumption of olive oil with different concentrations of phenolic compounds (refined,
296 common and virgin olive oils) resulted in important effects on the TG composition of VLDL
297 [17]. After the intake of the olive oil with the highest phenolic content (virgin olive oil),
298 VLDL presented higher concentrations of linoleic acid–containing TG and lower
299 concentrations of those TG containing palmitic acid. Interestingly, a significant positive
300 correlation between the phenolic content in the oil and linoleic acid-rich moieties was
301 observed. This phenomenon was associated with a possible effect of phenolics on the
302 modulation of the gene and protein expression or activities of key enzymes involved in TG
303 transport and metabolism, like LPL [18], apo B48, microsomal TG transfer protein (MTP)
304 [19], or receptors involved in the uptake of VLDL by the liver [20].

305 The TG composition of TRL is decisive for their clearance through hydrolysis by LPL and/or
306 liver uptake. Previous work has shown that the TRL uptake by the liver is modulated by the
307 fatty acid composition of the TG in the particle [21]. Sato et al. [5, 22] suggested that
308 modifications in lipoprotein fluidity by means of changing the polarity of the TG present in
309 TRL might modulate the affinity between the particles and LPL. In a previous work [16], we
310 observed that the rate of hepatic uptake of high-oleic sunflower oil-TRL was significantly
311 higher than that of TRL derived from virgin olive oil, probably due to the up-regulation of
312 mRNA expression for LDL receptor-related protein (LRP), one of the main receptors involved
313 in lipoprotein uptake by the liver. However, the observed effect might also involve changes in
314 the TG molecular species of TRL causing differential interaction with the receptors, and/or
315 differences in the content of the unsaponifiable fraction.

316 TRL clearance also depends on particle size. Karpe et al. [23] demonstrated that large TRL are
317 cleared from the plasma at a faster rate than small VLDL-sized TRL. In a previous study,
318 compared to OLIVE, POMACE led to higher TG/Apo B-48 and TG ratio/Apo B-100 ratios in
319 TRL obtained at 2h after the intake of the oils, leading to larger particles [10]. This effects
320 was associated to faster clearance rates and was attributed to the influence of the minor
321 components present in POMACE [10]. The higher estimated size (TRL/Apo B ratio) of
322 POMACE-TRL, compared to OLIVE-TRL was confirmed in the present study for all TG
323 molecular species (data shown for the main TG only, OOO, POO, POL and OOL). We also
324 plotted the variation of the TG concentrations and TG/Apo B ratios from 2h to 4h (TRL
325 clearance) against the ECN of each TG molecular species analyzed, finding a very significant
326 correlation. The ECN is used in chromatography as a determinant of the elution order of TG
327 and, thus, of their polarity [24]. Because this parameter is inherent to TG molecules, the ECN
328 can also be used as an indicator of TG polarity in lipoproteins, which can, in turn, determine
329 their propensity to be hydrolyzed by LPL. The correlation showed that TG with higher ECN
330 values (more apolar TG, containing mainly SFA) disappear more rapidly from each TRL
331 particle compared to those with lower ECN values (more polar TG, rich in MUFA and
332 PUFA). This is in agreement with previous observations in rats by Sato et al. [5], who
333 demonstrated that the V-max of LPL for chylomicrons and VLDL increased linearly with the
334 increased palmitic acid content in the lipoprotein TG, hence with more apolar TG molecular
335 species. These authors had previously suggested that lipoprotein catalysis by LPL is
336 modulated by the palmitic acid content of the lipoprotein triglyceride, which reduces the
337 fluidity of lipoproteins, enhancing the LPL-lipoprotein contact, compared to oleic and linoleic
338 acids [22]. Unfortunately, they did not include stearic acid-rich TG in the comparison. In

339 addition, we found that the variation of TG concentrations between 2h and 4h was higher in
340 the postprandial TRL obtained after the intake of POMACE for all TG molecular species,
341 which suggests that the high presence of minor components might be exerting an effect in TG
342 clearance. We observed this phenomenon for all TG molecular species, which indicates that
343 the effect the minor components is not selective.

344 In conclusion, the unsaponifiable fraction of POMACE influenced the clearance rate of TG
345 molecular species in postprandial TRL, which was related to their size, estimated as TG/Apo
346 B ratio. This conclusion is consistent with the hypothesis postulated at the beginning of the
347 study. Importantly, TG were cleared from TRL in a polarity-dependent manner, with the most
348 apolar TG molecular species being removed in the first place. Nevertheless, the study has
349 some limitations. Firstly, despite being in agreement with similar postprandial studies [7,8,10-
350 12], the number of participants was rather low and focused in one gender only. Secondly, TRL
351 particle size was estimated from the Apo B and TG content. Direct methods, such as dynamic
352 light scattering [25], can give a more precise estimation of particle size. Finally, the ECN is
353 not a continuous variable for which it should not be used to predict the actual TG clearance
354 from TRL.

355

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359

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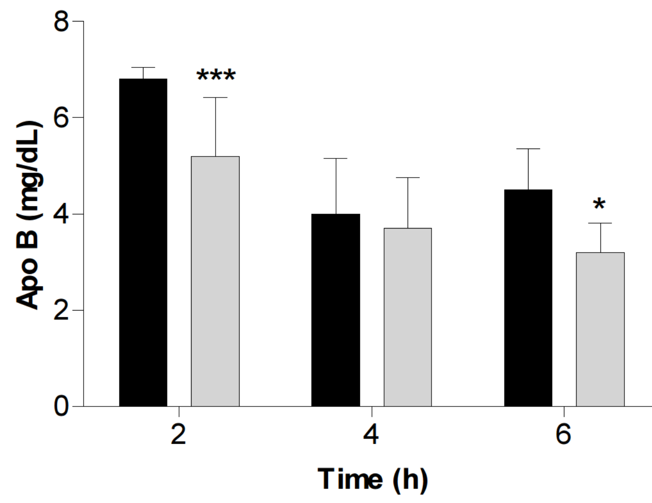
436

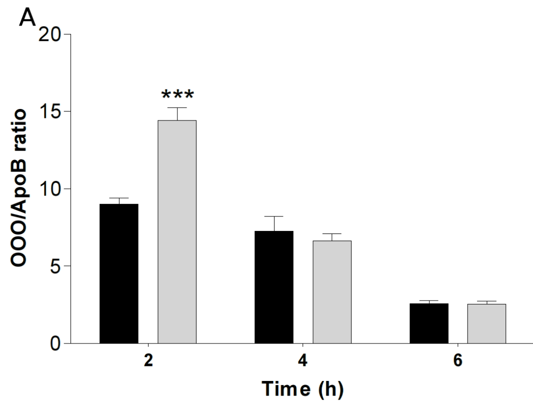
Fig. 1 Apolipoprotein B (Apo B) concentrations in triglyceride-rich lipoproteins collected at 2, 4 and 6 hours after the intake of refined olive oil (OLIVE, black bar) or pomace olive oil (POMACE, grey bar). Statistical significance of differences between the effects of both experimental oils was evaluated by paired t-test analyses. *: $p < .05$, **: $p < .01$, vs. OLIVE. Data are expressed as means ± SD, n = 10.

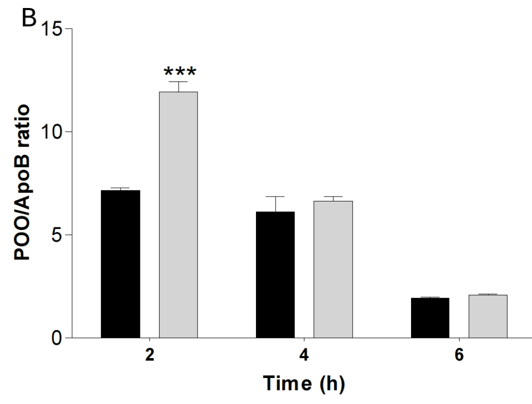
Fig. 2 Triacylglycerol molecular species to apolipoprotein B ratio (TG/Apo B) in triglyceride-rich lipoproteins collected at 2, 4 and 6 hours after the intake of refined olive oil (OLIVE, black bar) or pomace olive oil (POMACE, grey bar). Statistical significance of differences between the effects of both experimental oils was evaluated by paired t-test analyses.*: p < . 05, **: $p < .01$, ***: $p < .001$, vs. OLIVE. 2A: trioleoyl-glycerol, OOO; 2B: palmitoyl-dioleoyl-glycerol, POO; 2C: palmitoyl-oeloyl-linoleoyl-glycerol, POL; 2D: dioleoyl-linoleoyl-glycerol, OOL. Data are expressed as means ± SD, n = 10

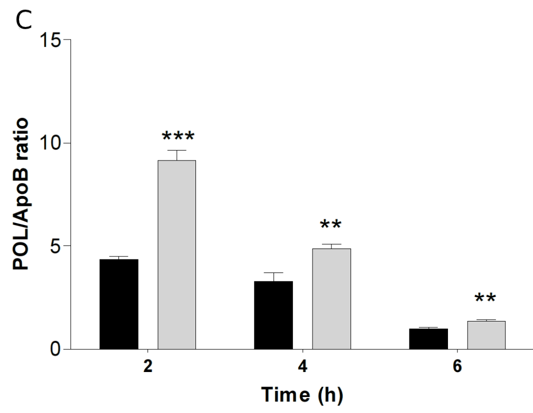
Fig. 3 Variations from 2h to 4h after the intake of pomace olive oil (POMACE, black dots) or refined olive oil (OLIVE, white dots) of triglyceride (TG) concentrations (3A) and TG to apolipoprotein B ratios (TG/Apo B, 3B) against the equivalent carbon number (ECN). Correlations between variables were assessed using Pearson's correlation coefficients.

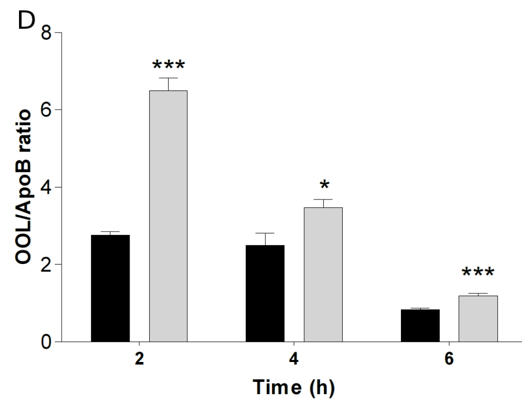


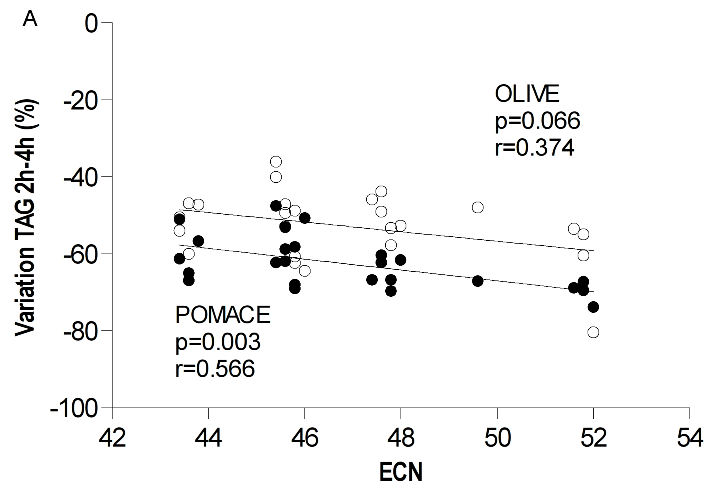
Apo B (mg/dL)
8
6
4
2
0
***
*
2
4
6
Time (h)

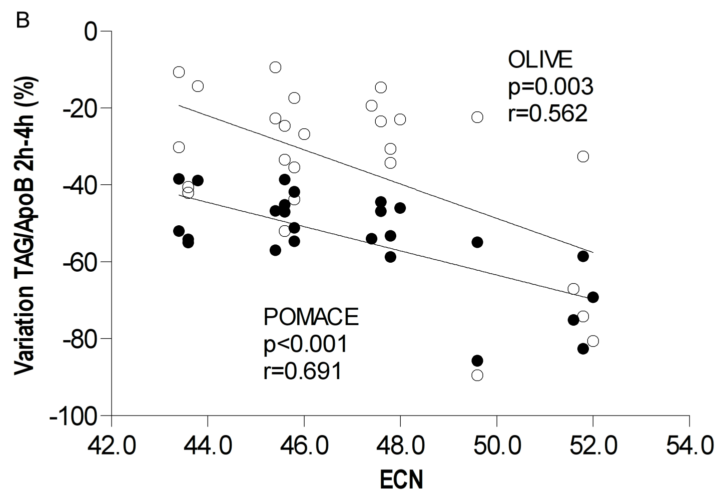

A
OOO/ApoB ratio
20
15
10
5
0
***
2
4
6
Time (h)


B
POO/ApoB ratio
15
10
5
0
***
2
4
6
Time (h)


C
POL/ApoB ratio
15
10
5
0
***
**
**
2
4
6
Time (h)


D
OOL/ApoB ratio
8
6
4
2
0
***
*
***
2
4
6
Time (h)


A
OLIVE
p=0.066
r=0.374
POMACE
p=0.003
r=0.566
Variation TAG 2h-4h (%)
ECN
0
-20
-40
-60
-80
-100
42
44
46
48
50
52
54


B
OLIVE
p=0.003
r=0.562
POMACE
p<0.001
r=0.691
Variation TAG/ApoB 2h-4h (%)
ECN
0
-20
-40
-60
-80
-100
42.0
44.0
46.0
48.0
50.0
52.0
54.0