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1 **Divergent anti-inflammatory effects of different oil acute consumption on**
2 **healthy individuals**

3

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22

23 **Abstract**

24 **Objective:** Inter-cellular adhesion molecule 1 (ICAM-1), vascular cell adhesion
25 molecule 1 (VCAM-1) and tumor necrosis factor alpha (TNF-a), are implicated in
26 atherogenesis. Additionally, several types of oil as part of different types of diet are
27 associated with the initiation of atherosclerosis and consequently with the risk of
28 cardiovascular disease. However, the exact role of oil consumption on vascular
29 inflammation remains unknown. In the present parallel study, we assessed the acute
30 effects of extra virgin olive oil, soy oil, corn oil and cod liver oil on circulating
31 soluble (s) forms of adhesion molecules and TNF-a.

32 **Methods:** Sixty seven healthy volunteers were randomized to receive 50 ml of oil.
33 Soluble forms of VCAM-1, ICAM-1 and TNF-a were measured by enzyme-linked
34 immunosorbent assay (ELISA) at baseline and 3 hours post oil consumption.

35 **Results:** All types of oil had no significant effect on sVCAM-1 levels ($p=NS$ for all).
36 On the contrary, all oil types decreased ICAM-1 levels ($p<0.01$). Olive oil ($p<0.05$),
37 soy oil and cod liver oil ($p<0.01$ for both) reduced TNF-a levels significantly, in
38 contrast to corn oil, which induced a non-significant decrease ($p=NS$). Moreover,
39 there was a significant correlation between the absolute change in ICAM-1 and TNF-a
40 levels ($\rho=0.379$, $p<0.05$), but not between the absolute changes in VCAM-1 and
41 TNF-a levels ($\rho=0.019$, $p=NS$).

42 **Conclusions:** Acute consumption of all types of oil decreased significantly ICAM-1
43 levels. In addition, olive oil, soy oil and cod liver oil decreased significantly TNF-a
44 levels. Moreover, the absolute change in TNF-a levels was correlated with the

45 absolute change in ICAM-1 levels. These findings indicate that acute consumption of
46 specific types of oil is associated with specific significant anti-inflammatory effects.

47 **Keywords:** oil; ICAM-1; VCAM-1; TNF-a; inflammation

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64 **Introduction**

65 Evidence suggests that inflammatory process contributes to the development
66 of atherosclerosis (Libby *et al.*, 2009). The role of several inflammatory biomarkers,
67 such as acute phase proteins, proinflammatory cytokines and adhesion molecules, is
68 nowadays under investigation as regards the relation with cardiovascular disease
69 (Biasucci *et al.*, 2004).

70 Tumor necrosis factor alpha (TNF- α) is known to be released by macrophages,
71 as well as vascular endothelial and smooth muscle cells, promoting the expression of
72 cell adhesion molecules (CAMs) and the migration of leukocytes into and through the
73 vascular wall (Klein *et al.*, 1995). Migration to the arterial wall, rolling and tethering
74 of leukocytes to the endothelium (adhesion) play a crucial role in atherogenesis.
75 Adhesion is a complex process depending on interactions between molecules such as
76 the vascular adhesion molecule-1 (VCAM-1) and the intercellular molecule-1 (ICAM-
77 1) on the endothelium and the integrins on neutrophils (Meydani, 2003). Interestingly,
78 studies have shown that despite the similar structure and function of VCAM-1 and
79 ICAM-1, these two molecules may participate at different stages of atherogenesis
80 (Blann *et al.*, 2000).

81 Recently, it is widely reported that dietary habits may modify cardiovascular
82 disease rates in diverse populations. Adhesion molecules and proinflammatory
83 cytokines are implicated as important mediating pathways for cardiovascular diseases,
84 activated through different intakes of food groups, such as alcohol (Bau *et al.*, 2007),
85 as well as obesity (Forsythe *et al.*, 2008). Accordingly, despite the fact that it is not
86 easy to specify substances with anti-atherosclerotic properties comprising diets,
87 studies have shown that components such as olive oil or n-3 fatty acids may play a

88 key role in cardiovascular protection, by especially inhibiting inflammatory process
89 (Perez-Martinez *et al.*, 2010; Massaro *et al.*, 2008). However, the impact of acute
90 consumption of different types of oil on proinflammatory cytokines and adhesion
91 molecules remains unknown.

92 Thus, the aim of the present study was to examine the acute effects of extra
93 virgin olive oil, soy oil, corn oil and cod-liver oil on circulating levels of sVCAM-1,
94 sICAM-1 and TNF- α in young, healthy individuals.

95

96 **Materials and methods**

97 *Study population and protocol*

98 A total number of 66 healthy young subjects were recruited randomly for this
99 parallel study according to their age, gender and body mass index (BMI). Exclusion
100 criteria were the presence of clinical evidence for atherosclerotic peripheral vascular
101 disease or coronary atherosclerosis, the presence of any acute or chronic inflammatory
102 disease (eg. liver disease). Subjects with any classic risk factor for atherosclerosis
103 such as smoking, as well as those receiving any systemic medication, were also
104 excluded from the study. Apart from the questionnaire given asking clinical and
105 medical variables, anthropometric and blood pressure assessments were made.

106 All the participants were randomly allocated into 4 groups to receive 4
107 different types of oil, in order to receive an equivalent amount of 50 ml (and almost
108 equal kilocalories) (Tousoulis *et al.*, 2010 and Vogel *et al.*, 2000). Each type of oil
109 had a known macronutrients composition commercially available. Thus, the
110 composition (provided by the manufacturer) per 100ml of each type of oil was: extra

111 virgin olive oil (824 kcal or 3449.9 kJ, 91.6gr fatty acids, 12.8gr saturated, 70.5gr
112 monounsaturated, 8.3gr polyunsaturated), corn oil (825 kcal or 3454.1 kJ, 92gr fatty
113 acids, 12gr saturated, 25gr monounsaturated, 55gr polyunsaturated), soy oil (825 kcal
114 or 3454.1 kJ, 92gr fatty acids, 14gr saturated, 23gr monounsaturated, 55gr
115 polyunsaturated) and cod liver oil (830 kcal or 3475.0 kJ, 92gr cod oil, 0.00828gr
116 eicosapentaenoic acid, 0.00736gr docosahexaenoic acid, 46gr monounsaturated fatty
117 acids, 30gr polyunsaturated fatty acids, 0.0112gr vitamin A, 0.0001gr vitamin D,
118 0.068gr vitamin E). It is worth to mention that extra virgin olive oil was administered,
119 as minor components (eg. β -carotene, α -tocopherol, β -sitosterol, tyrosol, oleuropein,
120 hydroxytyrosol) of virgin olive oil are thought to be responsible for much of its
121 beneficial effects. Oils were administered directly from their commercial form (liquid)
122 and not as part of a meal. Measurements were performed at baseline (0 hours) and 3
123 hours post oil consumption. None of the participants followed any specific
124 recommendation regarding diet or physical activity prior to the study. All subjects
125 participated normally to all their daily activities without modifications. Briefly, all
126 measurements were performed in the morning in a dark quiet room under controlled
127 temperature (22-24°C) and after patients were at a rest for at least 10 minutes. All
128 subjects abstained from food, tobacco, alcohol and caffeine-containing drinks or
129 antioxidant supplements such as juices for at least 8 hours before oil ingestion
130 (baseline measurement). In addition all subjects abstained from the aforementioned
131 parameters during the 3 hours (before the second measurement) post oil consumption.
132 The study protocol was approved by the Institutional Ethics Committee and written
133 informed consent was obtained from all subjects. In addition, the study was carried
134 out in accordance with the Declaration of Helsinki II and European Union guidelines
135 for good clinical practice.

136

137 *Laboratory measurements*

138 Venous blood samples were obtained at the beginning of each protocol and at
139 3 hours post intervention. Routine chemical methods were used to determine serum
140 concentrations of total cholesterol, triglycerides, high density lipoprotein and low-
141 density lipoprotein cholesterol. After centrifugation at 1150g at 4° C for 10 minutes,
142 serum was collected and stored at -80° C until assayed. Quantitative sandwich
143 enzyme immunoassay technique was used to determine serum sVCAM-1 (ng/mL)
144 (Quantikine human sVCAM-1 ELISA kit, R&D Systems), sICAM-1 (ng/mL)
145 (Quantikine human sICAM-1/CD54 ELISA kit, R&D Systems), as well as TNF- α
146 levels (pg/ml) (Quantikine HS, human TNF- α /TNFSF1A ELISA kit, R&D systems).

147

148 *Statistical analysis*

149 All variables were tested for normal distribution by Kolmogorov–Smirnov
150 test. Normally distributed variables are presented as mean values with standard errors
151 (mean \pm SD), whereas not normally distributed data are presented as median (25–75th
152 percentiles values). Correlations were assessed by Spearman’s correlation coefficient
153 (rho) due to not normally distributed data. Between groups comparisons were
154 performed by X^2 test for categorical variables or one-way ANOVA, followed by
155 Bonferoni post hoc correction for multiple comparisons of normally distributed
156 continuous variables or by Kruskal-Wallis followed by Dunn’s post test for not
157 normally distributed variables. Moreover, the effect of oil consumption on each
158 variable was examined by performing paired t-test or Wilcoxon signed-rank test as
159 appropriate. Our study was powered based on the data regarding to sICAM-1: pilot

160 data suggested that the difference in the response of matched pairs is normally
161 distributed with standard deviation 13.0. If the true difference in the mean response
162 of matched pairs is 66.77, we would need to study 4 pairs of subjects to be able to
163 reject the null hypothesis that this response difference is zero with probability (power)
164 0.99. The Type I error probability associated with this test of this null hypothesis was
165 0.01. All analyses were carried out by using the SPSS v18.0 (SPSS, Chicago, IL)
166 statistical package.

167

168

169 **Results**

170 The demographic characteristics and lipid profile of the participants are presented
171 in Table 1. There were no significant differences between the four groups at baseline.

172

173 *Effect of oil consumption on sVCAM-1 and sICAM-1*

174 Acute consumption of olive oil, soy oil, cod liver oil as well as corn oil had no
175 significant effect on sVCAM-1 levels ($p=NS$ for all) (Figure 1).

176 Interestingly, cod liver oil ($p<0.01$), olive oil ($p<0.01$), as well as soy oil
177 ($p<0.01$) decreased sICAM-1 levels significantly. Controversially to the
178 neutral/slightly negative effect of corn oil on sVCAM-1, the latter had also a
179 significant effect on sICAM-1 levels ($p<0.01$) (Figure 2).

180

181 *Effect of oil consumption on TNF-a*

182 Extra virgin olive ($p<0.05$), soy oil ($p<0.01$) and cod liver oil intakes ($p<0.01$)
183 reduced significantly TNF-a levels, whereas corn oil induced a non-significant
184 decrease in TNF-a levels ($p=NS$) (Figure 3).

185 Interestingly, the absolute change in TNF-a levels (39 pairs) was significantly
186 correlated with the absolute change in ICAM-1 levels (66 pairs) after oil consumption
187 ($\rho=0.379$, $p<0.05$) (Figure 4a). However, there was no significant correlation
188 between absolute changes in TNF-a levels and VCAM-1 levels (59 pairs) ($\rho=0.019$,
189 $p=NS$) (Figure 4b).

190

191

192 **Discussion**

193 In the present study we assessed the acute effect of extra virgin olive oil, soy
194 oil, corn oil and cod liver oil consumption on adhesion molecules and
195 proinflammatory cytokines in healthy individuals. We found that all types of oil
196 induced a significant reduction in ICAM-1 levels. In addition, extra virgin olive oil,
197 soy oil and cod liver oil reduced also TNF-a levels. Moreover, we observed a
198 significant correlation between the absolute change of TNF-a and ICAM-1 levels.
199 These findings indicate that specific types of oil exert significant anti-inflammatory
200 effects by affecting proinflammatory cytokines and specific adhesion molecules.

201

202 *Effect of oil acute consumption on adhesion molecules*

203 It is a fact that adhesion molecules participate in the development of
204 atherosclerosis. In addition, it has been shown that dietary habits or modifications
205 may prevent atherogenesis by affecting inflammatory molecules such as VCAM-1
206 and ICAM-1.

207 Mena et al (Mena *et al.*, 2009) while assessing the relationship between
208 Mediterranean diet (supplemented with virgin olive oil) and adhesion molecules,

209 showed a significant inverse correlation of this dietary pattern with ICAM-1 and
210 VCAM serum levels. Interestingly, in the PREDIMED study (Salas-Salvadó *et al.*,
211 2008) among middle aged individuals at high cardiovascular risk , the highest
212 consumption of nuts and virgin olive oil was associated with the lowest
213 concentrations of VCAM-1, ICAM-1, albeit only for ICAM-1 was this difference
214 statistically significant in the case of nuts and for VCAM-1 in the case of virgin olive
215 oil. The results were not in the same direction when analyzing n-3 fatty acids; in a
216 prospective, randomized, double-blind study enrolling patients with myocardial
217 infarction to receive highly concentrated n-3 polyunsaturated fatty acids (PUFAs) or
218 corn oil, inflammatory markers, such as E-selectin and ICAM-1 were unaffected 1
219 year later (Grundt *et al.*, 2004). Importantly, Fuentes et al (Fuentes *et al.*, 2008)
220 showed that different types of fat composition meals affected differentially (in 4
221 hours) VCAM-1 levels in healthy men, as monounsaturated fatty acids appeared to be
222 more beneficial than a-linoleic by reducing VCAM-1 levels. Data seem more
223 conflicting, when referring to healthy adults. Studies such as this by Eschen et al
224 (Eschen *et al.*, 2004) have shown a dose dependent effect as it was observed a
225 significant decrease in ICAM-1 in the PUFA 2.0gr group and a significant increase in
226 VCAM-1 in the PUFA 6.6gr group. However, other studies conducted in young
227 healthy subjects, showed that diets rich in lean fish significantly decreased ICAM-1
228 levels, but had had no effect on VCAM-1 levels (Paulo *et al.*, 2008).

229 In the present study we have shown that VCAM-1 levels are unaffected after
230 ingestion of all types of oil, a finding which is in line with our previous observation in
231 a smaller cohort (Tousoulis *et al.*, 2010). Importantly, we have demonstrated a
232 significant non specific acute effect of olive, soy, corn and cod liver oil on circulating

233 levels of sICAM-1. It is likely that a common ingredient in all types of oil may
234 modify the cleavage rate of sICAM-1 on cell membranes leading to rapid changes of
235 its circulating levels.

236

237 *Effect of oil acute consumption on TNF- α*

238 Tumor necrosis factor alpha has a crucial role in the inflammatory process
239 implicated in atherogenesis. Thus, dietary modification targeting TNF- α could be
240 beneficial in preventing atherosclerosis, as suggested by studies focusing on olive oil,
241 soy oil and polyunsaturated lipids.

242 Consumption of olive oil as a single component reduced significantly not only
243 VCAM-1, but also TNF- α serum levels in coronary venous blood, as reported by
244 Serrano-Martinez et al (Serrano-Martinez *et al.*, 2005). In addition, not only extra
245 virgin olive oil, but also refined olive oil has been found to downregulate TNF- α
246 (Marquez-Martin *et al.*, 2006). Moreover, dietary intervention with n-3 PUFA and
247 plant sterols reduced systemic inflammation in hyperlipidemic individuals, reflected
248 in a decrease of TNF- α levels by 10% (Micallef *et al.*, 2009). Interesting results have
249 been reported by studies of soy consumption. Thus, TNF- α levels were significantly
250 lower in the soy nut group compared to the soy protein and control groups as this was
251 demonstrated by Azadbakht et al (Azadbakht et al., 2008) implying a role in the soya
252 lipid fraction. In contrast to these findings, Jenkins et al (Jenkins et al., 2002) showed
253 that there was no significant effect on interleukin-6 or TNF- α in middle aged men and
254 women on the high-isoflavone versus the low-isoflavone soy food diet.

255 In the present study we have shown that acute consumption of extra virgin
256 olive oil, soy oil and cod liver oil reduced significantly TNF- α levels, while corn oil
257 induced a non significant decrease in TNF- α levels. It is likely that the different types

258 of oils may exert different intracellular “antioxidant” effects, which are then reflected
259 to a variable activation of intracellular redox sensitive pathways (such as nuclear
260 factor- κ B and activator protein-1). This different effect on intracellular redox
261 signaling could perfectly explain the differences in the circulating levels of soluble
262 adhesion molecules, which represent a marker of the expression of these molecules on
263 the cellular surface, a process regulated by these redox-sensitive transcriptional
264 pathways. We have also found a significant association between the absolute changes
265 (d) in TNF-a levels and ICAM-1 levels. These findings indicate that the expression of
266 the proinflammatory cytokine TNF-a which was modified by oil consumption may
267 regulate the subsequent expression of ICAM-1. Therefore, a potential pathway linking
268 these molecules may be affected by the anti-inflammatory effects of specific oils.
269 Although our findings point-out a significant role for oil acute consumption in
270 inflammatory markers, it is difficult to extrapolate our results to clinical practice, as
271 our study was mainly a pathophysiological study designed to assess the potential
272 pathways mediating the effect of different oil acute consumption on inflammatory
273 process.

274 In conclusion, acute consumption of all types of oil decreased significantly
275 ICAM-1 levels. In addition, olive oil, soy oil and cod liver oil decreased significantly
276 TNF-a levels. Moreover, the degree of change in TNF-a levels was correlated with the
277 degree of change in ICAM-1 levels, indicating that the expression of the
278 proinflammatory cytokine TNF-a after oil consumption, may regulate the subsequent
279 expression of ICAM-1 level. These findings suggest that soy oil, cod liver oil and
280 virgin olive oil exert specific significant anti-inflammatory effects.

281

282

283

284 **Acknowledgements:**

285 None

286 **Conflict of interest:**

287 The authors declare that there is no conflict of interest

288 **Figure legends**

289 *Figure 1*

290 All types of oil had no significant effect on sVCAM-1 levels (ng/mL) (p=NS for
291 all). Values are expressed as mean±SD.

292 *Figure 2*

293 Cod liver oil, olive oil, soy oil and corn oil decreased sICAM-1 levels (ng/mL)
294 significantly (p<0.01 for all). Values are expressed as mean±SD.

295 *Figure 3*

296 Extra virgin olive (p<0.05), soy oil and cod liver oil consumption (p<0.01 for both)
297 reduced significantly TNF-a levels (pg/ml). No effect was observed for corn oil
298 (p=NS). Values are expressed as mean±SD.

299 *Figure 4*

300 *a)* Acute oil consumption resulted in a significant correlation between the absolute
301 changes(d) in TNF-a levels (pg/ml) and ICAM-1 levels (ng/mL) *b)* (rho=0.379,
302 p<0.05). *b)* There was no significant correlation between the absolute changes(d) in
303 TNF-a levels and VCAM-1 levels (rho=0.147, p=NS).

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380

Table 1. Demographic characteristics of the participants

381

	Olive oil	Soy oil	Corn oil	Cod liver oil	p value
N (males/females)	19 (12/7)	17 (10/7)	15 (7/8)	15 (9/6)	p=0.796
Age (years)	25.8 ± 3.09	26.2 ± 6.39	24.7 ± 2.35	29.0 ± 8.62	p=0.196
BMI (kg/m²)	23.0 ± 2.83	23.2 ± 4.18	23.1 ± 3.04	22.6 ± 2.74	p=0.960
Systolic Blood Pressure	110 ± 11.7	114 ± 11.9	113 ± 10.8	111 ± 9.80	p=0.714
Diastolic Blood Pressure	72 ± 6.80	76 ± 5.00	76 ± 4.94	73 ± 6.87	p=0.129
Cholesterol (mg/dl)	172 ± 33.1	182 ± 25.9	168 ± 29.4	178 ± 35.3	p=0.597
Triglycerides (mg/dl)*	79 (48-127)	84 (31-191)	66 (42-138)	76 (45-134)	p=0.773
HDL (mg/dl)	50 ± 7.76	51 ± 9.02	55 ± 7.82	54 ± 14.7	p=0.437
LDL (mg/dl)	109 ± 27.3	108 ± 20.9	107 ± 27.5	102 ± 28.5	p=0.884

382

All values are expressed as mean±SD, No significant differences were observed for all variables among groups.

383

BMI: body mass index; HDL: high density lipoprotein cholesterol; LDL: low density lipoprotein cholesterol.

384

*Values expressed as median (25–75th percentile).

385

386

Figure 1. Effect of different types of oil acute consumption on sVCAM-1 levels.

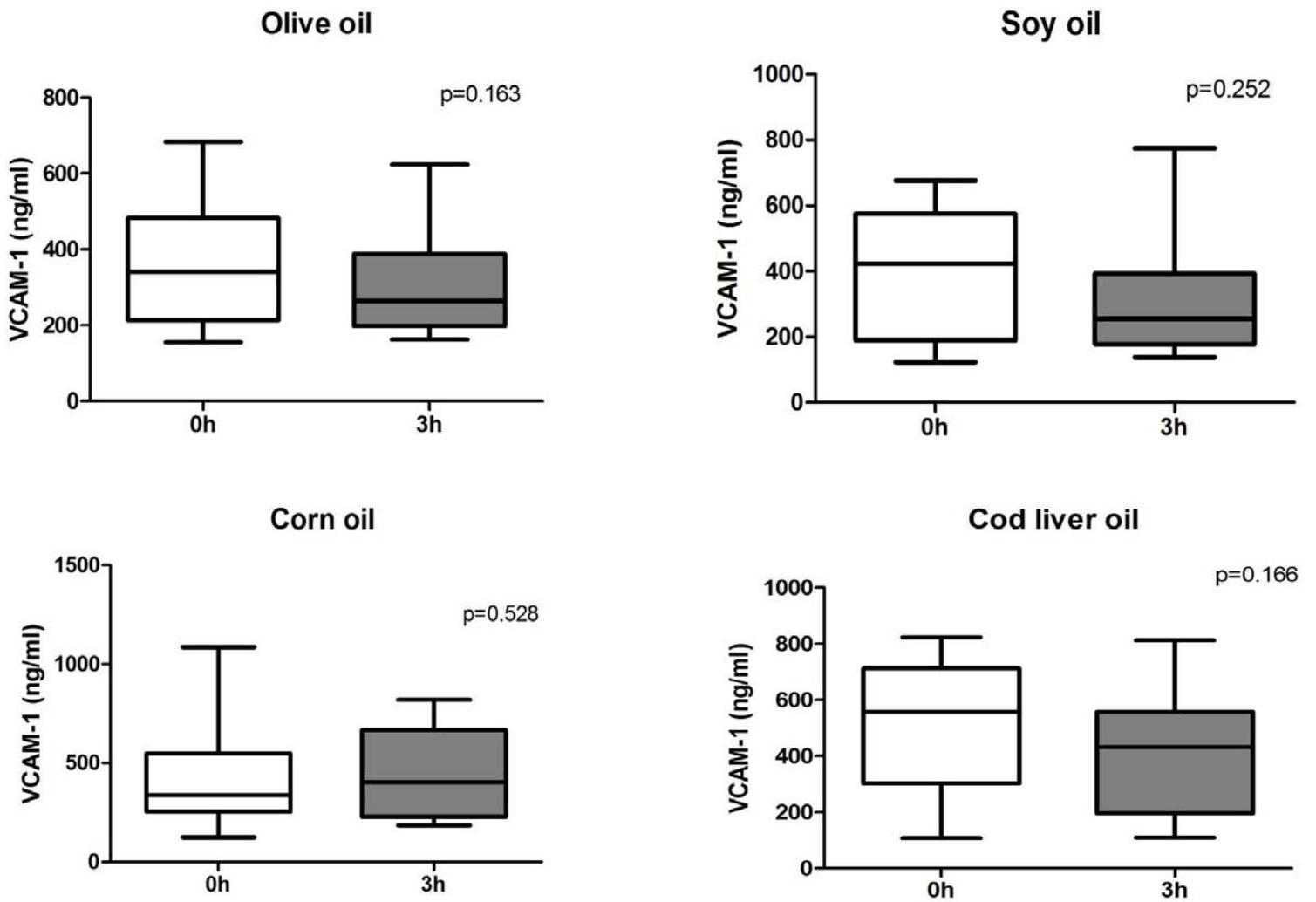


Figure 2. Effect of different types of oil acute consumption on sICAM-1 levels.

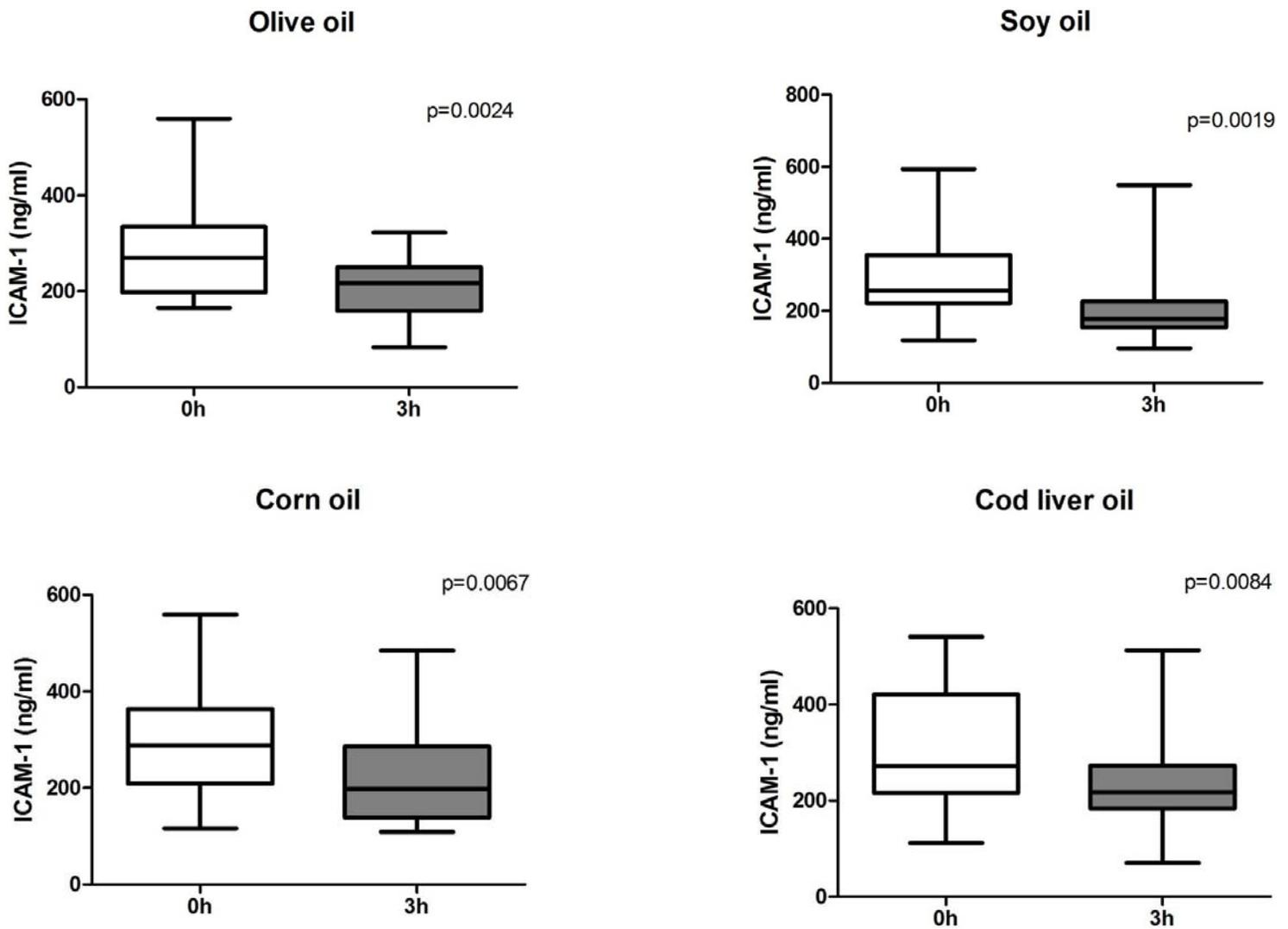


Figure 3. Effect of different types of oil acute consumption on TNF-a levels.

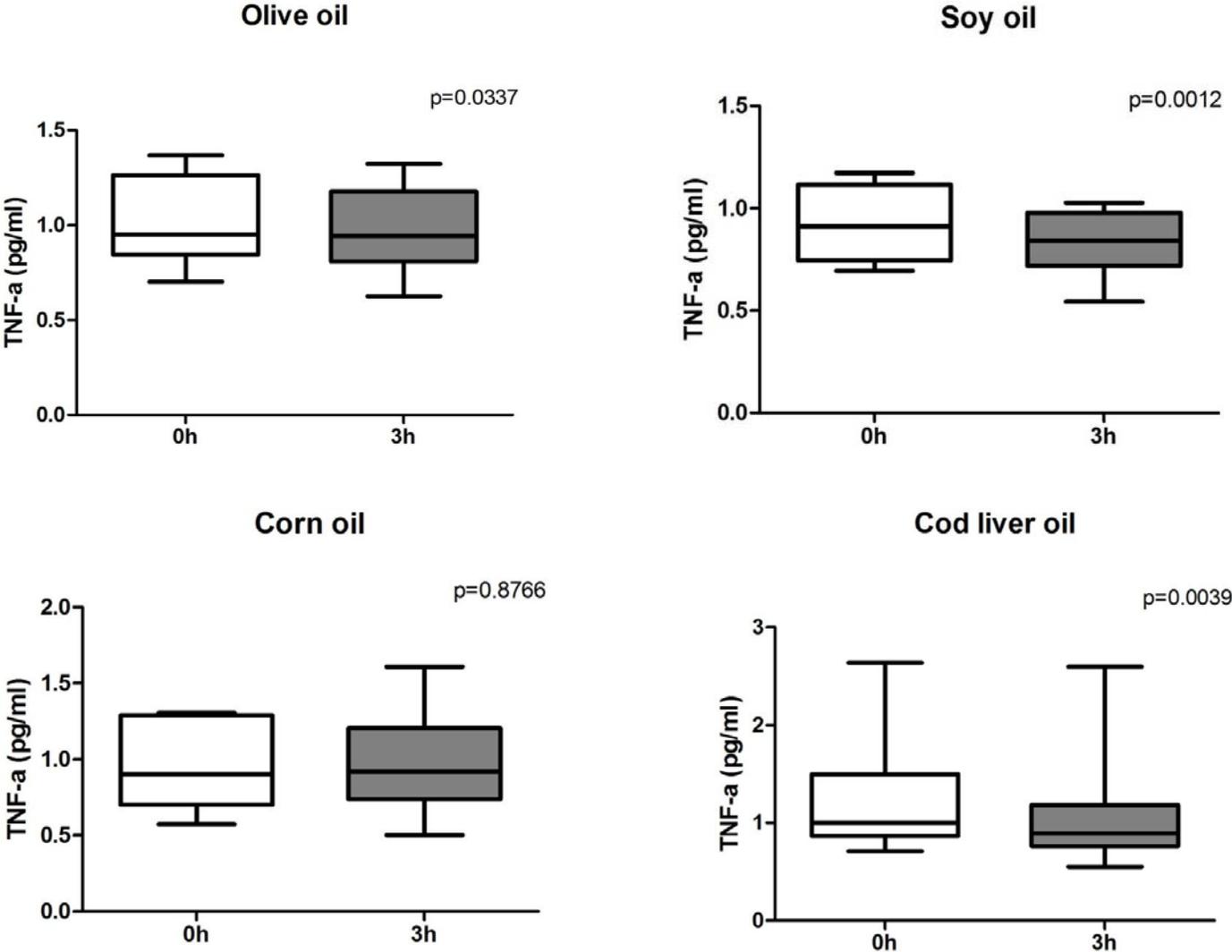


Figure 4a. Correlation between dTNF-a and dICAM-1

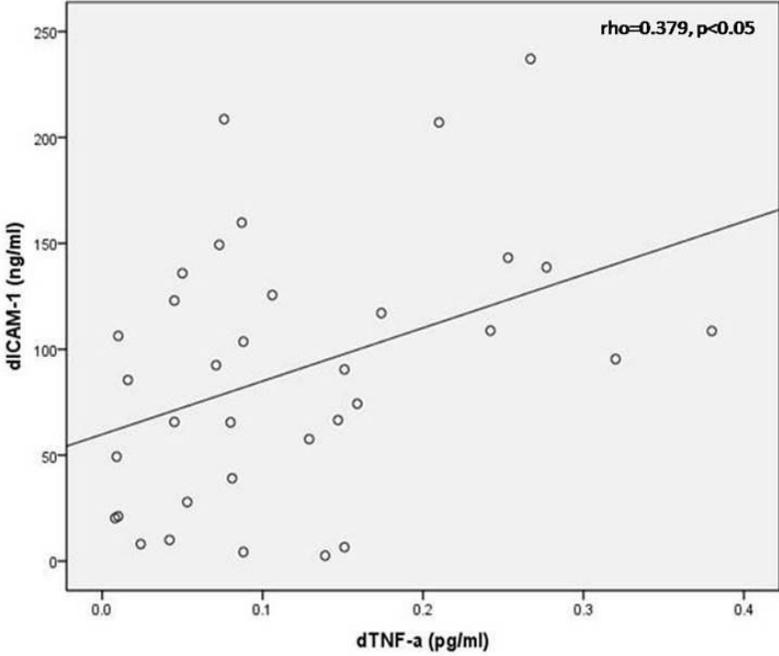


Figure 4b. Correlation between dTNF-a and dVCAM-1

