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FOOD & FUNCTION

The postprandial inflammatory response after ingestion of heated oils in obese persons is reduced by the presence of phenol compounds

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Scope: Heating during the process of cooking alters the chemical properties of foods and may affect subsequent postprandial inflammation. We tested the effects of four meals rich in different oils subjected to heating on the postprandial inflammatory metabolism of peripheral blood mononuclear cells (PBMCs).

Methods and results: Twenty obese participants received four breakfasts following a randomized crossover design, consisting of milk and muffins made with different oils (virgin olive oil (VOO), sunflower oil (SFO), and a mixture of seeds oil (SFO/canola oil) with added either dimethylpolysiloxane (SOD), or natural antioxidants from olive mill wastewater alperujo (phenols; SOP)), previously subjected to 20 heating cycles. Postprandial inflammatory status in PBMCs was assessed by the activation of nuclear NF-κB, the concentration in cytoplasm of the NF-κB inhibitor (IκB-α), the mRNA levels of NF-κB subunits and activators (p65, IKKβ, and IKKα) and other inflammatory molecules (TNF-α, IL-1β, IL-6, MIF, and JNK), and lipopolysaccharide (LPS) levels. VOO and SOP breakfasts reduced NF-κB activation, increased IκB-α, and decreased LPS plasma concentration. SFO increased IKKα, IKKβ, p65, IL-1b, IL-6, MIF, and JNK mRNA levels, and plasma LPS.

Conclusion: Oils rich in phenols, whether natural (VOO) or artificially added (SOP), reduce postprandial inflammation, compared with seed oil (sunflower).

Keywords:
Inflammation / Mononuclear cells / Olive mill wastewater / Phenols / Postprandial state

Human vascular homeostasis is altered during the postprandial state due to the rise in pro-atherogenic lipid fractions, the generation of a pro-coagulant environment, and the activation of peripheral mononuclear white cells (PBMC (monocytes and macrophages)) in response to several stimuli, mainly inflammatory/oxidative, through the NF-κB

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Abbreviations: PBMCs, peripheral blood mononuclear cells; **SFO,** sunflower oil; **SOD,** sunflower oil/canola oil enriched with 2 mg/kg dimethylpolysiloxane; **SOP,** sunflower oil/canola oil enriched with VOO-phenol compounds extracted from the residue of olive-oil production–alperujo; **VOO,** virgin olive oil

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pathway [1, 2]. While some of these processes appear to be inherent to the fact of eating, and regardless of the composition of the ingested foods, others are clearly related to the composition of the meal [2–4]. It has been stated that meals rich in saturated fats provoke a higher inflammatory response in the postprandial state than other sources of fatty acids [1, 5–8]. The cooking process may affect the physical and chemical properties of the foods and the oils used, resulting in an increase in the oxidative products in the frying oil, such as polymers and polar compounds, that may be incorporated into the foods and ingested, especially after several heating processes, activating the PBMC [9–11]. Seed oils are least resistant to this oxidative process due to the relative low antioxidant concentration of seed oils [9], while antioxidants from virgin olive oil (VOO) may act as a buffer, avoiding in part the production of the oxidative molecules at the cost of reducing the concentration of antioxidants in the final product [9, 12].

In summary, the postprandial state induces a variable grade of inflammation that mainly depends on the postprandial redox balance, but also depends on the relationships between the intestinal bacteria, the intestinal cells, and the PBMC [13]. Our aim was to investigate the effects of several heating processes on the chemical properties of different oils, the subsequent effects of the intake of these oils on the postprandial inflammatory response driven by the PBMC and the absorption of bacterial products.

The study protocol was approved by our hospital's Human Research Review Committee, and complied with both institutional and ''Good Clinical Practice'' guidelines. Twenty obese subjects (age of 56 ± 7.1 years, BMI of $37.3 \pm 4.2\,kg/m^2$, waist perimeter of 113.7 ± 13.9 cm, total cholesterol (TC) of 201.1 ± 34.7 mg/dL, triacylglycerols (TG) of 102.9 ± 34.0 mg/dL, low-density lipoprotein cholesterol (LDLc) of 130.2 ± 28.6 mg/dL, high-density lipoprotein cholesterol (HDLc) of 50.8 ± 10.2 mg/dL, glucose of 100.9 ± 10.5 mg/dL and insulin levels of 10.7 ± 5.1 mU/L, all values mean $\pm$ SD) were included in this study. No subjects neither showed evidence of chronic diseases (hepatic, renal, thyroid, or cardiac) nor they were taking medication or vitamins. They received four breakfasts of skimmed milk and muffins prepared with four different oils, in a randomized crossover design following a latin square design, and receiving one breakfast every two weeks. Each breakfast contained 0.45 mL of oil/kg of body weight (56% fat, 9% protein, and 35% carbohydrates). The oils used were as follows: VOO with 400 ppm of natural phenol antioxidants: 70.5% MUFA, 11.1% PUFA, 18.4% SFA; Sunflower oil (SFO): 34.3% MUFA, 58.3% PUFA, and 7.3% SFA; A mixed seed oil (30% high-oleic sunflower and 70% of canola oil) enriched with 2 mg/kg of the antioxidant dimethylpolysiloxane (SOD): 71.8% MUFA, 18% PUFA, and 10.2% SFA and a mixed seed oil (30% high-oleic SFO and 70% canola oil) enriched with 400 ppm of phenolic compounds extracted from the residue of olive-oil production – alperujo – (SOP): 76.7% MUFA, 17.6% PUFA, and 5.8% SFA.

Exhaustive experimental procedures protocols used have been previously reported, and they are provided as a supplemental file (Supporting Information file 1). The oils (2 L of each) were heated to $180 \pm 5^{\circ}C$ for 5 min in a stainless deep fryer ten times every day for two days (total heating cycles: 20). The enrichment of edible oils was produced as previously described in [14]. Measurement of phenolic content was determined for both the overall (Folin-Ciocalteu method), and individually (following chromatographic separation) after extraction of the target analytes. Blood samples, isolation of PBMCs, and protein extraction were performed by standard methods (shown in Supporting Information file 1).

Lipid analysis and biochemical measurements were also performed by well-validated methods. Electrophoretic mobility shift assays (EMSA) to test the NF-κB-binding activity was performed using consensus oligonucleotides 5′-AGTTGAGGGGACTTTCCCAGGC-3′ and 3′-TCAACTCCC CTGAAAGGGTCCG-5′, and subsequent gel shift assays were. For western blot analyses, we used primary polyclonal anti-IκB antibodies and the secondary antibody was (anti-rabbit IgG, conjugated to HRP). Real-time PCR reactions were carried out

Table 1. Concentration of phenols in oils (μg/g)

		VOO		SOP	
Phenol	%RSD[a)]	Cycle 0	Cycle 20	Cycle 0	Cycle 20
Hydroxytyrosol	(5.5)	12.39	ND	13.51	4.10
Tyrosol	(7.3)	7.98	4.89	11.76	8.46
Oleuropein aglycone	(6.6)	5.88	1.13	1.16	1.70
Oleuropein aglycone dialdehyde form	(3.2)	1.52	1.01	1.82	2.57
Luteolin	(2.2)	3.02	0.13	2.43	2.10
Apigenin	(3.0)	1.64	1.28	2.18	2.14
p-Coumaric acid	(4.8)	1.29	1.17	1.97	1.51
Vanillinic acid	(2.2)	1.22	1.42	4.61	3.33
Ferulic acid	(6.0)	1.14	ND	1.69	1.64

VOO: virgin olive oil; SOP: Sunflower oil/Canola oil enriched with phenols from olive mill wastewater alperujo; ND: non detected.
a) The experimental error for each phenol is expressed as relative standard deviation (%RSD).

by using the OpenArray™ NT Cycler platform. Finally, measurement of LPS was performed by limulus amebocyte lysate test. We used SSPS 19 for Windows (SPSS, Chicago, IL, USA) for the statistical analysis, using analysis of variance (ANOVA) for repeated measures, and subsequent Bonferroni's correction where applicable. A p-value < 0.05 was considered significant. All the data presented are expressed as mean values and typical error.

When analyzing the results of the enrichment method for the SOP, we observed a well-balanced profile of phenols in the final product. Table 1 shows the characteristics of the phenolic compounds of the VOO and the SOP, both at the beginning and at the end of the heating cycles. In our study the behaviour of the different phenols in response to the heating is compatible with the previous data [9, 12], with a critical decrease of hydroxytyrosol and a higher resistance to oxidation of tyrosol.

With the test meal, we created a situation of postprandial lipemia, as assessed by the increase in the TG concentrations at 4 hs of the intake (all diets $p < 0.05$ for postprandial TG versus fasting TG concentration) (Supporting Information Table 1). The intake of VOO ($p = 0.029$) and SOP breakfasts ($p = 0.009$) decreased the postprandial NF-κB activation of PBMC (-4 ± 1.7 and -7 ± 3.1 arbitrary units, respectively), while no changes were observed after consumption of SFO and SOD breakfasts (Supporting Information Fig. 1A). The intake of VOO ($p = 0.002$) and SOP breakfasts ($p = 0.028$) induced a postprandial increase in PBMC IκB-α protein levels (4.2 ± 1.8 and 2.1 ± 0.9 arbitrary units, respectively) (Supporting Information Fig. 1B). No changes in the IκB-α protein level were found after the intake of SFO or SOD breakfasts.

When evaluating the analysis of the relative expression of genes involved in the development of the inflammatory process, we demonstrated an over-expression of the complex IKK (IKKβ ($p = 0.043$) and IKKα ($p = 0.005$) PBMC mRNA levels) and an increase in the relative PBMC mRNA level of p65 ($p = 0.038$) at the 4th hour after consumption of SFO breakfasts compared with a fasting value (with no significant changes in the other three breakfasts). Finally, we observed no significant postprandial change differences in the relative level of expression of IκB-α mRNA in the four oils consumed (Fig. 1).

There were no significant postprandial changes in the relative expression of PBMC TNF-α mRNA in the four breakfasts. IL-1β increased from the 2nd–4th hour after SFO ($p = 0.037$) and SOD ($p = 0.025$). At this last time point (4th hour), PBMC IL-6 mRNA levels after SFO were higher than after SOP ($p = 0.018$). The SFO intake was followed by an increase in PBMC MIF mRNA levels at the 4th hour related to fasting values ($p = 0.04$). The expression of the JNK gene increased after the SFO in the two postprandial measurements (2nd and 4th hour) with respect to fasting (all $p < 0.05$). At the 4th hour, the expression of the JNK gene was higher after SFO than SOP ($p = 0.038$; Fig. 2).

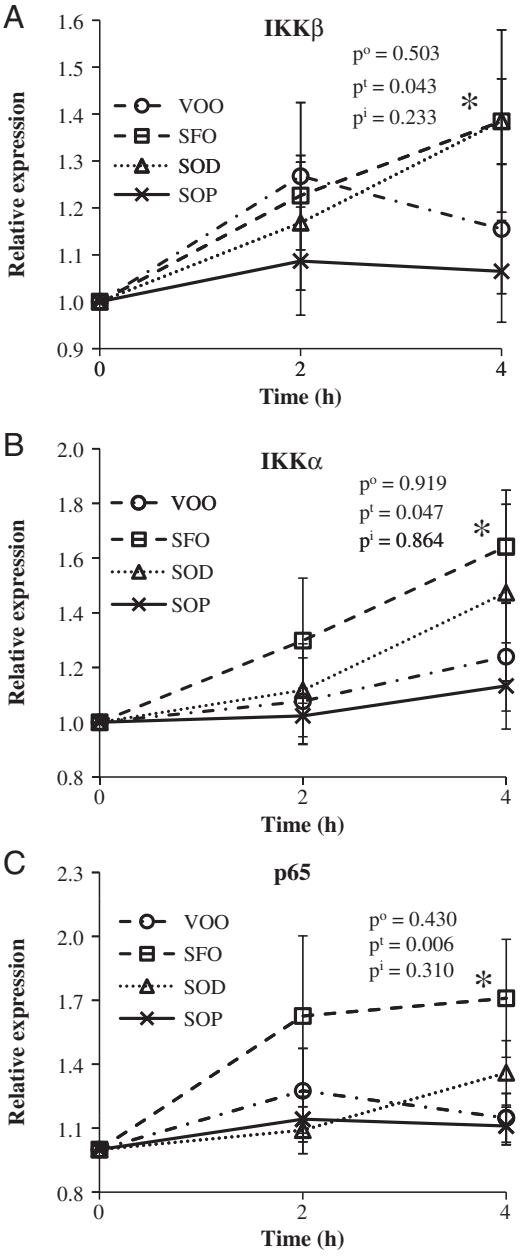

Figure 1. Relative gene expression of IKKβ, IKKα and p65 in PBMC obtained after the intake of four frying oils. Values are mean ± SEM. p^o effect of oil. p^t effect of time. p^i interaction time and oil. $^*p < 0.05$: SFO at 4th hour versus its basal time.

Finally, we found a decrease in the LPS plasma concentration at the 2nd hour of the VOO (relative value of 0.55 ± 0.11 versus the fasting value) and the SOP (relative value of 0.53 ± 0.09 versus the fasting value) consumption (all $p < 0.05$); Supporting Information Fig. 2), and a return to the fasting values at the 4th hour. Conversely, the SOD and SFO did not produce a postprandial reduction of circulating LPS, and SFO showed an increase above the fasting plasma

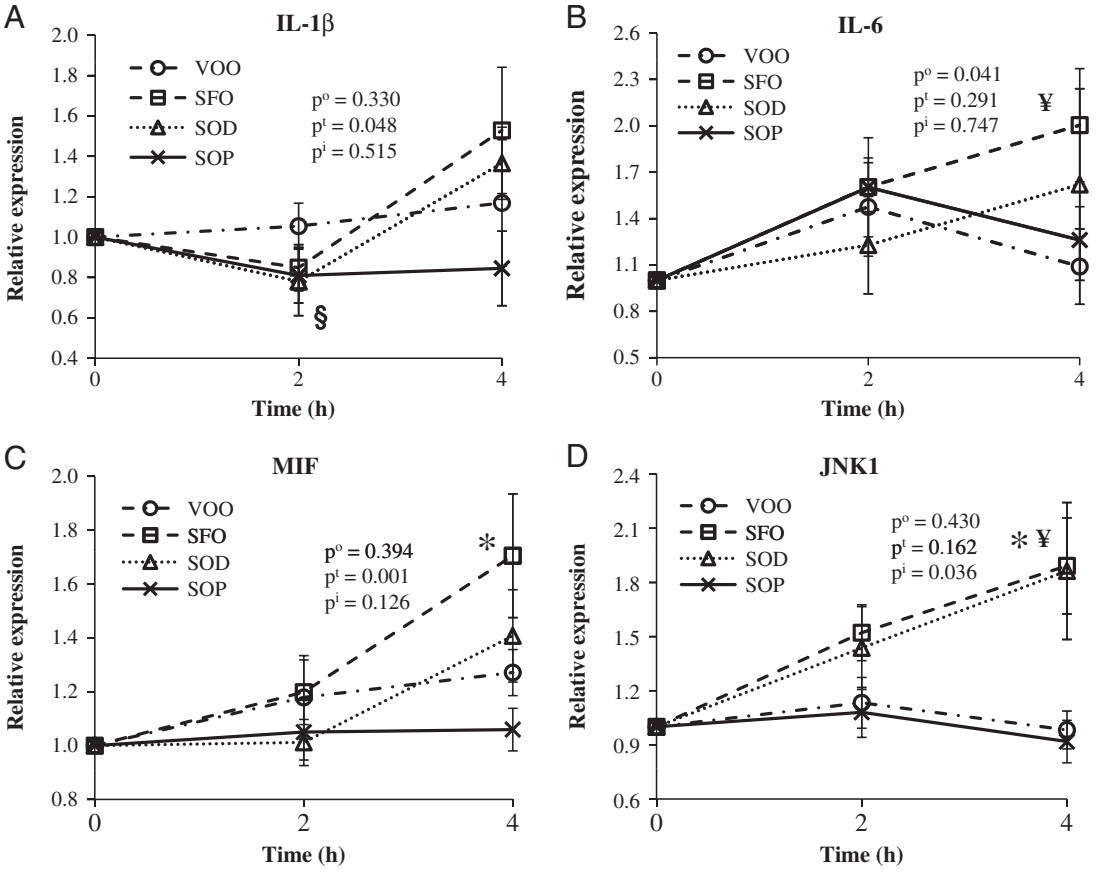

Figure 2. Relative gene expression of IL-1β, IL-6, MIF, JNK1 in PBMC obtained after the intake of four frying oils. Values are mean ± SEM. p^o effect of oil. p^t effect of time. p^i interaction time and oil. $^*p<0.05$: SFO at 4th hour versus its basal time. $^¥p<0.05$: SFO at 4th hour versus SOP at 4th hour. $^§p<0.05$: SFO and SOD at 2th hour versus 4th hour.

values at the 4th hour VOO (relative value of 1.38 ± 0.23 versus the fasting value). When comparing the different diets at each time point, we found that VOO and SOP exhibited lower plasma values than SFO at the 2nd and 4th hour (all $p<0.05$) (Supporting Information Fig. 2).

Eating out regularly, especially in industrialized countries, is becoming a common feature, due to the work schedule and/or other social factors. In many cases, these meals contain a food which has been subjected to deep-frying cooking process, like French fries or other side dishes. The fat vehicle used for deep-frying varies highly, but in many cases economic factors derive in the choosing of vegetal or seed oils for this use, despite other fat sources, like VOO, regardless the lower oxidant insult induced by this latter oil [9, 11]. Our study shows that the content in phenols of different oils subjected to heating determines the postprandial inflammatory status. Ingestion of a multi-heated VOO or SFO/canola oil enriched in natural antioxidants of olive oil origin (SOP) reduced the postprandial inflammatory response when compared with regular SFO. These facts may be related by the different fatty acid compositions of the different oils and to the different contents in antioxidants. We showed for the first time how the biological disadvantage of seed oils to oxidation, LPS increase and eventual NF-κb activation is blunted when natural antioxidants (phenols) from olive oil origin are added to seed oils.

In our study, we chose obese persons based on that obesity and elevated consumption of fried products are clearly linked [15]; and on that obese persons are an excellent model to study low-grade inflammation in human studies, in whom there is an abnormal cytokine production and activation of inflammatory signaling pathways, such as the NF-κB pathway [16].

The results implications for nutrition, food industry, and population health of this study may be significant. On the one hand, we show how seed oils may be provided with healthy antioxidant load by enriching them with natural antioxidants, which may open the door for food industry manufacturers interested in providing a ''healthier'' frying product. On the other hand, people consuming such products may take advantage of suffering a lower inflammation, and, hereby, a lower atherogenic environment in the postprandial state.

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The authors have declared no conflict of interest.

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