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Title: Effects of functional olive oil enriched with its own phenolic compounds on endothelial function in hypertensive patients. A Randomized Controlled Trial.

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Abstract: The additional healthy properties of functional olive oil (FVOO) enriched with its own phenolic compounds (OOPC) versus the parental standard natural virgin olive oil (VOO) must be tested in appropriate human clinical trials. Our aim was to assess FVOO effects on the endothelial function of hypertensive patients.
Thirteen pre-and stage-1 hypertensive patients, received a single dose of 30 mL of FVOO (OOPC=961 mg/kg) or VOO (OOPC=289 mg/kg) in a postprandial randomized, double-blind, crossover trial. Endothelial function, measured as ischemic reactive hyperemia (IRH), and related biomarkers were determined until 5 h.
Compared to VOO, FVOO increased IRH at 2 h ($P<0.05$), and plasma Cmax of hydroxytyrosol sulphate, a metabolite of OOPC, at 2 h postprandial ($P=0.05$). After FVOO ingestion, oxidized LDL decreased ($P=0.010$) in an inverse relationship with the IRH AUC values ($P = 0.01$). FVOO provided more benefits on endothelial function than a standard natural virgin olive oil in pre- and hypertensive patients.
Trial registration: isrctn.org. Identifier ISRCTN03450153

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Reus, April 2, 2013

Dear Editor,

Please find, enclosed, our manuscript entitled: **"Effects of functional olive oil enriched with its own phenolic compounds on endothelial function in hypertensive patients. A Randomized Controlled Trial"** which we would like to be considered for publication in the *Food Chemistry*. The trial is registered as ISRCTN03450153.

The main points addressed in the manuscript are:

1) Whether a functional olive oil (FVOO) enriched with its own phenolic compounds (OOPC) can provide additional healthy properties in front of the parental standard natural virgin olive oil (VOO), must be tested in appropriate human clinical trials.
2) To the best of our knowledge we report here for the first time that a FVOO can provide more benefits on human endothelial function than a standard VOO.
3) Olive oil, a recognized healthy food, can not be consumed in great quantities. The enrichment of olive oil with its phenolic compound is a way of increasing its healthy properties of the olive oil while consuming the same amount of fat.

The authors have any involvement that can be construed as a conflict of interest. We also declare there have not been any other involvements such as employment, consultancy and

patent products that can be construed as a conflict of interest. All authors have approved the submitted version of the manuscript. The manuscript, including related data, figures and tables has not been previously published and the manuscript is not under consideration elsewhere.

Thank you in advance for your kind attention

Sincerely

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***Highlights (for review)**

1. Phenol enriched virgin olive oil (FVOO) improves human endothelial function.
2. Postprandial values of glucose and triglycerides were lower after FVOO versus VOO.
3. Postprandial values of PAI-I, and hsCRP were lower after FVOO versus VOO.
4. FVOO increases VOO healthy properties when consuming the same amount of fat.

Title: Effects of functional olive oil enriched with its own phenolic compounds on endothelial function in hypertensive patients

A Randomized Controlled Trial

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Short title: Functional olive oil and endothelial function

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1. Introduction

The Mediterranean diet, in which olive oil is the main source of fat, has been shown to be protective against cardiovascular diseases (López-Miranda et al., 2010). Olive oil, besides oleic acid, contains bioactive minor components, the most well known being antioxidant polyphenols. In human studies, olive oil rich in polyphenols has demonstrated *in vivo* antioxidant, anti-inflammatory, and anti-cell adhesion molecule generation effects in front of low-polyphenol olive oils (Fitó, De la Torre & Covas, 2007; Covas, 2007; López-Miranda et al., 2010). Recently, the European Food Safety Authority (EFSA) released a claim concerning the effectiveness of the ingestion of olive oil polyphenols (5 mg/day) on protecting low density lipoprotein (LDL) from oxidation (EFSA Panel, 2011).

Endothelial dysfunction, oxidative stress mediated, is one of the characteristic features of essential hypertension (Ghiadoni, Taddei & Virdis, 2012) and is considered one of the first pathological signs of atherosclerosis (Celermajer, Sorensen, Bull, Robinson & Deanfield, 1994). In human studies an improvement in endothelial function has been observed after the consumption of dietary flavanols (Heiss et al., 2010; Balzer et al., 2008) or natural virgin olive oil (VOO) versus a very low-polyphenol content one (Ruano et al., 2005). The concentration of phenolic compounds in an olive oil depends on some factors such as the cultivar, climate, and ripeness of the olives at harvesting as agronomic and also technologic aspects of oil preparation. A measure to ensure a regular dose of polyphenols through the daily intake of VOO is their enrichment with its own phenols (Suárez, Romero, Ramo, Macià & Motilva, 2009). Also, olive oil, a recognized healthy food, can not be consumed in large quantities. Thus, the enrichment of olive oil with its phenolic compounds is a way of increasing its healthy properties

whilst consuming the same amount of fat. Currently functional foods are developed in order to improve the healthy properties of natural food components. However, these functional foods must be tested in human clinical intervention trials with an appropriate design.

Endothelial-dependent vasodilatation is impaired during the postprandial state (Ghiadoni, Taddei & Virdis, 2012), due to this, our aim was to test, in a postprandial randomized, crossover, controlled trial, whether a high-polyphenol content functional virgin olive oil, enriched with its own polyphenols, leads to an improvement of the endothelial function in pre- and hypertensive subjects beyond the effects observed after the intake of a standard virgin olive oil with moderate polyphenol content.

2. Materials and methods

2.1. Olive oil characteristics

The FVOO was prepared by the addition of a phenolic-rich extract (oleuropein complex or secoiridoids: 89.4%; hydroxytyrosol, tyrosol and phenil alcohols: 3.5%; and flavonoids, 6.0%), obtained from the olive cake, to a natural VOO as previously described.[10] Briefly, olive cake phenolic extract (7 mg/mL oil) and 0.3% (p/v) of lecithin (Emulpur; Cargill, Barcelona, Spain) was dissolved in ethanol-water (50:50, v/v), and added to the VOO until full homogenization, with a Polytron (Kinematica, Littau, Switzerland). In order to ensure similarities among oils, VOO was also submitted to the process of lecithin addition. Total polyphenol content of the olive oils was 289 and 961 mg/kg oil for VOO and FVOO, respectively, measured by ultraperformance liquid chromatography coupled to a tandem mass detector (UPLC-

ESI-MS/MS) as previously described (Suarez et al., 2011). Fatty acids were measured by gas chromatography**. Table 1** shows the composition of the olive oils used in the study.

2.2. Participants

Between January and July 2009, 22 participants, aged 20 to 75, were recruited through a volunteer center database. Participants were community-dwelling with pre-hypertension (systolic blood pressure (SBP) ≥120 mmHg to 139 mmHg, and/or diastolic blood pressure (DBP) ≥80 mmHg to 89 mmHg) and stage 1 hypertension (SBP ≥140 mmHg to 159 mmHg and/or DBP ≥90 mmHg to 99 mmHg (Chobanian et al.,2003), without antihypertensive treatment. This population was chosen because hypertensive patients have been shown to have a high degree of oxidative stress which plays a key role in endothelial functionality (Fatehi-Hassanabad, Chan & Furman, 2010). Exclusion criteria included: LDL cholesterol >4.9 mmol/L, triglycerides (TG) >3.97 mmol/L, or current hypolipemic treatment; diabetes mellitus; any chronic disease; and body mass index (BMI) >30 kg/m^2. Participants provided written informed consent prior to their enrollment in the trial. After a screening visit, eligibility or exclusion was assessed by the attending physician on the basis of the clinical records. The study was approved by the Clinical Research Ethical Committee of the Hospital Universitari Sant Joan de Reus (Ref 08-04-24/4proj5), Spain. Protocols were according to the Helsinki Declaration.

2.3. Study desing

The trial was a postprandial randomized, controlled, double-blind, and crossover with a washout periods between interventions of 2 weeks**.** The randomization scheme was generated by using a web site (http://www.randomization.com). Participants received 30 mL of each one of the two types of olive oil, VOO or FVOO, with bread (80 g) (**Figure 1**). The day before the intervention participants followed a polyphenol-free diet avoiding olive oil, olives, fresh fruit or juices, vegetables, legumes, soya, chocolate, coffee, tea, wine, and beer. Participants were instructed to follow a stabilization diet with 10% of saturated fatty acids during the week before the first postprandial test and between tests (two week washout period). Compliance with the stabilization diet was assessed using a 3-day dietary record (2 working days and a holiday or weekend one) before the intervention day. Trained dieticians explained to participants how to complete these questionnaires. Participants were instructed to avoid intense physical activity 3 days prior to the intervention day. Physical activity was evaluated by the Minnesota Leisure Time Physical Activity Questionnaire validated for its use in Spanish men and women (Elosua, Marrugat, Molina, Pons & Pujol, 1994; Elosua, Garcia, Aguilar, Molina, Covas & Marrugat, 2000). Anthropometric data were obtained by standardized methods. Systolic and diastolic blood pressure were measured, after 15 min of resting, three times at 1-min intervals using an automatic sphygmomanometer (OMRON HEM-907; Peroxfarma, Barcelona, Spain).Venous blood was collected at the baseline of each intervention period (0 h) and at several time points after olive oil administration (**Figure 1**). Serum and plasma were obtained by centrifugation of blood at 1500 g at 4^{o}C for 20 min and stored at -80 ^{o}C.

2. 4. Endothelial function

Endothelial-dependent vasomotor function was measured as ischemic reactive hyperemia (IRH) by a Laser-Doppler linear Periflux 5000 flowmeter (Perimed AB, Järfälla, Stockholm, Sweden). Measurements were performed with the patient lying in the supine position in a room with stable temperature (20°C to 22°C). Patients were at rest during 15-20 min before the test. The blood pressure cuff (HG Erkameter 300, Erka, Bad Tolz, Germany) was placed above the elbow, while the laser probe was attached to the palmar surface of the second finger of the same dominant hand. After a 5-min resting period, basal capillary flow was measured for 1 mim (t_0). Thereafter, 4-min distal ischemia was induced by inflating the cuff to suprasystolic pressure (220 mmHg). Subsequently, the cuff was deflated and, after 30s, the flow was recorded during 1min (t_d). Data were recorded and stored using the PeriSoft 2.5 software for Windows. The system monitor showed how the perfusion units (PU) fell regularly to reach biological zero. Results were expressed as arbitrary units (AU). Measurements were performed at baseline and at 2 h, 4 h, and 5 h after olive oil intake (**Figure 1**). Calculations were performed by the formula: IRH = ((PUt_d - PUt_0) / PUt_0) x 100). The IRH value of the area under the curve (AUC) was calculated by Microsoft Excel for pharmacokinetic functions.

A reproducibility assay in a preliminary study, performed in ten healthy subjects with measurements two weeks apart, showed an inter-study variability of 9.05%. A total of 10 measurements within the same day in one single healthy volunteer rendered an intra-study variability of 8.5%.

2.5. Systemic biomarkers

158 Cardiovascular risk biomarkers were measured at baseline (0 h) and at 2 h, 4 h,
159 and 5 h after olive oil intake (**Figure 1**). Serum total cholesterol, TG, HDL cholesterol,
160 high sensitivity C-reactive protein (hsCRP), insulin, and glucose were measured by
161 standardized methods in a Beckman autoanalyzer (Beckman Coulter-Synchron, Galway,
162 Ireland). LDL cholesterol was calculated by the Friedewald formula. Plasma EDTA
163 circulating oxidized LDL (Mercodia AB, Uppsala, Sweden), vascular cell adhesion
164 molecule type 1 (VCAM-1) and intercellular adhesion molecule type 1 (ICAM-1)
165 (R&D Systems, Minneapolis, USA) were measured by ELISA. Plasminogen activator
166 inhibitor type 1 (PAI-1) (Technoclone GmbH, Vienna, Austria) was measured in citrate
167 plasma using ELISA kits.
168 Plasma olive oil polyphenols and their biological metabolites were measured by
169 UPLC-MS/MS (Suarez et al., 2011) at baseline (0 h) and after 1 h, 2 h, 4 h, and 5 h.
170
171 2.6. Sample size and power analysis
172
173 A sample size of 13 participants allows at least greater than or equal to 80% power
174 to detect a statistically significant difference between groups of 10 Units of IRH,
175 assuming a dropout rate of 15% and a type I error of .05 (2-sided). The common
176 standard deviation of the method is 11 Units (Ruano et al., 2005).
177
178 2.7. Statistical analyses
179
180 Normality of continuous variables was assessed by normal probability plots. Rank
181 transformation was applied to the dependent, non Gaussian, continuous variables.

Pearson's correlation analyses were used to evaluate the relationship among variables. The analyses were performed using SPSS 17.0 software (SPSS Inc, Chicago, IL, USA). A general linear model was used to assess the treatment group, time, and time-by-treatment interactions. Inter-treatment comparisons were performed by paired Student t-test. We checked the possible carryover effect by testing a sequence-by-treatment interaction term in the general linear models. The level of significance was set at the standard two-sided level of 5%. The analyses were performed using SAS (version 9.1.3) software (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Study population

From the 22 participants recruited, 16 were eligible. Three participants dropped out before starting the study due to an incompatible work timetable, and finally 13 participants (7 men and 6 women) completed the study**.** Participants' baseline characteristics are shown in **Table 2.** No differences in baseline characteristics were observed between the two sequences of olive oil administration**.** No changes in blood pressure, weight, dietary habits, and physical activity were registered throughout the study.

3.2. Endothelial function

The postprandial time-course changes in IRH after ingestion of the olive oils is shown in **Figure 2**. IRH time-course increased in a linear trend after FVOO ingestion

from baseline to 5 h postprandial (P<0.022). After FVOO, IRH, increased two- and five-fold at 2 h and 3 h postprandial (P<0.05). When changes from baseline were compared, the increase after FVOO at 2 h was significantly higher after FVOO than after VOO, reaching a borderline significance at 5 h postprandial (P<0.066). An inverse relationship was observed between IRH AUC and oxidized LDL plasma concentrations at 5 h postprandial (P=0.014).

3.3. Systemic biomarkers

The time-course of the systemic cardiovascular risk biomarkers is shown in **Table 3**. No carryover effect between the two intervention periods was detected. The postprandial 2h- increase in glucose and TG was lower after FVOO than VOO ingestion. Plasma oxidized LDL decreased in a linear trend after both olive oil treatments, reaching significance after FVOO intervention (P=0.010). A decreasing linear trend was observed in PAI-1 and hsCPR concentrations after VOO (P<0.05) and FVOO (P<0.01). At 4 h postprandial PAI and hsPCR concentrations were lower after FVOO versus VOO treatment (P<0.05). No changes were observed in VCAM and ICAM as showed in **Table 3**, as well as in total, LDL, or HDL cholesterol (data not shown). Hydroxytyrosol sulphate, the main biological metabolite of hydroxytyrosol, increased in a dose-dependent manner with the polyphenol content of the olive oil, reaching a peak at 2 h after oil ingestion (P<0.05) **(Figure 3)**.

4. Discussion

Our study shows that a virgin olive oil enriched with its own polyphenols, FVOO, can provide additional health benefits on human endothelial function during the postprandial state versus those obtained by a standard VOO with a moderate polyphenol content. Postprandial values of glucose, TG, PAI-I, and hsCRP were lower after FVOO in comparison with VOO ingestion. FVOO consumption improved the postprandial endothelium-dependent microvascular dilatation in patients with pre- and stage-1 hypertensive status in comparison with VOO.

Several studies have addressed the relationship between high versus low polyphenol intake and endothelial function assessment**.** Flavonoid consumption has been shown to improve the endothelial function, after both acute and sustained consumption, in diabetic (Balzer et al., 2008) and coronary heart disease patients (Heiss et al., 2010). Olive oil polyphenols have been shown to improve endothelial function in hyperlipemic (Ruano et al., 2005) and hypertensive (Moreno-Luna et al., 2012) patients. We performed a study comparing the benefits of a functional versus a natural virgin olive oil on endothelial function. To the best of our knowledge is the first time that a very high polyphenol-rich olive oil (>900 mg/kg) is tested in front of a natural virgin olive oil with an standard polyphenol content (200-300 mg/kg). We performed our study at postprandial state. In western populations, we spend most of the time in a non-fasting state, with continuous fluctuations in plasma lipids throughout the day. The postprandial state is an active field of research in cardiovascular disease due to the evidence indicating that it influences cardiovascular risk. Postprandial lipemia has been recognized as a risk factor for atherosclerosis development as it is associated with oxidative changes (López-Miranda, Pérez-Martínez, Marín, Moreno, Gómez & Pérez-Jiménez, 2006; Roche & Gibney, 2000). After a high-fat meal an oxidative stress occurs which has been linked with a concomitant impairment in the endothelial function

(Ceriello et al., 2002). However, the consumption of fatty meals with suitable sources of antioxidants, such as red wine (Natella, Ghiselli, Guidi, Ursini & Scaccini, 2001), or vitamin C (Ling, Zhao, Gao, Zhou, Li& Xia, 2002) has been shown to minimize postprandial oxidative stress.

In our pre-and hypertensive patients, consumption of FVOO reduced, in comparison with values after VOO, the postprandial hyperglycemia and hypertriglyceridemia peak. In agreement with this, a reduction of the LDL oxidation and inflammatory biomarkers was observed after FVOO versus VOO. Circulating oxidized LDL, one of the recognized methods for measuring oxidative damage mediated by reactive oxygen species, has been reported to be a predictor for cardiovascular disease development (Meisinger, Baumert, Khuseyinova, Loewel & Koenig, 2005). Oxidized LDL produces pro-atherogenic effects in endothelial cells by inducing the expression of adhesion molecules, stimulating apoptosis, inducing superoxide anion formation, and impairing protective endothelial nitric oxide formation (Yu, Wong, Lau, Huang & Yu, 2011). We have previously reported in acute and sustained consumption studies a decrease in oxidized LDL and hsCRP, linked to the polyphenol content of the olive oil, in healthy volunteers (Covas et al., 2006a; Covas et al., 2006b) and in stable coronary heart disease patients (Fitó et al., 2005; Fitó et al., 2008). The protection on LDL oxidation linked to FVOO consumption in our study could be mediated by the observed increase of olive oil phenolic compounds metabolites (i.e. hydroxytyrosol sulfate) in plasma. In our previous studies, we have observed a direct relationship between the increase of tyrosol and hydroxytyrosol in human plasma after VOO ingestion and the polyphenol content of the LDL (Covas et al., 2006b), which in turn has been shown to be inversely related to the degree of LDL oxidation (de la Torre et al., 2010).

The term endothelial dysfunction implies a loss of homeostasis resulting from a complex interaction of vasodilatory and vasoconstrictive factors, on which diet exerts a crucial influence (Turner, Belch & Khan, 2008; Nettleton et al., 2006). The literature is generally consistent that oxidative stress contributes to the five characteristic microvascular responses to inflammation: vasomotor dysfunction (impaired vessel dilation and constriction), leukocyte recruitment, increased vascular permeability, angiogenesis, and thrombosis (Nettleton et al., 2006). In the present study, the improvement in endothelial function, reflected in the increase in ischemic reactive hyperemia, after FVOO was inversely related to the LDL oxidative damage. Thus, the reduction in both the oxidative stress, reflected in a decrease of the oxidative damage to LDL, and that of the inflammation could account for the improvement in the endothelial function observed after FVOO ingestion. Our results point out a key role of the polyphenols in which the olive oil was enriched on the improvement of the endothelial function in our pre- and hypertensive patients.

One strength of the study is its design. Randomized, controlled, clinical trials are those able provide the first level of scientific evidence. In addition, the crossover design, in which each subject acts as the corresponding control, minimizes the interference of possible confounding variables. Our design, however, did not allow modeling the first- and second-order possible carryover effects. One potential limitation of the study was, although the trial was blinded, some participants might have identified the type of olive oil ingested by its organoleptic characteristics. Another limitation is the inability to assess potential interactions between olive oils and other diet components. The controlled diet followed by participants during the washout period could account for limiting the scope of these interactions.

In summary, a functional virgin olive oil enriched with its phenolic compounds promoted greater additional improvements in human endothelial function than those provided by a standard virgin olive oil. The observed increase in the biological metabolites of olive oil phenolic compounds in plasma, hydroxytyrosol sulphate, together with the decrease in oxidized LDL and hsCRP appear as possible mechanisms for explaining the improvement in endothelial function after ingestion of the polyphenol-enriched FVOO. From our results, functional olive oil polyphenol-rich olive oils can be an useful tool for improving the endothelial function in hypertensive status.

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(3) Statement of Authorship: Authors'contributions to manuscript: RS and M-JM designed research; R-MV, MS, SF-C, FF and JL-M conducted research; R S, R-M V, MF, M S, S F-C, MF, MG, M-I C, and M-JM provided essential reagents or provided essential materials; R-M V, M-I C, VK and MF analyzed data or performed statistical analysis; RS, R-M V, M-I C, MF, MF,M-JM wrote paper; RS and M-J M had primary responsibility for final content. All authors critically revised the manuscript for

important intellectual content and approved the manuscript being submitted for acknowledgement to contributors who do not meet authorship criteria publication.

(4) Statement of any conflicts of interest: The authors have declared that no competing interests exist.

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Table 1. Characteristics of the olive oils administered.

	FVOO	VOO
Quality parameters		
Free acidity (% of oleic acid)	0.19	0.26
Peroxide value ($mEqO_2/kg$)	16.76	6.10
Fatty acids (% of total)		
Monounsaturated	72	72
Polyunsaturated	11	11
Saturated	17	17
Total polyphenols (mg/kg of olive oil)	289	961
Free Hydroxytyrosol	0.37	6.64
Free Tyrosol	1.03	8.7
Secoroid derivatives	123	680
Vanillic acid	0.37	3.94
p-coumaric acid	0.08	0.84
Vanillin	0.16	1.44
Pinoresinol	116	173
Luteolin	1.44	6.28
Apigenin	0.27	0.80

Abbreviations: VOO, natural virgin olive oil; FVOO, polyphenol enriched virgin olive oil.

Table 2. Baseline characteristics of the participants.

Variable	Mean (SD)
Gender (male/female)	7/6
Age, years	50.6 (16.9)
Body mass index, kg/m^2	25.7 (1.7)
Waist circumference, cm	79.0 (6.0)
SBP, mm Hg	139 (13.6)
DBP, mm Hg	84 (10.2)
Total cholesterol, mmol/L	5.06 (0.75)
LDL cholesterol, mmol/L	2.84 (0.69)
HDL cholesterol, mmol/L	1.70 (0.40)

Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL, low density lipoprotein; HDL, high density lipoprotein.

Table 3

Table 3. Postprandial time-course of systemic biomarkers after virgin olive oil (VOO) and polyphenol-enriched virgin olive oil (FVOO).

Variable	Intervention	Baseline	2 hours	4 hours	5 hours	P linear trend
Glucose, mmol/L	VOO	5.44 (0.51)	6.31 (1.57)†	5.19 (0.58)	5.07 (0.35) †	0.006
	FVOO	5.37 (0.34)	6.08 (1.07)†$	5.04 (0.41) †	5.07 (0.31) †	0.001
Insulin, pmoL/L	VOO	36.7 (1.41)	99.4 (1.83) †	35.4 (2.09)	24.0 (1.80) †	0<.001
	FVOO	36.2 (1.41)	102.9 (1.89)	30.2 (1.41)†	23.1 (1.55)	0<.001
Triglycerides,* mmol/L	VOO	1.03 (1.61)	1.39 (1.62) †	1.29 (1.76) †	1.17 (1.63)	0.008
	FVOO	0.98 (1.69)	1.29 (1.82) †$	1.32 (1.77)	1.40 (1.71) †	0.002
Oxidized LDL, U/L	VOO	69.29 (18.37)	67.27 (11.61)	69.01 (22.59)	63.30 (14.09) †	0.057
	FVOO	70.59 (15.51)	67.71 (17.31) †	66.75 (15.49) †	65.41 (16.39) †	0.010
ICAM-1, ng/mL*	VOO	170.51 (1.25)	167.83 (1.25)	165.87 (1.35)	164.47 (1.29)	0.192
	FVOO	165.40 (1.25)	161.39 (1.32)	170.30 (1.37)	170.61 (1.31)	0.135
VCAM-1, ng/mL	VOO	612.29 (97.68)	609.56 (107.77)	626.27 (123.69)	635.71 (129.95)	0.180
	FVOO	633.28 (112.85)	589.70 (118.49) †	609.65 (107.58)	601.32 (97.29)	0.270

Table 3. Postprandial time-course of systemic biomarkers after virgin olive oil (VOO) and polyphenol-enriched virgin olive oil (FVOO) (continued).

Variable	Intervention	Baseline	2 hours	4 hours	5 hours	P linear trend
PA1-1, ng/mL*	VOO	13.18 (2.31)	14.40 (2.55)	6.56 (1.79) †	7.40 (1.59)	0.022
	FVOO	12.94 (2.34)	9.19 (1.55)	6.05 (2.09) †$	6.95 (1.81) †	0.002
hsCRP ng/mL*	VOO	0.71 (2.31)	0.66 (2.27) †	0.65 (2.30) †	0.66 (2.25) †	0.029
	FVOO	0.72 (1.85)	0.66 (1.84) †	0.64 (1.87) $	0.64 (1.84) †	0.006

Values expressed as mean (±SD). * Non normal variables are expressed by Geometric mean (logSD). General linear mixed model.

† $p<0.05$ versus baseline ; $ $p<0.05$ between treatments at the same time point.

Abbreviations: ICAM, inter-cellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1; PAI-1, plasminogen activator inhibitor-1; hsCRP, high sensitive C reactive protein.

FIGURE CAPTIONS

Figure 1. Study design.

FVOO, functional virgin olive oil; VOO, virgin olive oil; IRH, ischemic reactive hyperemia.

Figure 2. Ischemic reactive hyperemia (IRH) at postprandial, after the intake of virgin olive oil (VOO) and functional olive oil enriched with its own phenolic compounds (FVOO).

* P<0.05 versus baseline; † P<0.05) versus VOO at the same time-point.

Figure 3. Time-course of plasma concentrations of hydroxytyrosol sulphate (μmol/L) in human plasma after the intake of virgin olive oil (VOO) and functional olive oil enriched with its own phenolic compounds (FVOO).

* P<0.05 versus baseline; † P<0.05 versus VOO at the same time-point.

Figure 1

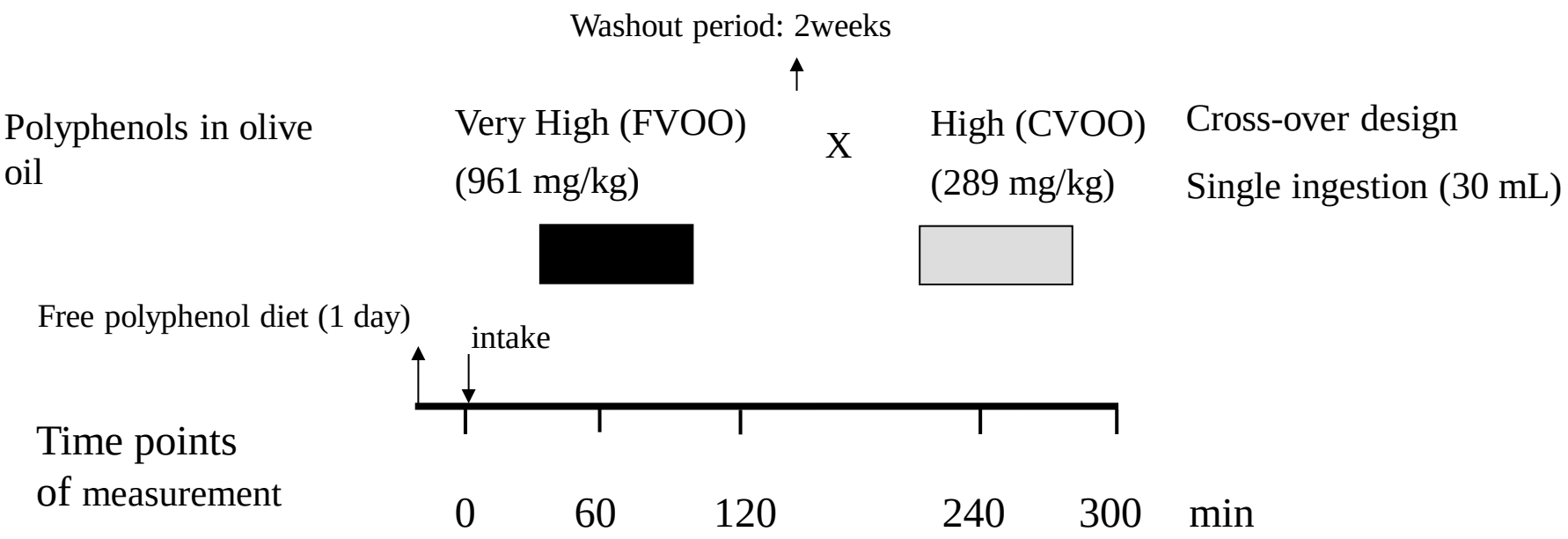
Washout period: 2weeks
Polyphenols in olive oil
Very High (FVOO)
(961 mg/kg)
X
High (CVOO)
(289 mg/kg)
Cross-over design
Single ingestion (30 mL)
Free polyphenol diet (1 day)
intake
Time points of measurement
0
60
120
240
300
min
IRH
Systemic biomarkers
Olive oil polyphenols in plasma

Figure 2

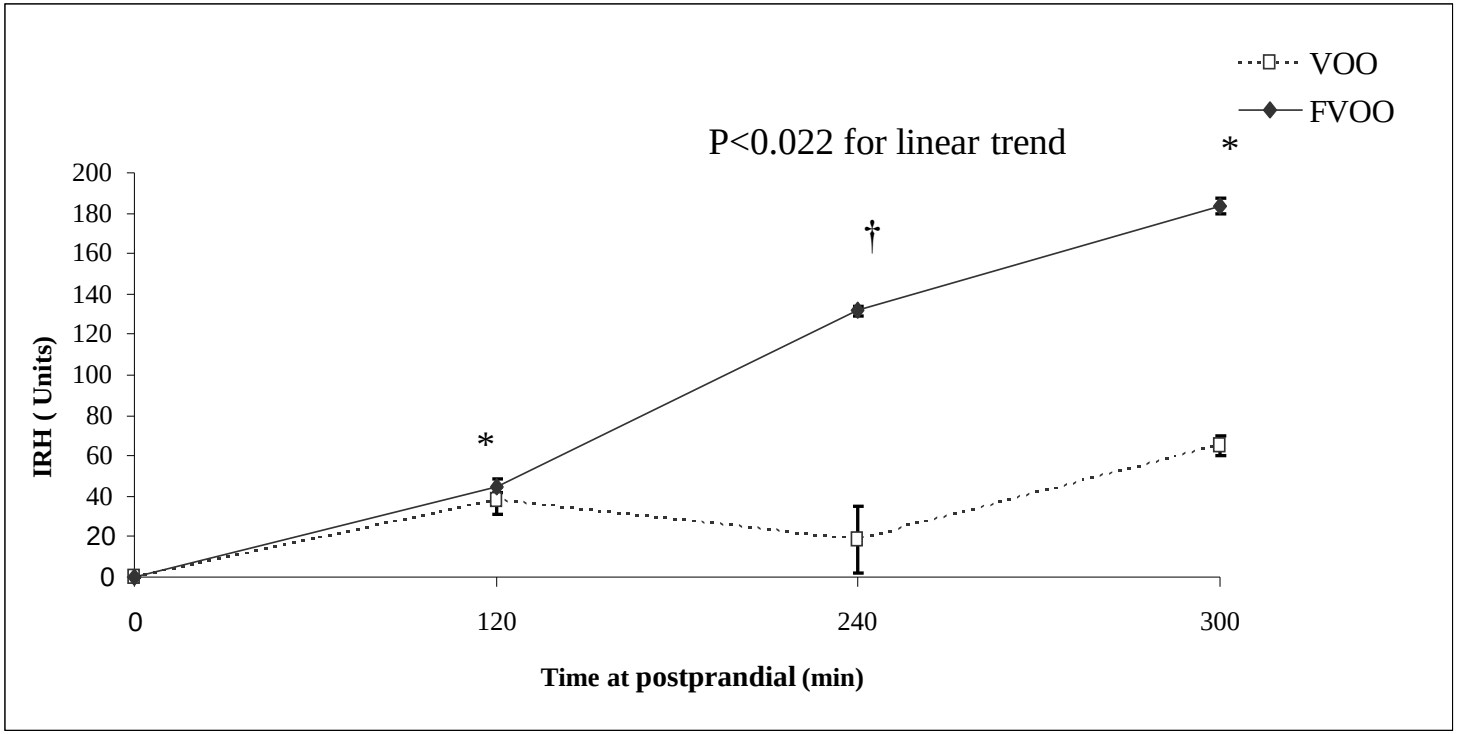
VOO
FVOO
P<0.022 for linear trend
*
†
*
200
180
160
140
120
100
80
60
40
20
0
IRH (Units)
0
120
240
300
Time at postprandial (min)

Figure 3

Figure 3