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Virgin Olive Oil Study (VOLOS): vasoprotective potential of extra virgin olive oil in mildly dyslipidemic patients

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■ **Summary** *Background* In vitro, olive phenols exert potent antioxidant and enzyme-modulating activities. *Aim of the study* We comparatively evaluate, in mildly dyslipidemic patients, the vasoprotective potential of extra virgin olive oil. *Methods* 22 patients were administered 40 mL/day of either extra-virgin, i. e. phenol rich, or refined, i. e. phenol poor, olive oils (EVOO or ROO, respectively, with nearly identical fatty acid composition), with a crossover design. Each treatment was carried out for seven weeks, with four weeks of washout in between. Plasma antioxidant capacity, serum thromboxane B₂ (TXB₂) formation, and urinary isoprostane excretion were evaluated as surrogate markers of cardioprotective potential and vascular function. *Results* No effects on plasma lipid/lipoprotein profile were ob-

served. Conversely, EVOO consumption was associated with favorable effects on circulating markers. Namely, decreased serum TXB₂ production and increased plasma antioxidant capacity were observed when EVOO was administered in both treatment arms. Neither treatment had any significant effect on isoprostane excretion. *Conclusions* EVOO consumption by mildly dyslipidemic patients is associated with favorable changes in circulating markers of cardiovascular condition. Based on current knowledge, these effects may be associated with cardioprotection.

■ **Key words** atherosclerosis – antioxidants – coronary disease – free radicals – olive oil – isoprostanes

Introduction

The incidence of major risk factors for coronary heart disease (CHD), e.g. high blood pressure and elevated serum cholesterol concentrations, is similar between populations of the Mediterranean area and those of other North European and Western countries, despite remarkable differences in the prevalence of CHD in these areas [1, 2]. This observation, together with experimental evidence of the involvement of lipid, namely low density lipoprotein (LDL), oxidation [3–5] in the onset of atherosclerosis, led investigators to propose a protec-

tive role for dietary antioxidants. Accordingly, the consumption of diets rich in antioxidants, e.g. vitamins and polyphenols, is associated with decreased oxidative stress [6] and lower incidence of CHD [7–9].

Olive oil is the principal source of fat in the Mediterranean area, where cardiovascular mortality is remarkably low [10–12]. Thus far, most of the cardioprotective effects of olive oil have been ascribed to its high content of the monounsaturated fatty acid (MUFA) oleic acid (18:1n-9). Whether the beneficial effects of olive oil on the cardiovascular system are exclusively due to oleic acid is, however, questionable. Indeed, intake of MUFAs lowers LDL susceptibility to oxidation compared to sup-

plementation with polyunsaturated fat [13–15]. Furthermore, oleic acid has been shown to exert endothelial vasomodulatory and antioxidant effects [16, 17]. Yet, the overall proportion of MUFAs in the Mediterranean diet is not much different from that of other diets, like the North American one, which are associated with higher cardiovascular mortality [18]. The high proportion of MUFAs, namely oleic acid, in food items such as poultry, pork, and recently-marketed seed oils accounts for this similarity. Moreover, the direct effects of MUFAs on serum lipid/lipoprotein profiles are, at present, equivocal [19]. Replacement of saturated fat with MUFA is, in fact, associated with favorable changes in plasma lipoprotein profile (reduction of LDL cholesterol and increase in HDL cholesterol concentrations) but this effect may partly be attributed to the concomitant reduction of saturates. Further, equicaloric replacement of carbohydrates with MUFAs leads to modest modifications of serum cholesterol and lipoprotein concentrations [20].

Extra virgin olive oil (EVOO) is rich in phenols; specifically, the catecholic compounds hydroxytyrosol (HT) and oleuropein (OE) act as potent antioxidants, free radical scavengers, and modulators of various oxygen-dependent enzymes [21–23]. Although most of these properties have been demonstrated *in vitro*, published data show that HT is dose-dependently absorbed after oral ingestion by rats [24–28] and humans [29–31] and that it retains its antioxidant properties *in vivo* [28, 32–34].

Based on such data, we sought to comparatively investigate the effects of extra virgin (phenol-rich) olive oil supplementation on oxidative and enzymatic markers of processes currently considered relevant for CHD, in mildly dyslipidemic patients. The Virgin Olive Oil Study (VOLOS) is a randomized, single-blind, crossover study aimed at comparing the effects of EVOO with those of refined (phenol-poor) olive oil, thus maintaining identical MUFA supplementation.

Materials and methods

■ Olive oils

Extra virgin olive oil (EVOO), naturally rich in phenolic compounds, and refined olive oil (ROO), very low in phenolics, were provided by Carapelli s. p. a. (Tavarnelle Val di Pesa, Italy) in unlabeled 500 mL dark glass bottles. A dietician instructed study subjects to consume 40 mL of raw olive oil/day, subdivided between lunch and dinner, to be eaten with pasta or vegetables. This amount is within the range of habitual consumption in Mediterranean populations [35]. Olive oil was provided to study subjects in large excess for consumption by the rest of their family.

EVOO had a total hydroxytyrosol content (as free

HT + HT esterified in oleuropein), of 166 mg/L, as assessed by gas-chromatography/mass spectrometry analysis of the phenolic extracts [29, 36], while the phenolic concentration of ROO was 2 mg/L. Estimated daily intakes of total HT were therefore 6.64 and 0.08 mg, respectively.

Fatty acid analysis of EVOO and ROO was performed by gas liquid chromatographic separation of the methyl esters [37] and revealed nearly identical fatty acid (FA) profiles. The predominant FAs in both oils were oleic (70.9% in EVOO vs. 72.7% in ROO), palmitic (11.5% vs. 10.7%), and linoleic (8.5% vs. 7.6%) acids.

All reagents were from Sigma (Milan, Italy) and were of the highest purity available.

■ Patient selection and study design

This study conforms with the principles outlined in the “Declaration of Helsinki”, and has been approved by the Niguarda Hospital Internal Review Board.

Study subjects were selected among those who were diagnosed for mild dyslipidemia at the Grossi Paoletti Center, University of Milan, before initiation of pharmacological therapy. Inclusion criteria were: total cholesterol 220–280 mg/dL, low-density lipoprotein cholesterol (LDL-C) 150–170 mg/dL, and triglycerides (TG) 130–180 mg/dL.

Twenty-two subjects (12 males and 10 females, aged 18 to 65 years, seven smokers and 15 nonsmokers, BMI < 25 kg/m²) were enrolled and gave informed consent to the study.

The study followed a crossover design. Three weeks (T –21 days, “run-in period”) before initiation of the two treatments, all subjects consumed 40 mL/day of ROO, in order to minimize potential body storage of olive oil phenolic compounds. This run-in period was followed by treatments with either one of the two oils, which lasted for a period of seven weeks (T49 days). During this period, patients were randomly divided into two groups. The first one, consisting of 13 individuals, was randomly assigned to the administration of EVOO. The second group (nine subjects) was initially given ROO. The treatment period was followed by four weeks of washout (WO), during which all subjects consumed ROO. After the washout period, subjects were switched treatment, which lasted for an additional seven weeks. Compliance was monitored by frequent interviews with the dietician, who also controlled the diet for other polyphenol-rich foods, e. g. tomato.

■ Blood analyses

Blood was drawn in the morning from the antecubital vein of fasting subjects at the following time points: T-

21 (recruitment), T0 (initiation of treatment), T28 and T49 (28 and 49 days, respectively, middle and end points of first treatment), washout, T28 and T49 of the second treatment.

One test tube was employed for lipid analysis (by routine laboratory methods), while another tube was placed in a 37 °C water bath for one hour, to allow for clotting [38]. Serum was then separated by centrifugation at 2000 x g for 10 min and subsequently stored at -80 °C. Thromboxane B₂ (TXB₂) in serum was quantified by immunoassay (Cayman Chemical, Ann Arbor, MI), following the manufacturer's instructions.

Blood was also collected into a third tube, containing Li⁺ heparin as anticoagulant. Total antioxidant capacity of plasma was evaluated by an established method based on the reduction, by antioxidants, of Cu⁺⁺ to Cu⁺, as previously described (Oxis Research, Portland, OR) [28, 39].

■ Urine analyses

24-hour urine was collected at the beginning and at the end of each treatment (including washout). Total collected volumes per individual (which varied very little from one collection to the other) were measured and 40 mL aliquots were stored at -80 °C. The F₂ isoprostane, namely 8-iso-PGF_{2α} (iPF_{2α}-III), concentration was evaluated by a mass spectrometry-validated immunoassay, as previously described [34, 40].

Laboratory personnel were blinded to treatments.

■ Statistical analyses

Data were analyzed for distribution using frequency histograms. Two separate analyses were performed: 1) a three periods crossover analysis with two treatments and two sequences and 2) repeated measures analysis of variance. The latter was performed to confirm the re-

sults of the crossover analysis and to identify potential trends in the outcomes. Software: Stata version 7 and SAS version 8.01. A p value less than 0.05 was considered statistically significant.

Results

Treatments with either EVOO or ROO were not associated with marked variations of serum lipid and lipoprotein parameters, such as total, LDL-, and HDL-cholesterol, and triacylglycerols (Table 1). Other indexes such as BMI, mean blood pressure, and glycemia were also unaffected by the administration of either oil (data not shown).

Serum TXB₂ concentrations, a sensitive index of thromboxane production by maximally activated platelets, were decreased (~21 %) by EVOO supplementation. This effect was noted both when EVOO was administered in the first and in the second arm of the study (Fig. 1). In particular, seven weeks of EVOO intake were associated with a reduction of serum TXB₂ of approximately 20 % (Fig. 1a). This effect was reverted upon subsequent ROO administration. These data were confirmed by those obtained following administration of EVOO in the second arm of the study, which led to a ~30 % reduction of serum TXB₂ production (Fig. 1b). On the other hand, ROO as first treatment did not affect TXB₂ production.

The antioxidant capacity of plasma increased after EVOO administration (from 210 to 291 μM Cu⁺⁺ reduced, i.e. by approximately 40 %), returning to basal levels after washout (Fig. 2a). A further reduction was observed after treatment with ROO. When ROO was administered during the first arm of treatment, a reduction of antioxidant capacity from 221 to 161 μM Cu⁺⁺ reduced (-28 %) was observed (Fig. 2b). Subsequent consumption of EVOO increased antioxidant capacity up to ~12 % over basal levels.

Table 1 Serum lipid profile of patients administered extra virgin olive oil or refined olive oil

	T0	T28EVOO	T49EVOO	WO	T28ROO	T49ROO
CHO	249.8±23.6	240.2±69.6	247.9±28.6	260.4±21.9	257.6±15.3	261.6±23.3
TG	129.1±62.1	117.0±49.3	101.0±29.8	121.1±53.7	118.0±43.3	135.8±62.3
HDL	53.4±15.1	54.7±15.6	53.3±14.4	57.5±16.6	52.9±10.8	63.8±21.9
LDL	170.4±19.7	177.5±27.2	175.7±28.8	178.9±22.2	180.5±10.1	170.3±28.1
	T0	T28ROO	T49ROO	WO	T28EVOO	T49EVOO
CHO	223.4±22.6	238.4±34.7	256.3±44.9	246.0±36.6	238.3±23.3	253.6±37.9
TG	140.3±42.8	164.5±54.7	213.3±78.5	123.5±43.3	148.9±88.9	162.3±81.4
HDL	51.1±13.2	53.1±18.9	51.0±18.8	57.4±18.4	54.8±15.4	60.3±18.4
LDL	144.2±32.8	152.3±35.5	170.4±49.3	162.9±30.8	153.2±28.6	160.4±42.1

CHO total cholesterol; TG triacylglycerols; HDL high density lipoprotein; LDL low density lipoprotein; EVOO extra virgin olive oil; ROO refined olive oil; T0 beginning of trial; T28 28 days of supplementation; T49 49 days of supplementation; WO washout (four weeks). n = 22. Data are means ± S. D. and are expressed as mg/dL.

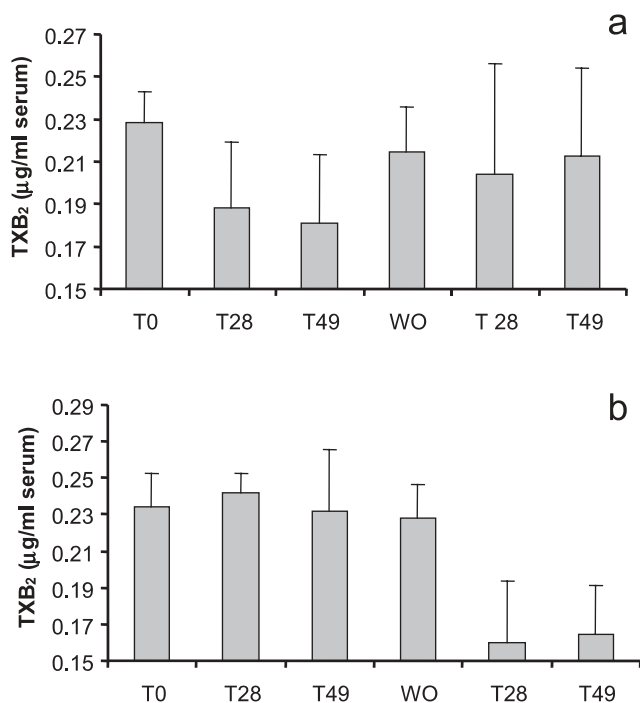


Fig. 1 Serum TXB₂ concentrations (µg/ml) in subjects administered EVOO or ROO for 49 days. **a** Subjects were first given EVOO and then, after washout, ROO. **b** Subjects were first given ROO and then, after washout, EVOO. Data are means ± S.D., n = 22

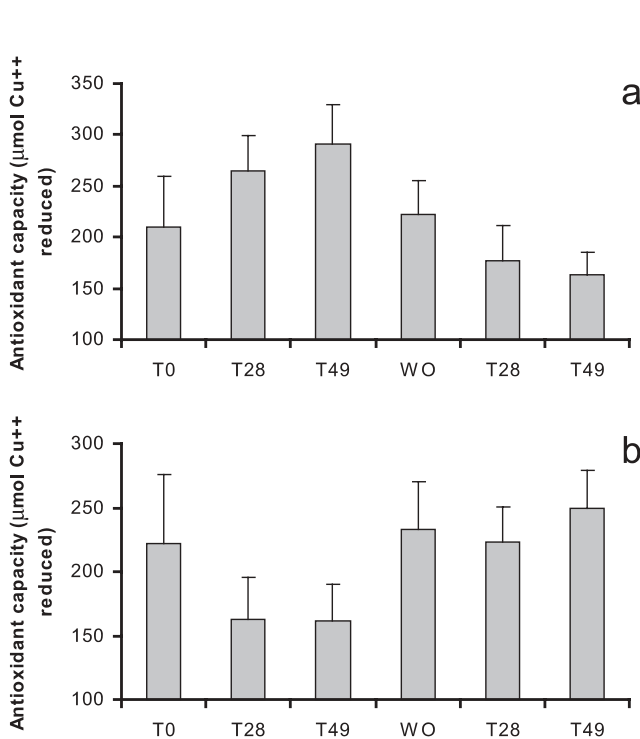


Fig. 2 Plasma antioxidant capacity (as µM Cu⁺⁺ reduced) in subjects administered EVOO or ROO for 49 days. **a** Subjects were first given EVOO and then, after washout, ROO. **b** Subjects were first given ROO and then, after washout, EVOO. Data are means ± S.D., n = 22

The urinary excretion of 8-iso-PGF_{2α} (Fig. 3) was not affected by the two different treatments (see later), although a trend toward an enhancement of total isoprostane excretion by ROO was recorded in both arms of the crossover study (Fig. 3a and b).

The statistical analyses of the crossover design excluded any carry-over effect.

As far as TXB₂ production was concerned, the crossover analysis showed two separate effects of treatment ($p < 0.0001$) and period ($p < 0.01$). The analysis of variance confirmed the treatment and period effects and highlighted the presence of a linear trend in the outcome, both overall ($p < 0.001$) and within treatments ($p < 0.001$).

The antioxidant capacity exhibited similar statistical significance. The crossover analysis showed again two separate effects of treatment ($p < 0.0001$) and period ($p < 0.0001$). The analysis of variance confirmed the treatment effect ($p < 0.0001$). A linear trend in the outcome was found only within treatments ($p < 0.001$).

Finally, the crossover analysis of 8-iso-PGF_{2α} excretions showed both treatment ($p < 0.05$) and period (0.0001) effects. The analysis of variance confirmed a period effect ($p < 0.01$) but did not reveal any effect due to the treatment.

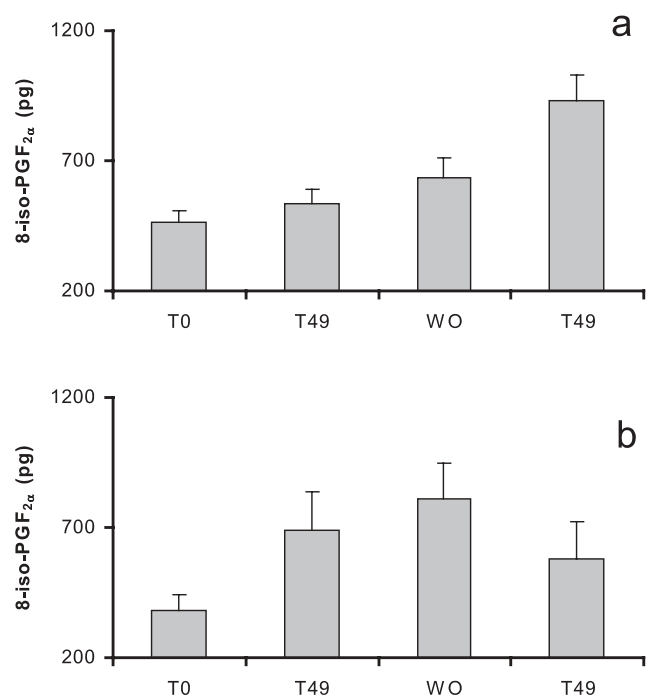


Fig. 3 Total, 24-hour urinary 8-iso-PGF_{2α} excretion (pg) in subjects administered EVOO or ROO for 49 days. **a** Subjects were first given EVOO and then, after washout, ROO. **b** Subjects were first given ROO and then, after washout, EVOO. Total volume of 24-hour urine was measured and 8-iso-PGF_{2α} concentration was evaluated by immunoassay. Data are means ± S.D., n = 22

Discussion

Interest in the biological activities of olive oil phenolics has been steadily growing over recent years, as their “pharmacological” properties might partly explain the low CHD mortality in the Mediterranean area. In fact, the low CHD mortality associated with olive oil consumption appears not to be exclusively related to its high monounsaturated fatty acid content. Currently, *in vivo* studies of the biochemical effects of natural and synthetic antioxidants are scarce, mostly due to the lack of appropriate markers of antioxidant effect [41, 42]. In the VOLOS study, we focused on the evaluation of biochemical indexes, either related to the presence of the compounds under investigation in the analyzed biological samples (antioxidant capacity and serum TXB₂) or direct markers of *in vivo* oxidative processes and their prevention (urinary isoprostanes).

These markers are currently considered relevant to the onset and development of CHD: it is therefore of interest that the administration of extra virgin olive oil (EVOO), naturally abundant in phenolic compounds, was associated with improvement of these indexes. The crossover design of VOLOS further strengthens these observations. In particular, consumption of EVOO increased plasma antioxidant capacity, consistent with previous data obtained in the rat but in disagreement with what was reported by Visser et al. [43], who reported no antioxidant effects after three weeks of EVOO administration (providing 18 mg/day of total phenols with an unknown proportion of catechols). Also consistent with *in vitro* studies [44] reporting antithrombotic potential of olive oil phenols is the observation of reduced production of TXB₂ by maximally activated platelets, such as those in serum allowed to clot at 37 °C for one hour. Other *in vitro* and *in vivo* studies suggested antiinflammatory activities of olive oil phenols [45–49], reinforcing the notion that these compounds can modulate enzymatic activities related to eicosanoid metabolism. Possibly, olive oil phenolics are able to maintain reduced intracellular environment, thus inhibiting the activities of peroxide tone-dependent enzymes [50, 51].

The prolonged administration of olive oil appears to increase 8-iso-PGF_{2α} excretion, regardless of the kind of olive oil supplied to the patients. This finding is apparently in contrast with results obtained after a single administration of a phenol-enriched (providing higher doses of phenol) EVOO to humans [33] or of pure HT to sidestream smoke-exposed rats [34]. Yet, the significant period effect does not allow any inference on the effects of olive oil phenolics on this parameter. Moreover, it should be noted that, unlike blood sampling, collection of urine was performed only twice for each arm of the treatment, making it impossible to statistically assess

trends. Thus, the period effect might be masking a possible treatment effect.

The lack of differences in the effects of both oils on the patients’ lipid/lipoprotein profiles – except for the non-significant favorable changes in TG concentrations after EVOO intake during the first arm of the treatment – was expected, due to the relatively limited duration of the study and to the nearly identical fatty acid compositions of the two oils. Relevant to these results, it is noteworthy that the Lyon Diet Heart Study observed a dramatic reduction in CHD mortality following dietary intervention that did not affect cholesterol concentrations [52, 53].

One of the most interesting aspects of the results obtained in the VOLOS study is that they were obtained with a daily ingestion of a food item (EVOO) in amounts (40 ml/day) and with a phenolic content (providing 6.6 mg/day) that are comparable to those currently consumed by many population groups in the Mediterranean area [54]. In contrast, available data on the biological effects of other antioxidants, namely vitamins, in animals and humans have been obtained by administering high doses of such molecules, up to 100-fold the average dietary intakes, i.e. with a pharmacological approach [5, 55].

As opposed to epidemiological studies of antioxidant intake and CHD mortality, recent reports of large-scale clinical trials have shown that administration of antioxidant vitamins, namely vitamin E and β-carotene, does not reduce coronary events in CHD patients [56]. Proposed interpretations include correct selection of individuals at risk for oxidative stress [57], administration of right antioxidants at right dosages for the right time [5, 58], and lack of *in vivo* markers of antioxidant effect [5, 42]. Furthermore, the possibility that dietary rather than pharmacological intake of antioxidants is accompanied by higher absorption [59–61] and ingestion of “cooperating” antioxidants [62] should be taken into account. To date, human studies based on the administration of oligonutrients from foods, e.g. from cocoa and tea, are still scarce [51, 63–66].

In conclusion, data from VOLOS demonstrate that inclusion of practical quantities of extra virgin, phenol-rich olive oil in the diet can positively modify surrogate biological markers – associated with lower oxidative stress and thrombotic potential – for vascular function and cumulative cardiovascular risk, in mildly dyslipidemic patients. Based on current knowledge, these modifications may be associated with cardioprotective effects.

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References

- Parfitt VJ, Rubba P, Bolton C, Marotta G, Hartog M, Mancini M (1994) A comparison of antioxidant status and free radical peroxidation of plasma lipoproteins in healthy young persons from Naples and Bristol. *Eur Heart J* 15: 871–876
- Simopoulos AP, Sidossis LS (2000) What is so special about the traditional diet of Greece. The scientific evidence. *World Rev Nutr Diet* 87:24–42
- Navab M, Hama SY, Ready ST, et al. (2002) Oxidized lipids as mediators of coronary heart disease. *Curr Opin Lipidol* 13:363–372
- Witztum JL, Steinberg D (2001) The oxidative modification hypothesis of atherosclerosis: does it hold for humans? *Trends Cardiovasc Med* 11:93–102
- Steinberg D, Witztum JL (2002) Is the oxidative modification hypothesis relevant to human atherosclerosis? Do the antioxidant trials conducted to date refute the hypothesis? *Circulation* 105: 2107–2111
- Miller ER III, Appel LJ, Risby TH (1998) Effect of dietary patterns on measures of lipid peroxidation: results from a randomized clinical trial. *Circulation* 98:2390–2395
- Stocker R (1999) Dietary and pharmacological antioxidants in atherosclerosis. *Curr Opin Lipidol* 10:589–597
- Hu FB, Willett WC (2002) Optimal diets for prevention of coronary heart disease. *JAMA* 288:2569–2578
- Bazzano LA, He J, Ogden LG, et al. (2002) Fruit and vegetable intake and risk of cardiovascular disease in US adults: the first National Health and Nutrition Examination Survey Epidemiologic Follow-up Study. *Am J Clin Nutr* 76:93–99
- Ferro-Luzzi A, Branca F (1995) Mediterranean diet, Italian-style: prototype of a healthy diet. *Am J Clin Nutr* 61: 1338S–1345S
- Willett WC, Sacks F, Trichopoulos A, et al. (1995) Mediterranean diet pyramid: a cultural model for healthy eating. *Am J Clin Nutr* 61:1402S–1406S
- Trichopoulos A, Costacou T, Bamia C, Trichopoulos D (2003) Adherence to a Mediterranean diet and survival in a Greek population. *N Engl J Med* 348: 2599–2608
- Reaven P, Parthasarathy S, Grasse BJ, et al. (1991) Feasibility of using an oleate-rich diet to reduce the susceptibility of low-density lipoprotein to oxidative modification in humans. *Am J Clin Nutr* 54:701–706
- Bonanome A, Pagnan A, Biffanti S, et al. (1992) Effect of dietary monounsaturated and polyunsaturated fatty acids on the susceptibility of plasma low density lipoproteins to oxidative modification. *Arterioscler Thromb* 12:529–533
- Berry EM, Eisenberg S, Friedlander Y, et al. (1992) Effects of diets rich in monounsaturated fatty acids on plasma lipoproteins – the Jerusalem Nutrition Study. II. Monounsaturated fatty acids vs carbohydrates. *Am J Clin Nutr* 56: 394–403
- Fuentes F, Lopez-Miranda J, Sanchez E, et al. (2001) Mediterranean and low-fat diets improve endothelial function in hypercholesterolemic men. *Ann Intern Med* 134:1115–1119
- Massaro M, Basta G, Lazzerini G, et al. (2002) Quenching of intracellular ROS generation as a mechanism for oleate-induced reduction of endothelial activation and early atherogenesis. *Thromb Haemost* 88:335–344
- Dougherty RM, Galli C, Ferro-Luzzi A, Iacono JM (1987) Lipid and phospholipid fatty acid composition of plasma, red blood cells, and platelets and how they are affected by dietary lipids: a study of normal subjects from Italy, Finland, and the USA. *Am J Clin Nutr* 45:443–455
- Hegsted DM, Ausman LM, Johnson JA, Dallal GE (1993) Dietary fat and serum lipids: an evaluation of the experimental data. *Am J Clin Nutr* 57:875–883
- Mensink RP, Katan MB (1992) Effect of dietary fatty acids on serum lipids and lipoproteins. *Arterioscler Thromb Vasc Biol* 12:911–919
- Visioli F, Poli A, Galli C (2002) Antioxidant and other biological activities of phenols from olives and olive oil. *Med Res Rev* 22:65–75
- Visioli F, Galli C (2002) Biological properties of olive oil phytochemicals. *Crit Rev Food Sci Nutr* 42:209–221
- Stark AH, Madar Z (2002) Olive oil as a functional food: epidemiology and nutritional approaches. *Nutr Rev* 60: 170–176
- Bai C, Yan X, Takenaka M, Sekiya K, Nagata T (1998) Determination of synthetic hydroxytyrosol in rat plasma by GC-MS. *J Agric Food Chem* 46: 3998–4001
- Ruiz-Gutierrez V, Juan ME, Cert A, Planas JM (2000) Determination of hydroxytyrosol in plasma by HPLC. *Anal Chem* 72:4458–4461
- Tuck KL, Freeman MP, Hayball PJ, Stretch GL, Stupans I (2001) The in vivo fate of hydroxytyrosol and tyrosol, antioxidant phenolic constituents of olive oil, after intravenous and oral dosing of labeled compounds to rats. *J Nutr* 131: 1993–1996
- D'Angelo S, Manna C, Migliardi V, et al. (2001) Pharmacokinetics and metabolism of hydroxytyrosol, a natural antioxidant from olive oil. *Drug Metab Dispos* 29:1492–1498
- Visioli F, Caruso D, Plasmati E, et al. (2001) Hydroxytyrosol, as a component of olive mill waste water, is dose-dependently absorbed and increases the antioxidant capacity of rat plasma. *Free Rad Res* 34:301–305
- Visioli F, Galli C, Bornet F, et al. (2000) Olive oil phenolics are dose-dependently absorbed in humans. *FEBS Lett* 468:159–160
- Caruso D, Visioli F, Patelli R, Galli C, Galli G (2001) Urinary excretion of olive oil phenols and their metabolites in humans. *Metabolism* 50:1426–1428
- Vissers MN, Zock PL, Roodenburg AJ, Leenen R, Katan MB (2002) Olive oil phenols are absorbed in humans. *J Nutr* 132:409–417
- Bonanome A, Pagnan A, Caruso D, et al. (2000) Evidence of postprandial absorption of olive oil phenols in humans. *Nutr Metab Cardiovasc Dis* 10:111–120
- Visioli F, Caruso D, Galli C, Viappiani S, Galli G, Sala A (2000) Olive oils rich in natural catecholic phenols decrease isoprostane excretion in humans. *Biochem Biophys Res Commun* 278:797–799
- Visioli F, Galli C, Plasmati E, et al. (2000) Olive phenol hydroxytyrosol prevents passive smoking-induced oxidative stress. *Circulation* 102:2169–2171
- Boskou D (2000) Olive oil. *World Rev Nutr Diet* 87:56–77
- Caruso D, Colombo R, Patelli R, Giavarini F, Galli G (2000) Rapid evaluation of phenolic component profile and analysis of oleuropein aglycon in olive oil by atmospheric pressure chemical ionization-mass spectrometry (APCI-MS). *J Agric Food Chem* 48:1182–1185
- Marangoni F, Angeli MT, Colli S, et al. (1993) Changes of n-3 and n-6 fatty acids in plasma and circulating cells of normal subjects, after prolonged administration of 20:5 (EPA) and 22:6 (DHA) ethyl esters and prolonged washout. *Biochim Biophys Acta* 1210: 55–62
- Van Hecken A, Juliano ML, Depre M, et al. (2002) Effects of enteric-coated, low-dose aspirin on parameters of platelet function. *Aliment Pharmacol Ther* 16:1683–1688

39. Visioli F, Riso P, Grande S, Galli C, Porrini M (2003) Protective activity of tomato products on in vivo markers of lipid oxidation. *Eur J Nutr* 42:201–206
40. Wang Z, Ciabattini G, Creminon C, et al. (1995) Immunological characterization of urinary 8-epi-prostaglandin F₂ alpha excretion in man. *J Pharmacol Exp Ther* 275:94–100
41. Halliwell B (1999) Establishing the significance and optimal intake of dietary antioxidants: the biomarker concept. *Nutr Rev* 57:104–113
42. Halliwell B (2000) Lipid peroxidation, antioxidants and cardiovascular disease: how should we move forward? *Cardiovasc Res* 47:410–418
43. Vissers MN, Zock PL, Wiseman SA, Meyboom S, Katan MB (2001) Effect of phenol-rich extra virgin olive oil on markers of oxidation in healthy volunteers. *Eur J Clin Nutr* 55:334–341
44. Petroni A, Blasevich M, Papini N, Papini N, Montedoro GF, Galli C (1995) Inhibition of platelet aggregation and eicosanoid production by phenolic components of olive oil. *Thromb Res* 78:151–160
45. Petroni A, Blasevich M, Papini N, Salami M, Sala A, Galli C (1997) Inhibition of leukocyte leukotriene B₄ production by an olive oil-derived phenol identified by mass-spectrometry. *Thromb Res* 87:315–322
46. de la Puerta R, Ruiz G, V, Hoult JR (1999) Inhibition of leukocyte 5-lipoxygenase by phenolics from virgin olive oil. *Biochem Pharmacol* 57:445–449
47. de la Puerta R, Martinez-Dominguez E, Ruiz-Gutierrez V (2000) Effect of minor components of virgin olive oil on topical antiinflammatory assays. *Z Naturforsch* 55:814–819
48. Martinez-Dominguez E, de la Puerta R, Ruiz-Gutierrez V (2001) Protective effects upon experimental inflammation models of a polyphenol-supplemented virgin olive oil diet. *Inflamm Res* 50:102–106
49. Kohyama N, Nagata T, Fujimoto S, Sekiya K (1997) Inhibition of arachidonate lipoxygenase activities by 2-(3,4-dihydroxyphenyl)ethanol, a phenolic compound from olives. *Biosci Biotechnol Biochem* 61:347–350
50. Diaz MN, Frei B, Vita JA, Keaney JF (1997) Antioxidants and atherosclerotic heart disease. *New Engl J Med* 337:408–416
51. Halliwell B, Gutteridge JMC (1999) Free radicals in biology and medicine. Oxford, UK: Oxford University Press
52. de Lorgeril M, Renaud S, Mamelle N, et al. (1994) Mediterranean alpha-linolenic acid-rich diet in secondary prevention of coronary heart disease. *Lancet* 343:1454–1459
53. de Lorgeril M, Salen P, Martin J-L, Monjaud I, Delaye J, Mamelle N (1999) Mediterranean diet, traditional risk factors, and the rate of cardiovascular complications after myocardial infarction. Final report of the Lyon diet heart study. *Circulation* 99:779–785
54. Visioli F (2000) Antioxidants in Mediterranean Diets. In: Simopoulos AP, Visioli F (eds) *Mediterranean diets*. Basel: Karger, pp 43–55
55. McCall MR, Frei B (1999) Can antioxidant vitamins materially reduce oxidative damage in humans? *Free Radic Biol Med* 26:1034–1053
56. Kritharides L, Stocker R (2002) The use of antioxidant supplements in coronary heart disease. *Atherosclerosis* 164:211
57. Meagher EA, Barry OP, Lawson JA, Rokach J, FitzGerald GA (2001) Effects of vitamin E on lipid peroxidation in healthy persons. *JAMA* 285:1178–1182
58. Bellomo G (1999) Is it really time to perform large intervention trials with antioxidant vitamins to prevent cardiovascular disease? *Pharmacol Res* 40:207–208
59. Burton GW, Traber M, Acuff RV, et al. (1998) Human plasma and tissue alpha-tocopherol concentrations in response to supplementation with deuterated natural and synthetic vitamin E. *Am J Clin Nutr* 67:669–684
60. Visioli F, Rise P, Plasmanti E, Pazzucconi F, Sirtori CR, Galli C (2000) Very low intakes of N-3 fatty acids incorporated into bovine milk reduce plasma triacylglycerol and increase HDL-cholesterol concentrations in healthy subjects. *Pharmacol Res* 41:571–576
61. Visioli F, Galli C, Grande S, et al. (2003) Hydroxytyrosol excretion differs between rats and humans and depends on the vehicle of administration. *J Nutr* 133:2612–2615
62. Thomas SR, Leichtweis SB, Pettersson K, et al. (2001) Dietary cosupplementation with vitamin E and coenzyme Q(10) inhibits atherosclerosis in apolipoprotein E gene knockout mice. *Arterioscler Thromb Vasc Biol* 21:585–593
63. Vinson JA (1998) Flavonoids in foods as in vitro and in vivo antioxidants. *Adv Exp Med Biol* 439:151–164
64. Visioli F, Borsani L, Galli C (2000) Diet and prevention of coronary heart disease: the potential role of phytochemicals. *Cardiovasc Res* 47:419–425
65. Kris-Etherton PM, Keen CL (2002) Evidence that the antioxidant flavonoids in tea and cocoa are beneficial for cardiovascular health. *Curr Opin Lipidol* 13:41–49
66. Linus Pauling Institute website (2003)