Some nutritional benefits of extra virgin olive oil

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SUMMARY

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The benefits of olive oil could be related to its antioxidant potential. The aim of this study was to evaluate the "in vivo" antioxidant activity of different extra virgin olive oils (EVOO) produced in the Mediterranean area. One hundred and twenty rats were fed diets containing oxidised and refined olive oil (400 mEq O_2 kg) for 11 weeks, a period of time sufficient to induce globular fragility and marginal plasma tocopherols deficiency.

The animals were then fed 5 diets containing EVOO with content of tocopherols and phenols ranging from 165 to 335 and from 62 to 389 ppm respectively, in different tocopherols/phenols ratios, for 4 weeks.

Results show that plasma tocopherols concentration after EVOO feeding was directly related to dietary intake.

Moreover, globular resistance improved to a different extent with EVOO diets compared to the oxidised oil diet. The most favourable antioxidant response was obtained by feeding the oil high in both tocopherols and phenols; the action of phenols was considered synergetic to that of tocopherols in restoring normal conditions, impaired by the oxidised oil diet.

KEY-WORDS: Globular resistance - Nutritional quality - Phenol - Tocopherol - Virgin olive oil.

1. INTRODUCTION

The use of vegetable oils has been recommended to increase the ratio of unsaturated to saturated fatty acids and to lower serum cholesterol. Among vegetable oils, extra virgin olive oil (EVOO) offers good protection against lipid oxidation due to the prevalence of monounsaturated with respect to polyunsaturated fatty acids and to the presence of natural antioxidants, in particular tocopherols and phenols (Nardini et al., 1993). The intake of olive oil is considered responsible for low serum cholesterol and coronary risk profile in Mediterranean countries (Trevisan et al., 1990; Masana et al., 1991). The low prevalence of coronary disease could be related either to the fatty acid profile of olive oil or to the presence of minor components with biological activity, or both. The composition of dietary fatty acids is known to influence the composition and thus fluidity of cellular membranes (Lin et al., 1993 and Hillier et al., 1991).

In red blood cells the changes introduced by dietary lipids may be considerable because the fluidity of red blood cells affects flexibility, oxygen transport and active receptors that depend on fluidity (Hagve et al., 1991). The role of fatty acids is important not only because they are structural components of membranes but also because they are precursors of components such as eicosanoids that play a major role in the regulation of coagulation. On the other hand, tocopherols, phenols and other minor compounds, which are present in appreciable amounts in EVOO, may have a positive influence on lipoprotein composition and oxidative status in different population groups (Mata et al., 1992; Aviram et al., 1993). The present study was undertaken to assess the comparative effect of minor compounds (tocopherols and phenols) present in EVOO produced in the Mediterranean area on oxidative stress indexes in rats.

2. EXPERIMENTAL

The EVOOs used for the experiment were selected, to have different tocopherols/phenols ratios, from 16 EVOO produced in different countries during the winter of 1992. The content of tocopherols and phenols of the oils is shown in Figure 1.

We studied 120 1-month-old female Sprague-Dawley rats. Four were killed immediately as described below. The other 116 were fed diet AIN-76 (American Institute of Nutrition 1976) with added strongly oxidised refined olive oil (OX-ROO) (peroxide level 400 mEq 0_2/kg of fat), for 11 weeks, in order to produce detectable modifications or damage in tissues (liver and blood); 4 were killed at 5 weeks and 4 at 11 weeks. The remaining animals were then divided into 7 groups and fed for 4 additional weeks on the same diet as above with OX-ROO; refined olive oil (ROO); high tocopherols-low phenols Spanish EVOO (S602CE); high tocopherols-high phenols Spanish EVOO (S502CE); low tocopherols-high phenols Italian EVOO (I302PR); medium tocopherols-medium phenols Greek EVOO (G101CE); or low tocopherols-low phenols EVOO made in our laboratory from I302PR (EVOO). The composition of the diets and the peroxide, tocopherols and phenols content of oils are reported in tables I, and II, respectively.

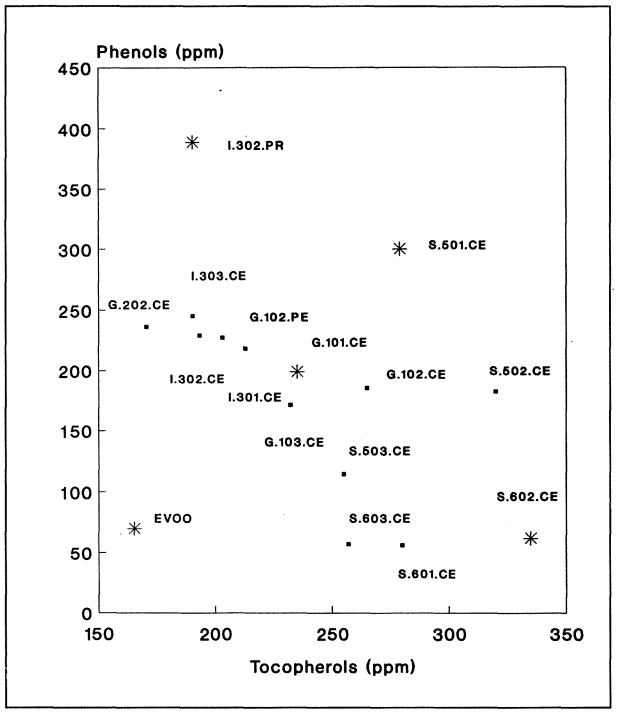

Figure 1
Tocopherols and phenols composition of extra virgin olive

Table I
Compositiom (%) of experimental diets

Casein	20
d-l-methionine	0.3
Corn starch	15
Sucrose	50
Solka floc	3
Cellulose	2
Minerals	3.5
Vitamins* + choline	1.2
Lipids**	5

* No addition of vit. A and E
** See table II

The animals were housed in plastic cages with stainless-steel grid floors and fed the diet and water ad libitum. Four animals per group were killed at various time points (11, 12, 13 and 14 weeks) by bleeding from the abdominal aorta under ether anaesthesia, and tocopherols and malondialdehyde (MDA) in plasma, cytochromes P450 and b5 in liver, and erythrocyte fragility were measured. The liver was perfused in situ with 1.15% KCl saline, removed, blotted and homogenized for cytochrome analyses. The heparinized blood was centrifuged at 1,000xg for 10 minutes; erythrocytes were washed 3 times with an equal volume of isotonic saline and the hemoglobin assayed according to Crosby et al. (1954). Plasma MDA was

Table II
Tocopherol, phenols and peroxide content of the olive oils studied.

		Peroxide (meq O_2/kg)	Tocopherols (ppm)	Phenols (ppm)
** OX-ROO	:	330	>1	5
** ROO	:	8.1	22	4
** EVOO	:	10.4	165	70
** S62CE	: Spanish EVOO	10.2	335	62
** S52CE	: Spanish EVOO	7.7	235	199
** I32PR	: Italian EVOO	6.2	190	389
** G11CE	: Greek EVOO	11.2	279	300

OX-ROO: Oxidized refined olive oil
ROO : Refined olive oil
EVOO : Extra virgin olive oil

determined by the thiobarbituric acid reaction (Ohkawa et al., 1979). Hepatic microsomal cytochromes P450 and B5 were measured by spectrophotometry (Omura et al., 1964; Eriksson et al., 1982) and plasma tocopherols were measured by high performance liquid chromatography (Vuilleumier et al., 1983). The globular resistance of erythrocytes to lysis was assayed using 2.5% hydrogen peroxide in saline as oxidant compound (Clemens et al., 1989).

3. RESULTS

After 11 weeks of the OX-ROO diet, the plasma tocopherols level of the animals was dramatically depleted (from 4.8 to 1.5 mcg/ml) (Figure 2). The level continued to drop in the animals still fed the OX-ROO diet and in those given the ROO and EVOO diets, reaching a minimum of 0.5 mcg/ml. Feeding with the other diets increased plasma tocopherols concentrations starting from the first week, although the basal value was not reached. Concentrations at the end of experiment were related to the concentrations of tocopherols in the diet. Liver cytochromes P450 and B5 increased after the diet containing OX-ROO or the low tocopherols oils (Figures 3 and 4).

Plasma MDA rose significantly ($p<0.01$) only after the diet containing OX-ROO, whereas with the other diets it increased to a lesser extent or remained unmodified (Figure 5). The resistance of erythrocytes to lysis decreased dramatically at 11 weeks of the OX-ROO diet, with 20% intact erythrocytes after oxidative stress (Figure 6). Resistance to lysis was fully restored by feeding the S501CE diet, which is rich in both tocopherols and phenols. It was increased (up to 60 % intact cells) with the S602CE diet rich in tocopherols only, I302PR rich in phenols only, and G101CE with medium tocopherols and phenols content, whereas with the ROO, EVOO and OX-ROO diets it remained depressed at around the 11 week value.

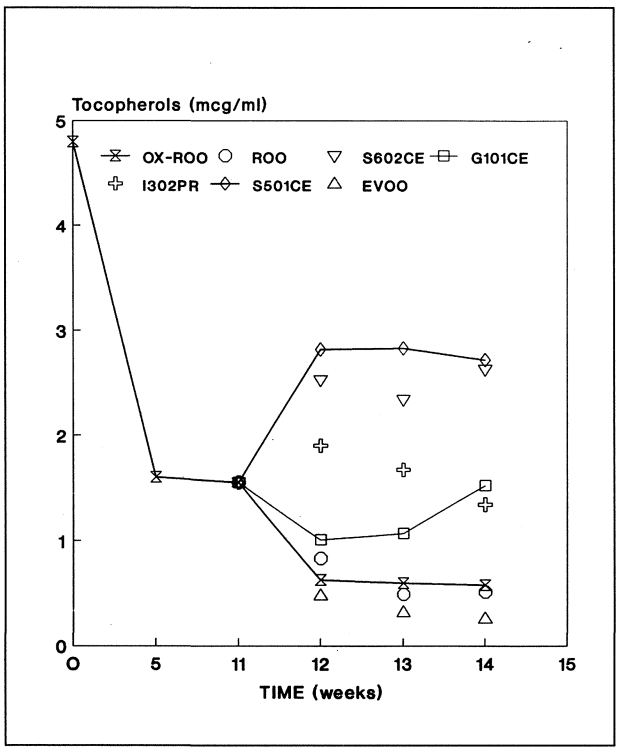

Figure 2
Plasma tocopherols content (mcg/ml) in rats fed OX-ROO, ROO, EVOO and S602CE, G101CE, I302PR, S501CE oils.

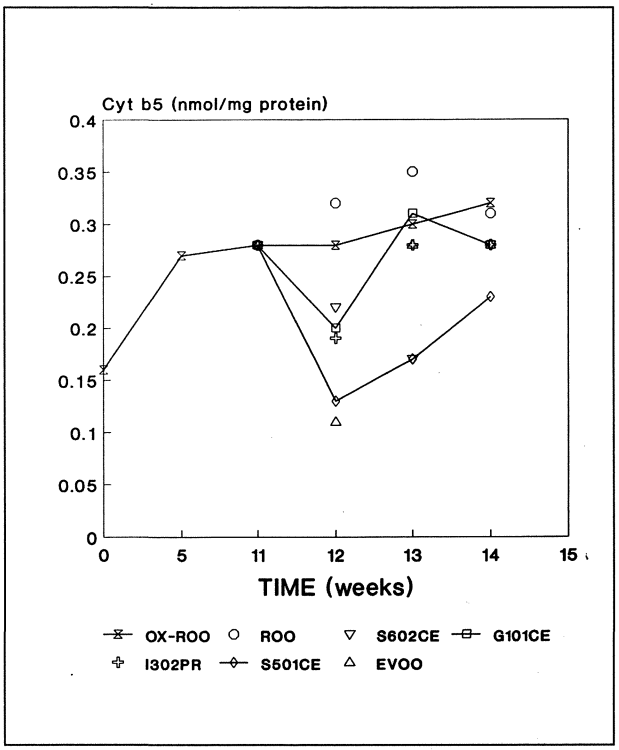

Figure 4
Liver b5 cytochrome content (nmol/mg protein) in rats fed different oils (for details see Figure 2).

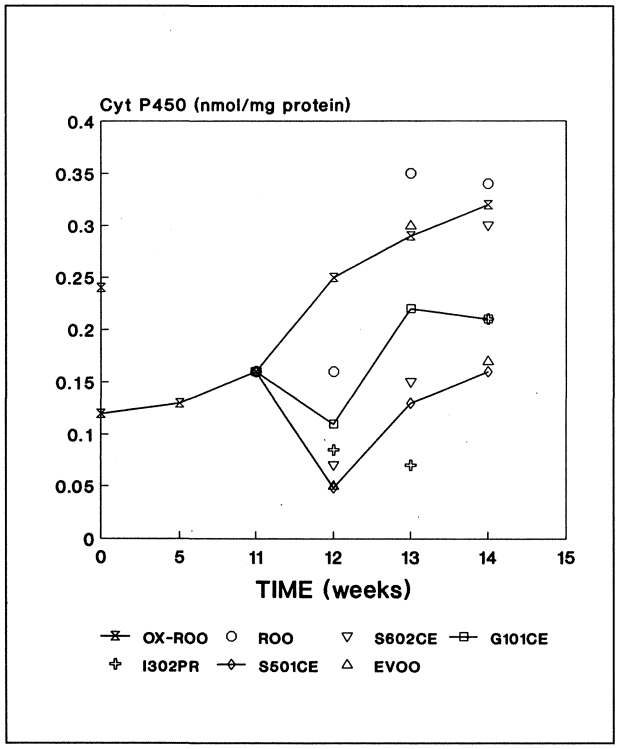

Figure 3
Liver P450 cytochrome content (nmol/mg protein) in rats fed oils different oils (for details see Figure 2).

4. DISCUSSION

The beneficial effects of changing the quality of lipids in the diet have been investigated in a number of experimental studies in the last decade. The principal aspects considered were the nature of fatty acids, particularly the effect of specific lipid classes such as n-3 polyunsaturated and n-9 monounsaturated fatty acids, (Lin et al., 1993). Some experimental evidence has been also reported indicating that high monounsaturated oils, because of their resistance to oxidative stress, could play an important role in maintaining optimal nutritional status in both animals and humans (Mensink et al., 1987; Trevisan et al., 1990).

However, vegetable oils contain a number of minor compounds which may have a positive effect in protecting the fatty acids from oxidative damage. This could result not only from a lesser peroxide intake with the diet but also from enhanced protection of membrane lipids if the compounds maintain their antioxidant activity once absorbed by the intestinal tract.

Among vegetable oils, EVOO is a good source of both monounsaturated fatty acids and antioxidants such as tocopherols and phenols, which increase its stability while conferring the typical aroma and taste. Moreover, the differences in the relative content of tocopherols and phenols, which depend on the variety of olive, degree of

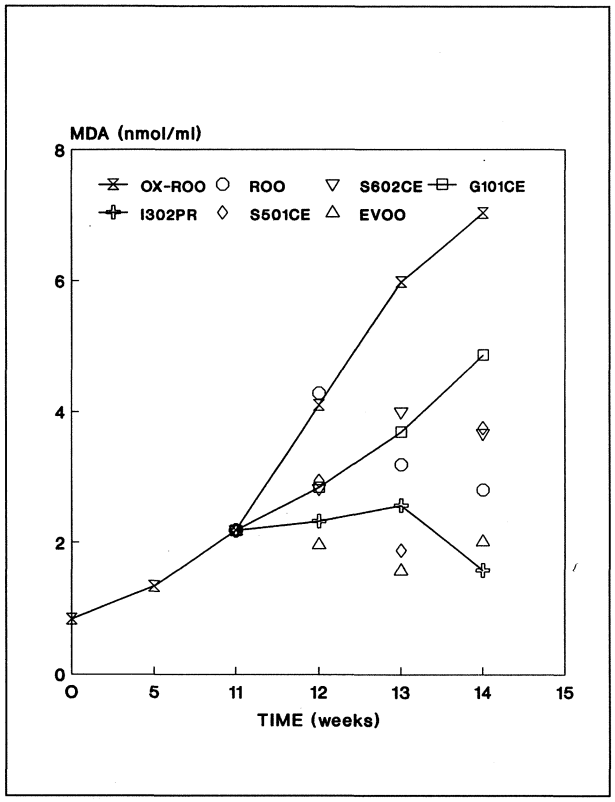

Figure 5
Plasma malondialdehyde (MDA) content (nmol/ml) in rats fed different oils (for details see Figure 2).

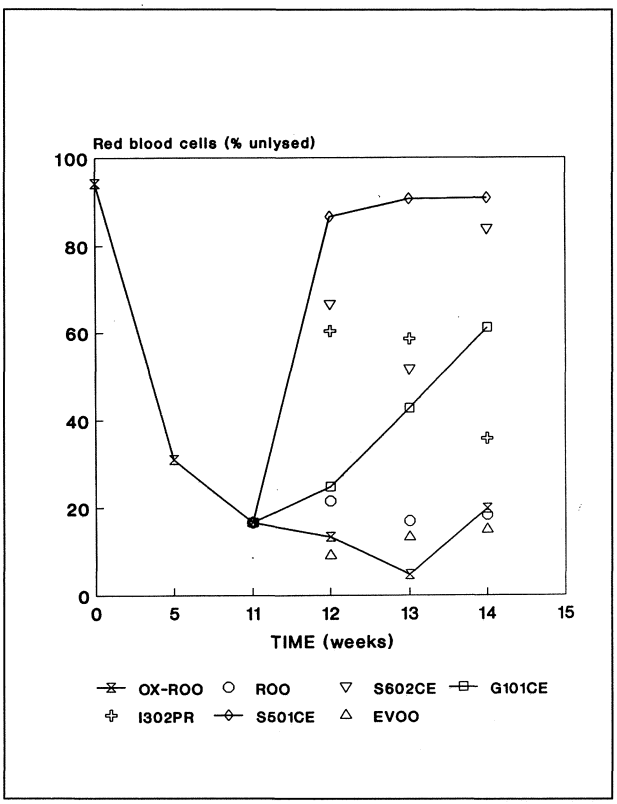

Figure 6
Globular fragility (% of unlysed red blood cells), in rats fed different oils (for details see Figure 2).

ripening, and technology of extraction, make olive oil a good model to test the relative effect of these two classes of antioxidants. We investigated the effect of different variables which could influence the nutritrional status related to oxidative damage of membranes induced by highly peroxidized lipids: 1) simple suspension of intake of peroxides, and 2) suspension of peroxide intake coupled with high intake of tocopherols, phenols or both.

The results indicate that the simple suspension of the intake of peroxidized lipids is not sufficient to restore a normal antioxidant status if feeding is continued with refined oil (ROO) or antioxidant-depleted EVOO. On the contrary, the presence of high tocopherols-high phenols EVOO in the diet reversed the picture of oxidative damage within a few days. As the fatty acid composition of the oils was the same, this effect must be ascribed to the unsaponifiable fraction, and in particular to the presence of natural antioxidants.

Moreover, the experimental protocol adopted by us, was able to discriminate between the effect of different olive oils on the reversal of oxidative damage. The globular resistance and plasma MDA observed by us indicate that the effect depends on the tocopherols content of oils, whereas phenolic compounds have only a minor or synergetic effect.

We may conclude that EVOO is effective in reducing some metabolic effects of oxidative stress, and that this activity may be improved by selecting olive varieties and technological procedures able to maximise the content of tocopherols and phenols of the oils.

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