CLINICAL STUDY

Effects of dietary extra virgin olive oil on serum lipid resistance to oxidation and fatty acid composition in elderly lipidemic patients

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Abstract

An inverse relation between high consumption of olive oil and low incidence of coronary heart disease among the people living in Mediterranean countries has been proposed. It has been shown, that an oleic acid-rich diet could increase the resistance of human LDL to in vitro oxidation which is postulated to play an important role in the development of atherosclerotic lesions. The aim of this study was to assess the effect of extra virgin olive oil consumptiom on the resistance of serum lipids to in vitro oxidation and on fatty acid composition in the serum of elderly lipidemic patients. A total of 26 patients (mean age 69 years) with combined hyperlipidemia consumed daily 2 table spoons (approx. 20 g) of extra virgin olive oil for 6 weeks. Plasma lipids, total antioxidant capacity, indices of serum lipid oxidizability (lag time and maximal rate of oxidation) and the content of fatty acids in serum phospholipids were determined before and after dietary supplementation with olive oil. Plasma total cholesterol and LDL cholesterol decreased significantly after 6 weeks of dietary intervention. A significant increase in the lag time of conjugated diene formation (p=0.026) and the decrease in the rate of lipid oxidation (p=0.030) were observed after olive oil consumption. The changes in the fatty acid profile were characterized by an increase in oleic acid content (p=0.005) as well as by a decline in the content of linoleic acid (p=0.020) and arachidonic acid (p=0.022). Linear regression analysis revealed some interesting and significant correlations between indices of serum lipid resistance to oxidation and individual fatty acids, suggesting a protective effects of olive oil in lipoprotein oxidation. In conclusion, the daily consumption of extra virgin olive oil in elderly lipidemic patients favourably affected serum lipoprotein spectrum and fatty acid composition that probably contributed to the increased resistance of serum lipids to oxidation. ***(Tab. 2, Ref. 18.)***

Key words: olive oil, lipoprotein oxidation, fatty acid composition, oleic acid.

An inverse relation between high consumption of olive oil and low incidence of coronary heart disease is supposed among the people living in Mediterranean countries. High content of monounsaturated fatty acids (MUFAs) such as oleic acid, may reduce the levels of atherogenic LDL cholesterol (1). The beneficial effects of oleic acid on lipoprotein spectrum were also observed in our previous study in elderly subjects (2).

The oxidation of LDL in the vascular wall is postulated to play an important role in the development of atherosclerotic lesions (3). It has been shown, that an oleic acid-rich diet could increase the resistance of human LDL to in vitro oxidation and hence could prevent or slow down the onset of the disease (4, 5, 6). An early step in the oxidation of LDL is peroxidation of polyunsaturated fatty acids (PUFAs) of membrane phospholipids, which results in the formation of lipid hydroperoxides. The estimation of PUFA peroxidation can be measured by the formation of lipid hydroperoxides, conjugated dienes and malondialdehyde levels. The major substrate for the oxidation of LDL is linoleic acid. Diets enriched in linoleate increase linoleic acid content in LDL and in this way could increase the susceptibility of LDL to oxidation. On the other hand, LDL isolated from subjects consuming oleate-rich diets appear to be more resistant to oxidation (5). The intake of a diet balanced for MUFAs and PUFAs content is therefore recommended to avoid the pro-oxidative effects

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Tab. 1. Serum lipid levels (mmol/l), lipid oxidizability and fatty acid content of elderly lipidemic patients supplemented with extra virgin olive oil.

	Supplementation (n=26)		
	before	after	p=
Age (years)	69±8	69±8	NS
Total cholesterol (mmol/L)	7.10±0.96	6.28±1.09	0.006
HDL cholesterol (mmol/L)	1.18±0.21	1.22±0.36	NS
LDL cholesterol (mmol/L)	4.98±0.98	4.20±1.07	0.009
Triacylglycerols (mmol/L)	2.20±1.07	1.97±1.09	NS
Lag time (min)	87±20	102±27	0.026
Maximal rate of oxidation (mabs/min)	7.47±2.13	6.46±1.80	0.030
FRAP (μmol/L)	990±210	979±202	NS
Oleic acid (C18:1n9)	10.5±1.7	11.5±1.88	0.005
Linoleic acid (C18:2n6)	22.5±3.3	21.1±2.1	0.020
α-Linolenic acid (C18:3n3)	0.19±0.09	0.20±0.07	NS
Arachidonic acid (C20:4n6)	11.1±2.05	10.4±1.93	0.022
Eicosapentaenoic acid (EPA; C22:5n3)	0.84±0.39	0.86±0.49	NS
Docosahexaenoic acid (DHA; C22:6n3)	3.15±0.92	3.08±0.93	NS

Values are means±SD. Fatty acid values are expressed as percentage of total.

of a PUFAs rich diet. The most frequent method used to determine the resistance of LDL to oxidation is the method of in vitro copper-mediated oxidation of isolated LDL by the measuring of the kinetics of conjugated diene formation (7). Recently, several authors have used the whole serum or plasma to measure the lipid resistance to oxidation where water-soluble antioxidants are not artificially removed during the isolation (8, 9, 10).

We have chosen this method to test the effect of extra virgin olive oil consumption on the resistance of lipids to oxidation, measured directly in serum by monitoring the change in absorbance at 245 nm, and find out a relationship between lipid oxidizability and fatty acid composition in serum of elderly lipidemic patients.

Subjects and methods

Twenty six elderly lipidemic patients (mean age 69 years; 2 men and 24 women) participated in this study to compare the effects of extra virgin olive oil on serum lipids, lipid resistance to oxidation and fatty acid composition in serum phospholipids. Patients consumed daily 2 table spoons (approx. 20 g) of extra virgin olive oil (Aceites Borges Pont, Spain) over a period of 6 weeks. They were advised not to change their usual dietary habits for the duration of the study. Informed consent was obtained from all participants and the study was approved by the Institute's Ethics Committee.

Plasma and serum were isolated from blood samples collected after an overnight fast. Serum lipids were measured using standard laboratory methods and LDL cholesterol was calculated from the Friedewald formula. The resistance of lipids to copper-induced oxidation was measured in fresh serum by continuous recording of absorbance at 245 nm (9). The lag time and the maximal rate of oxidation (V_{max}) were calculated from the oxidation curve. Plasma total antioxidant capacity (FRAP) was measured by spectrophotometric method (11) and fatty acid content in serum phospholipids was determined by gas chromatography in the form of methylesters after one-step transesterification. Individual fatty acids were identified by comparison of their retention times with those of fatty acids standards (12).

Statistical evaluation was performed using unpaired Student's t-test and linear regression analysis. The minimal acceptable level of significance was $p<0.05$.

Results and discussion

A beneficial effect of olive oil consumption on lipoprotein spectrum and a significant increase in the serum lipid resistance to in vitro oxidation were the most relevant findings of our study. In addition, serum fatty acid composition correlated well with the indices of lipid oxidizability.

As shown in Table 1, a significant decreases were observed in plasma total cholesterol and LDL cholesterol (by 11 % and 15 %, resp.) after 6-weeks of dietary supplementation with olive oil. Triacylglycerol concentrations were also decreased (by 10%), but the difference was not significant. It has been shown that the composition of fatty acids in a diet and the replacement of saturated fatty acids with monounsaturated (MUFAs) or polyunsaturated (PUFAs) fatty acids significantly reduce serum cholesterol levels (1, 2). According to the recommendations, the proportion of saturated fatty acids should not exceed 8 % of total energy intake and the proportion of monounsaturated and polyunsaturated fatty acids should be in the range 10–13 % and 8–10 %, respectively (13). Our results concerning the changes in serum total and LDL cholesterol confirmed the hypocholesterolemic effect of MUFAs enriched diet observed in subjects who traditionally consume olive oil.

Another potential health benefit of olive oil consumption is its effect on the susceptibility of LDL to oxidative modification (4, 5, 6). The content of linoleic and oleic acid in LDL, the size of LDL particles and vitamin E and other antioxidant concentra-

Tab. 2. Significant and interesting correlations between lipid oxidizability parameters and fatty acids cacid content in serum of elderly lipidemic patients supplemented with extra virgin olive oil.

Variable analyzed	Results of correlation analysis (n=26)			
	before supplementation		after supplementation	
	r=	p=	r=	p=
Lag time vs oleic acid	0.18	NS	0.56	0.003
Lag time vs arachidonic acid	-0.36	NS	-0.38	0.050
Lag time vs docosahexaenoic acid	-0.41	0.048	-0.23	NS
Lag time vs FRAP	0.40	0.045	0.68	0.000
Vmax vs oleic acid	-0.33	NS	-0.45	0.035
Vmax vs linoleic acid	0.44	0.026	0.10	NS
Oleic acid vs linoleic acid	-0.65	0.000	-0.52	0.007
Oleic acid vs α-linolenic acid	0.51	0.007	0.11	NS
Oleic acid vs arachidonic acid	-0.33	NS	-0.62	0.001
Oleic acid vs docosahexaenoic acid	-0.19	NS	-0.53	0.006

tions all of them can modulate the susceptibility of LDL to oxidation. The protective effects of phenolic compounds with antioxidative properties present in extra virgin olive oil on LDL oxidation resulted mostly from studies in in vitro experiments (14). Our results show that olive oil consumption increase the resistance of serum lipids to oxidation reflected in a prolonged lag time (p=0.026) and by the decrease in rate of oxidation (p=0.030). Between the oxidizabilities of plasma and LDL (isolated from the same samples) a positive correlation was found (8, 10). Furthermore, plasma oxidizability correlated negatively with MUFAs and saturated fatty acid content and positively with plasma PUFAs content (10). From this aspect, our results are consistent with the results of above mentioned authors and indirectly also support the observations about the beneficial effects of olive oil on LDL oxidizability. A significant correlations between lag time, rate of oxidation and oleic acid after olive oil consumption found in this study strongly suggest that increased intake of MUFAs in the diet can also favourably influence the oxidizability of whole serum lipids where the conditions for the oxidation may be different from those of LDL (Tab. 2).

As was expected, the content of oleic acid in serum was significantly increased after olive oil consumption, while the content of n-6 fatty acids (linoleic and arachidonic acid) was significantly lower (Tab. 1). Linoleic acid is a precursor for arachidonic acid and other fatty acids with 3–6 double bonds produced by elongation and desaturation in the human organism. These two fatty acids are the main substrates for lipidperoxidation and therefore the decrease in their content could contribute to the decreased oxidizability of serum lipids. This is supported by the fact, that we found a significant negative correlations between serum lipid oxidizability and n-6 fatty acid content. Many factors can affect the resistance of serum lipids to oxidation. Not only disturbances in lipid metabolism and lipid composition but changes in plasma antioxidant capacity or the activities of protective antioxidant enzymes could be also important from this respect. Our results show no significant changes in plasma total antioxidant capacity (FRAP) but more significant positive correlation between FRAP and lag time was observed after olive oil treatment. In this study, the consumption of olive oil did not affect plasma n-3 fatty acids content (α-linolenic acid, EPA and DHA). On the contrary, in our previous study we observed the decrease in plasma oleic acid content after supplementation with long-chain n-3 PUFAs in patients with NIDDM and we emphasized the importance for the balanced ratio between n-3 PUFAs and MUFAs with special respect to its hypolipidemic effects (15). It has been shown, that beside their hypolipidemic effects, n-6 and n-3 fatty acids are also precursors for the synthesis of prostaglandins with cardioprotective effects and eicosanoids (16). The values of n-6/n-3 ratio in phospholipids of thrombocytes in relation to cardiovascular mortality was published by Weber et al (17). For Eskimos the ratio n-6/n-3 was 1:1 and cardiovascular mortality 7 %, while the mortality in Japanese population (the ratio n-6/n-3 12:1) was 12 %. In Europe and North America the n-6/n-3 ratio was 50:1 and the death rate for cardiovascular diseases was 45 %. Higher fatty acids produced in the organism by elongation and desaturation can be also derived from the diet, however exclusively of animal origin (meat n-6, fish n-3). This may represents a health risk for certain groups with alternative nutrition (18). Similarly, the requirement of balanced ratio of saturated fatty acids and MUFAs is important to avoid increased lipid peroxidation, especially under conditions of reduced intake of antioxidants such as vitamin C, vitamin E, ß-carotene and others.

Our results show the importance of fatty acid composition in the resistance of serum lipids to oxidation and from this aspect, serum fatty acid profile after olive oil consumption seems to be more favourable, suggesting a protective effects of olive oil on lipoprotein oxidation.

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Received June 14, 2003.
Accepted July 3, 2003.