Olive Oils Improve Lipid Metabolism and Increase Antioxidant Potential in Rats Fed Diets Containing Cholesterol

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The effect of olive oils on lipid metabolism and antioxidant activity was investigated on 60 male Wistar rats adapted to cholesterol-free or 1% cholesterol diets. The rats were divided into six diet groups of 10. The control group (control) consumed the basal diet (BD) only, which contained wheat starch, casein, cellulose, and mineral and vitamin mixtures. To the BD were added 10 g/100 g virgin (virg group) or Lampante (Lamp group) oils, 1 g/100 g cholesterol (chol group), or both (chol/virg group) and (chol/Lamp group). The experiment lasted 4 weeks. Plasma total cholesterol (TC), LDL-cholesterol (LDL-C), HDL-cholesterol (HDL-C), triglycerides (TG), total phospholipids (TPH), HDL-phospholipids (HDL-PH), total radical-trapping antioxidative potential (TRAP), malondialdehyde lipid peroxidation (MDA), and liver TC were measured. Groups did not differ before the experiment. In the chol/virg and chol/Lamp vs chol group, the oil-supplemented diets significantly ($P < 0.05$) lessened the increase in plasma lipids due to dietary cholesterol as follows: TC (25.1 and 23.6%), LDL-C (39.3 and 34.7%), TG (19.3 and 17.0%), and TC in liver (36.0 and 35.1%) for the chol/virg and chol/Lamp group, respectively. The chol/virg and chol/Lamp diets significantly decreased the levels of TPH (24.7 and 21.2%; $p < 0.05$ in both cases) and HDL-PH (22.9 and 18.0%; $p < 0.05$ in both cases) for the chol/ virg and chol/Lamp group, respectively. Virgin and Lampante oils in rats fed basal diet without cholesterol did not affect the lipid variables measured. Virgin, and to a lesser degree Lampante, oils have increased the plasma antioxidant activity in rats fed BD without cholesterol (an increase in TRAP, 20.6 and 18.5%; and a decrease in MDA, 23.2 and 11.3%, respectively). In the rats of chol/ virg and chol/Lamp vs Chol diet groups the added oils significantly hindered the decrease in the plasma antioxidant activity (TRAP, 21.2 and 16.7%; and MDA, 27.0 and 22.3%, respectively). These results demonstrate that virgin, and to less degree Lampante, oils possess hypolipidemic and antioxidant properties. It is more evident when these oils are added to the diets of rats fed cholesterol. These positive properties are attributed mostly to the phenolic compounds of the studied oils.

KEYWORDS: Olive oils; phenols; rats; lipoproteins; antioxidant potential

INTRODUCTION

Coronary atherosclerosis is still one of the most dangerous diseases in humans; it is the principal cause of death in Western civilization (*1*). Some authors have shown that the Mediterranean alpha-linoleic acid-rich diet is effective in prevention of this disease (*2*, *3*): this diet controls blood lipid levels (*4*) and decreases the plasma cholesterol (*5*).

Experiments in vitro and in vivo on laboratory animals have demonstrated that LDL-C oxidation was inhibited by olive oil constituents (*6*, *7*). It was also observed that vegetable oils affect lipid peroxidation and antioxidant parameters, and lead to favorable changes in the plasma lipid status (*6*, *8*). It is known that sudden cardiac death accounts for 50% of total coronary

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disease mortality (*9*). Replacement of saturated animal fat with either n-6-rich sunflower seed oil or n-3-rich fish oil in experiments on rats reduced the incidence and severity of arrhythmias (*9, 10*). Thrombosis of coronary arteries plays a major role in pathogenesis of fatal myocardial infarction (*11*). Phenolics of olive oils inhibit platelet aggregation (*12*). Therefore, the above-mentioned data demonstrate that vegetable oils could be very important in prevention and treatment of coronary atherosclerosis.

The main aim of the present investigation was to study the effect of olive oils on lipid metabolism and antioxidant activity in rats adapted to cholesterol-free or cholesterol diets. It was claimed that the positive influence of the Mediterranean diet is connected to its low saturated and high monounsaturated fatty acids content (*4, 5*). However, oils rich in monounsaturated fatty acids do not have the same positive effect (*13*). At present, there is no unified explanation of this finding. Some authors have reported that antioxidant capacity of oils in vitro is directly connected to their phenolic content (*14, 15*). Maybe this is the answer to the question why oils rich in monounsaturated fatty acids do not have the same positive effect.

The additional aim of our investigation is to prove this hypothesis. Therefore, five different Spanish olive oils were investigated in vitro and two of them with high and low antioxidant potentials were used in experiment in vivo on rats fed cholesterol-containing and cholesterol-free diets.

As far as we know there have been no such previous investigations.

MATERIALS AND METHODS

Oil Samples. Arbequina, Hojiblanca, extra virgin, Picual, and Lampante Spanish olive oils were investigated. These olive oil samples were purchased in the same Spanish supermarket and were produced by various Spanish oil factories. The names of the oils correspond to different olive varieties grown in Spain. Picual and Hojiblanca are from the South of Spain, and Arbequina is grown in Catalonia.

Fatty Acids. Fatty acids were extracted from the oils by *n*-hexane/diethyl ether (98:2, v/v), and were measured as their methyl esters and analyzed by gas chromatography.

Phenolic Compounds. Phenols were extracted from the oils as described by Espin et al. (*14*), and Pellegrini et al. (*15*), and the content was determined by the Folin–Ciocalteu method (*16*), using gallic acid as a standard for the calibration curve.

Stability. Stability was evaluated by the oxidation induction time, with the use of the Rancimat apparatus (Metrohm CH 9100). A flow of air (10 L/h) was bubbled through the oil heated at 100° C, and the volatile compounds were collected in cold water, increasing the water conductivity. The time to reach a fixed level was recorded (*17*).

Total Radical-Trapping Antioxidative Potential (TRAP). TRAP was determined in all oil samples before extraction of fatty acids, and phenolic and orthodiphenolic compounds. TRAP measurements and the kinetics of the oil samples were done as described previously (*18, 19*). Peroxyl radicals produced at a constant rate by thermal decomposition of 2,2-azo-bis-2-amidinopropane hydrochloride (ABAP, Polyscience, Warrington, PA) were monitored by luminol-enhanced CL. The reaction was initiated by mixing 475 μL of phosphate buffered saline, 50 μL of 10 mM luminol in 100 mM borate buffer (pH 10.0), and 50 μL of ABAP. This mixture was incubated (37 °C) in the temperature-controlled sample carousel of the luminometer BioOrbit 1251 (BioOrbit, Finland) for 15 min. Then 20 μL of sample extracted with acetone was added directly into the cuvette and the samples were measured for another period of time. Time needed for a 50% recovery of the original steady-state signal (so-called half peak-time, τ) was identified for each sample. 8.0 nM Trolox (Aldrich Chemical Co., Milwaukee, WI) was used as a reference inhibitor instead of sample. The results obtained were expressed as nmol of peroxyl radicals trapped by 1 mL of sample. Solvents were verified to have negligible TRAP.

Table 1. Compositions of the Diets[a]

ingredients	g/kg diet					
	control	chol	virgin	chol/virg	Lamp	chol/Lamp
wheat starch	693	683	693	683	693	683
casein	150	150	150	150	150	150
peanut oil	100	100	0	0	0	0
virgin oil	0	0	100	100	0	0
Lampante oil	0	0	0	0	100	100
cholesterol	0	10	0	10	0	10
cellulose	10	10	10	10	10	10
vitamin mixt	10	10	10	10	10	10
mineral mixt	37	37	37	37	37	37

[a] Abbreviations: chol, cholesterol; Lamp, Lampante oil; mixt, mixture; virg, virgin oil./

Malondialdehyde (MDA) Assay. The concentration of MDA, an index of lipid peroxidation, was determined spectrophotometrically (*19*). The reaction mixture containing of 1% barbituric acid (Sigma, St. Louis, MO) in 10% trichloroacetic acid (Sigma) in the ratio of 1:2 (v/v) was added to the samples. The samples were incubated in a water bath (100 °C) for 20 min. After the samples cooled, 4 mL of *n*-butanol was added, and the mixture was shaken vigorously. The samples were centrifuged (10 min, 2000g), and the absorbance of the upper layer was measured spectrophotometrically at 532 nm. 1,1,3,3-Tetraethoxypropane (Sigma) in the final concentration of 0.1 μM was used as a standard. Lipid peroxidation was expressed in nmol of barbituric acid reactive substances per 1 mL of the sample.

Rats and Diets. The experiment lasted 4 wks (*20, 21*). The Animal Care Committee of Warsaw Agricultural University approved this study. The Institute of Animal Physiology and Nutrition, of the Polish Academy of Sciences (Jablonna, Poland) provided male Wistar rats (n = 60) with a mean weight of 120 g. They were housed individually in stainless steel metabolic cages and were divided into 6 groups of 10.

Six groups were fed a basal diet (BD) that included wheat starch, casein, cellulose, and mineral and vitamin mixtures. The control (control) group was fed only the BD. The other five groups were named virg, Lamp, chol, chol/virg, and chol/Lamp. To BD of these groups were added 10 g/100 g virgin (virg) or Lampante (Lamp) oils, 1 g/100 g nonoxidized cholesterol (chol), or both (chol/virg) and (chol/Lamp).

Peanut oil (Salvadori factory, Florence, Italy) with minimal antioxidant capacity as was determined by TRAP test was added as control oil to the diets for control and chol groups in a concentration of 10 g/100 g. Cholesterol of analytical grade (USP) was obtained from Sigma Chemical, St. Louis, MO. The cholesterol batches were mixed carefully with the basal diet (1:99) just before the diets were offered to the rats. The dietary cholesterol was checked according to the HPLC method and was found not to contain cholesterol oxides. The exact compositions of the diets are presented in **Table 1**.

The diets contained, as percentage of energy, 61% carbohydrates, 26% fat, and 13% protein. The calculated energy values of all diets were not significantly different.

All rats consumed food ad libitum once a day beginning at 10:00 a.m. and had unrestricted access to drinking water. Diet intake was monitored daily. Before the experiment the blood samples were drawn from the tail vein. At the end of the experiment the rats were anesthetized using diethyl ether. Blood samples were taken from the left atrium of the heart. Plasma was prepared and used for laboratory tests. After anesthesia, the abdomen was opened to take samples of the liver for determination of TC. The weight gain of the rats was recorded on a weekly basis. Two time points were used in this experiment: before and after 4 wks of feeding. At these points a wide range of laboratory tests was performed. Total cholesterol (TC), HDL-cholesterol (HDL-C), total phospholipids (TPH), HDL-phospholipids (HDL-PH), triglycerides (TG), TRAP and TC in liver were determined as previously described (*20*).

Statistics. Values are given as the means $\pm$ SD of five measurements. Where appropriate, data were tested by two-way ANOVA (chol/virg, chol/Lamp) using GraphPad Prism, version 2.0 (GraphPad Software, San Diego, CA), followed by Duncan's (*22*) multiple range

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Table 2. Content of Some Fatty Acids in Different Spanish Olive Oils[a]

Spanish olive oils	fatty acids						
	myristic 14:0	palmitic 16:0	palmitoleic 16:1	stearic 18:0	oleic 18:1	linoleic 18:2	linolenic 18:3
Arbequina	0.05 ± 0.004	12.47 ± 1.30	1.15 ± 0.10	1.98 ± 0.21	72.28 ± 7.31	9.63 ± 1.10	0.59 ± 0.06
Hojiblanca	0.05 ± 0.004	9.08 ± 1.00	0.48 ± 0.04	3.47 ± 0.33	75.57 ± 7.62	8.30 ± 0.90	0.76 ± 0.08
extra virgin	0.05 ± 0.004	9.45 ± 1.10	0.49 ± 0.04	3.42 ± 0.32	75.21 ± 7.61	8.49 ± 0.93	0.66 ± 0.07
Picual	0.05 ± 0.004	9.10 ± 1.00	0.49 ± 0.04	3.49 ± 0.33	76.60 ± 7.69	8.35 ± 0.91	0.78 ± 0.08
Lampante	0.05 ± 0.004	10.53 ± 1.20	0.72 ± 0.08	3.01 ± 0.30	78.14 ± 7.82	5.58 ± 0.60	0.51 ± 0.05

[a] Values are means of 5 measurements ± SD. Data are expressed as percentage of total fatty acids.

Table 3. Individual Antioxidants, Total Radical-Trapping Antioxidant Potential (TRAP), and Stability (Rancimat 120 °C) of Spanish Olive Oils[a]

olive oil	tocotrienols (ppm)	tocopherols (ppm)	polyphenols (ppm)	o-diphenols (ppm)	TRAP (nmol/mL)	stability (h)
Hojiblanca	324 ± 31.9	350 ± 34.1	4.4 ± 0.4	2.6 ± 0.3	660 ± 81	6.0 ± 0.6
extra virgin M.	329 ± 31.8	353 ± 23.6	4.6 ± 0.4	2.7 ± 0.2	668 ± 49	6.3 ± 0.6
Lampante	148 ± 15.1	169 ± 15.8	2.1 ± 0.2	1.3 ± 0.2	225 ± 42	3.0 ± 0.3
Picual	323 ± 31.9	349 ± 34.1	4.5 ± 0.5	2.5 ± 0.3	661 ± 102	6.2 ± 0.3
Arbequina	301 ± 29.7	247 ± 23.4	4.1 ± 0.4	2.1 ± 0.2	541 ± 67	5.9 ± 0.6

[a] Values are means ± SD of 5 measurements.
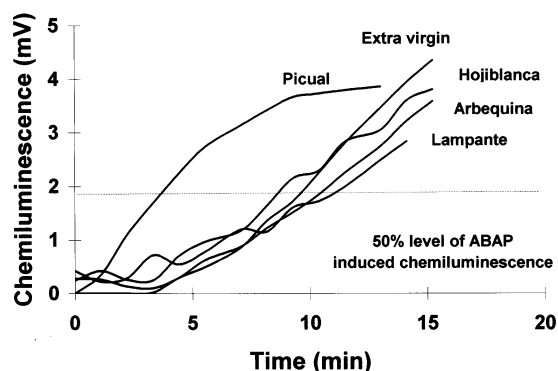
Figure 1. Kinetics of ABAP-induced, luminol-enhanced chemiluminescence in the presence of Picual, extra virgin, Hojiblanca, Arbequina, and Lampante acetonic extracts as well as of the reference antioxidant Trolox. Dashed line represents control ABAP-induced CL.

test to assess differences between group means. Differences of $P < 0.05$ were considered significant.

RESULTS

In Vitro. The results of the fatty acid analyses are summarized in **Table 2**. The statistically evaluated results of the contents of the major antioxidant compounds, stability, and TRAP values are summarized in **Table 3**. As shown in the tables, the virgin oil possesses the highest antioxidant capacity, whereas the Lampante oil has the lowest antioxidant capacity.

The kinetic studies of oil samples are reflected in **Figure 1**, showing the differences among them. The antioxidative potential (**Figure 1**) is displayed as diminishing of CL signal. Later recovery of CL signal is equal to higher antioxidative activity. There are significant differences between Arbequina, Hojiblanca, virgin, Picual samples, and Lampante oils; their antioxidative activity increases from Lampante to Virgin. This steady-state signal is shown in **Figure 1** as dashed line, ABAP-induced CL. High correlation levels were calculated between TRAP and stability ($R^2 = 0.957$, **Figure 2A**), and TRAP and polyphenols ($R^2 = 0.984$, **Figure 2B**). In contrast, the correlation of TRAP and the ratio of oleic/linoleic acids ($R^2 = 0.442$, **Figure 2C**) was low. The participation in total antioxidant activity of fatty acids was about 20% (**Figure 2D**, $R^2 = 0.442$), and of polyphenols was about 50% (**Figure 2D**, $R^2 = 0.996$).

To find out how oils with different antioxidant capacities influence lipid metabolism and antioxidant activity in rats fed cholesterol-containing and cholesterol-free diets, extra virgin and Lampante oils were used in this study.

In Vivo. The addition of oils or cholesterol, or both, to the diets did not affect food intake, body weight gain, or feed efficiency (data not shown). At baseline, the six groups did not differ from one another in plasma lipid concentration (data not shown). The statistically evaluated results (ANOVA) of the changes in plasma lipid concentration after the trial are summarized in **Table 4**.

As can be seen, the oil supplemented diet significantly ($P < 0.05$) hindered the increase in plasma lipids due to dietary cholesterol, as calculated in the percentages: TC (3.61 vs 4.82 mmol/L, −25.1%; and 3.73 vs 4.82 mmol/L, −23.6%), LDL-C (1.94 vs 3.20 mmol/L, −39.3%; and 2.09 vs 3.20 mmol/L, −34.7%), TG (0.71 vs 0.88 mmol/L, −19.3%; and 0.73 vs 0.88 mmol/L, −17.0%), and TC in liver (30.9 vs 48.3 μmol/g, −36.0%; and 31.3 vs 48.3 μmol/g, −35.1%) for the chol/virg and chol/Lamp groups vs the chol group, respectively. The oil supplemented diets significantly decrease the levels of HDL-PH due to dietary cholesterol (0.75 vs 0.61 mmol/L, −22.9%; and 0.72 vs 0.61 mmol/L, −18.0%; $p < 0.05$ in both cases) and TPH (1.31 vs 1.74 mmol/L, −24.7%; and 1.37 vs 1.74 mmol/L, −21.2%; $p < 0.05$ in both cases) for the chol/virg and chol/Lamp groups vs the chol group, respectively. Virgin and Lampante oils in rats fed basal diet without cholesterol did not significantly affect the lipid variables measured.

Liver weight was 4.35 g in all 6 groups. After 4 weeks of feeding, the liver TC concentration in the rats of chol/virgin, chol/Lamp and chol diet groups was 30.9, 31.3, and 48.3 mol/g; which was 5.27, 5.34, and 8.27 times higher than that in the control group, respectively. The liver TC concentration in the chol group was higher than that in chol/virgin and chol/Lamp, 56.3% and 44.3%, respectively ($p < 0.001$ in both cases). Therefore, oil-supplemented diets significantly hindered the increase of liver TC due to dietary cholesterol.

Virgin oil, and to a lesser degree Lampante oil, has increased the plasma antioxidant activity in rats fed basal diet without cholesterol (**Figure 3**): an increase in TRAP values (299.3 vs 248.2, −20.6%; and 287.1 vs 248.2 nmol/mL, −18.5%) and a decrease in MDA values (1.29 vs 1.68, −23.2%; and 1.49 vs

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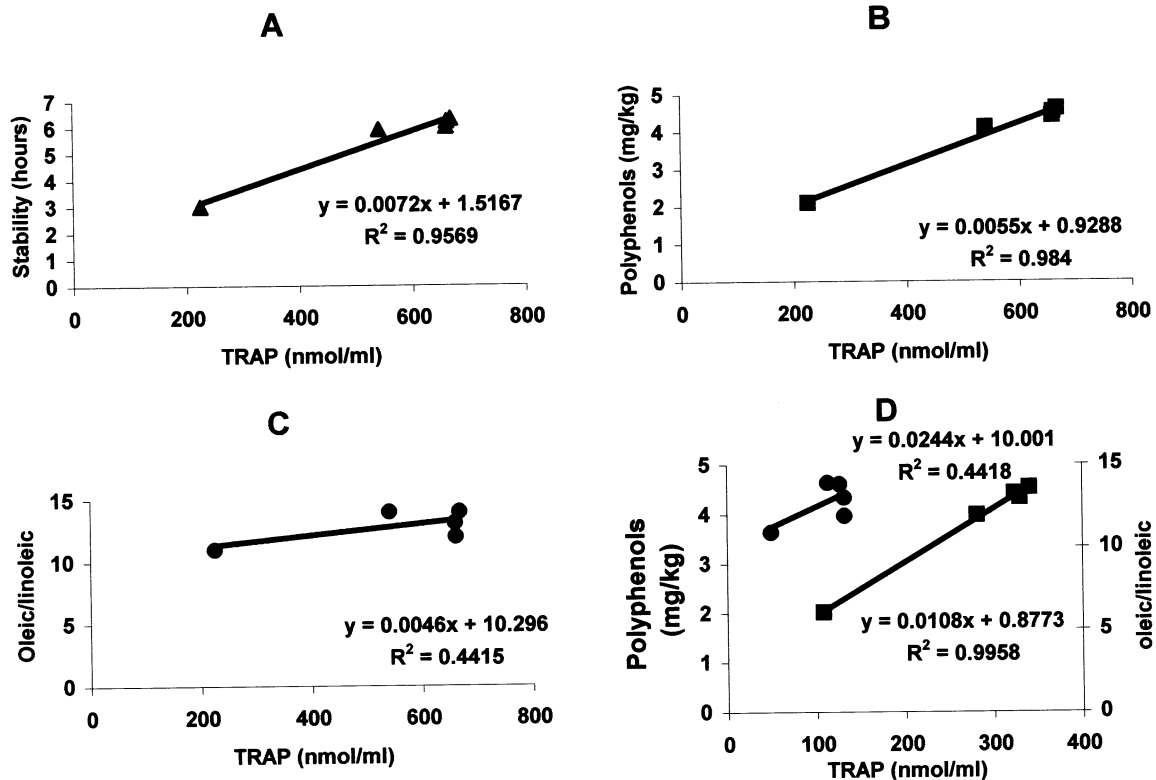*J. Agric. Food Chem.*, Vol. 50, No. 21, 2002 **6105**

Figure 2. Correlation between (A), total radical-trapping antioxidative potential (TRAP) and stability; (B), TRAP and polyphenols; (C), TRAP and oleic/linoleic acids; (D), correlation after extraction of individual components: TRAP and oleic/linoleic acids; TRAP and polyphenols.

Table 4. Plasma Lipids and Total Cholesterol Concentration in Liver of Rats Fed Diets With and Without 1% Cholesterol, and With and Without Virgin and Lampante Oils[a]

	plasma lipids						liver
diets	TC	LDL-C	HDL-C	TG	TPH	HDL-PH	TC
	concentration (mmol/L)						(μmol/g)
control	2.86 ± 0.15c	1.23 ± 0.05c	1.62 ± 0.07a	0.69 ± 0.04b	1.76 ± 0.08a	1.05 ± 0.06a	5.86 ± 0.24c
chol	4.82 ± 0.21a	3.20 ± 0.12a	1.61 ± 0.07a	0.88 ± 0.05a	1.74 ± 0.08a	0.61 ± 0.04c	48.3 ± 0.31a
Lampante	2.85 ± 0.15c	1.21 ± 0.05c	1.63 ± 0.07a	0.71 ± 0.05b	1.72 ± 0.08a	1.08 ± 0.06a	5.79 ± 0.24c
chol/Lamp	3.73 ± 0.18b	2.09 ± 0.05b	1.62 ± 0.07 a	0.73 ± 0.05b	1.37 ± 0.06b	0.72 ± 0.04b	31.3 ± 0.25b
virgin	2.81 ± 0.15c	1.19 ± 0.05c	1.61 ± 0.07 a	0.70 ± 0.05b	1.72 ± 0.08a	1.10 ± 0.06a	5.74 ± 0.24c
chol/virg	3.61 ± 0.18b	1.94 ± 0.05b	1.66 ± 0.07 a	0.71 ± 0.05b	1.31 ± 0.06b	0.75 ± 0.04b	30.9 ± 0.25b
	2-way ANOVA (*P*-value)						
Lampante	NS	NS	NS	NS	NS	NS	NS
virgin	NS	NS	NS	NS	NS	NS	NS
chol	<0.001	<0.001	NS	<0.001	NS	<0.001	<0.0005
chol/Lamp	<0.050	<0.050	NS	<0.050	<0.010	<0.050	<0.0005
chol/virg	<0.050	<0.050	NS	<0.050	<0.010	<0.050	<0.0005

[a] Values are means ± SD, *n* = 10. Means in columns without letters in common differ significantly (*P* < 0.05). Abbreviations used: chol, nonoxidized cholesterol; HDL-C, HDL cholesterol; HDL-PH, HDL phospholipids; Lamp, Lampante oil; LDL-C, LDL cholesterol; NS, not significant; TC, total cholesterol; TG, triglycerides; TPH, total phospholipids; virg, virgin oil.

1.68 nmol/mL, −11.3%) for virgin and Lampante groups vs the control group, respectively.

After four weeks of feeding, a decrease in plasma antioxidant activity in the chol/virg, chol/Lamp, and chol groups was registered. These results corresponded with the observations that cholesterol-supplemented diet leads to a decrease in blood antioxidant activity (*23, 24*). However, the diets supplemented with virgin, and to a less degree with Lampante, oil significantly hindered the decrease in the plasma antioxidant activity in rats fed added cholesterol. A decrease in TRAP (231.6 vs 191.1, −21.2%; and 223.1 vs 191.1, nmol/mL, −16.7%) and an increase in MDA (1.87 vs 2.56, −27.0%; and 1.99 vs 2.56 nmol/ml, −22.3%) for the chol/virg and chol/Lamp vs the chol group,

respectively, is shown in **Figure 4**. These results demonstrate that virgin, and to a lesser degree Lampante, oils positively affect antioxidant potential of rats fed both cholesterol-containing and cholesterol-free diets.

DISCUSSION

It is known that elevated levels of total cholesterol, LDL-C, triglycerides, apolipoproteins B and C-III, and reduced level of HDL-C and apolipoprotein A−I, are major risk factors for atherosclerosis (*25, 26*). Hypercholesterolemia remains an anatomic foundation of this disease (*27−29*). It was shown that only oxidized LDL-cholesterol particles are able to penetrate

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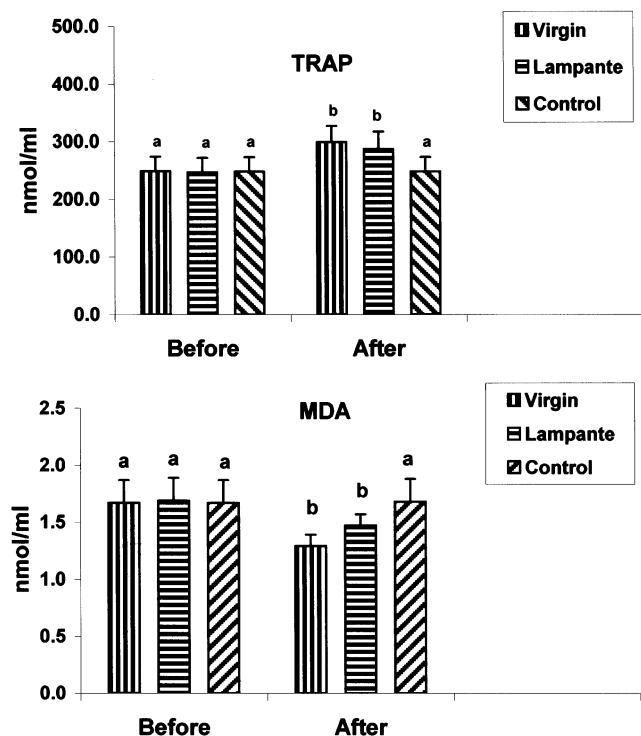
Figure 3. Total radical-trapping antioxidative potential (TRAP) and malondialdehyde (MDA) lipid peroxidation test in rats fed without added cholesterol: before the feeding period; after four weeks of feeding period (time points of the experiment, *X* axis) and concentrations of TRAP and MDA (nmol/mL, *Y* axis). M ± SD (vertical lines). Bars with different letters are significantly different ($p < 0.05$).

Figure 4. TRAP and MDA values in rats fed with added cholesterol: before the feeding period; after four weeks of feeding period (time points of the experiment, *X* axis) and concentrations of TRAP and MDA (nmol/mL, *Y* axis). M ± SD (vertical lines). Bars with different letters are significantly different ($p < 0.05$).

arterial walls and cause their occlusion (*30*, *31*). Therefore, hypocholesterolemic and antioxidant substances have to be an integral part of atherosclerosis-preventing diets (*2*, *3*, *32*−*36*).

It was proved that the Mediterranean diet with low saturated, and high monounsaturated, fatty acids is effective in prevention of atherosclerosis and other diseases (*2*−*5*). However, other researchers have shown that oils rich in monounsaturated fatty acids do not have the same effect (*13*). In the present trial we tried to show why this effect is different.

Some authors have studied the importance of the total or individual phenol contents with regard to virgin olive oil stability (*37*−*40*). However, very little was known about the percentage contribution of these compounds to the stability, and especially to the antioxidant capacity, of vegetable oils. Our results in vitro indicate that antioxidant compounds, and not the monounsaturated fatty acids, are the main bioactive factors of vegetable oils. We found a high correlation between TRAP and polyphenols ($R^2 = 0.98$). In contrast, the correlation of TRAP and the oleic/linoleic acids ratio ($R^2 = 0.44$) was low. The total antioxidant activity of oil samples was composed of about 50% of polyphenol antioxidants and 20% of fatty acids.

These results are in agreement with those of other authors who have found in vitro that not monounsaturated oleic acid, but the remarkable quantities of phenolic compounds, could account for the cardioprotective effect of the Mediterranean diet (*7*, *41*).

However, these suggestions were proved via in vivo experiments. Therefore, two olive oils with equal monounsaturated fatty acids content but different antioxidant capacity were used for the in vivo study.

Virgin, and to a lesser degree Lampante, oil positively affect lipid metabolism in rats fed a cholesterol-containing diet: the
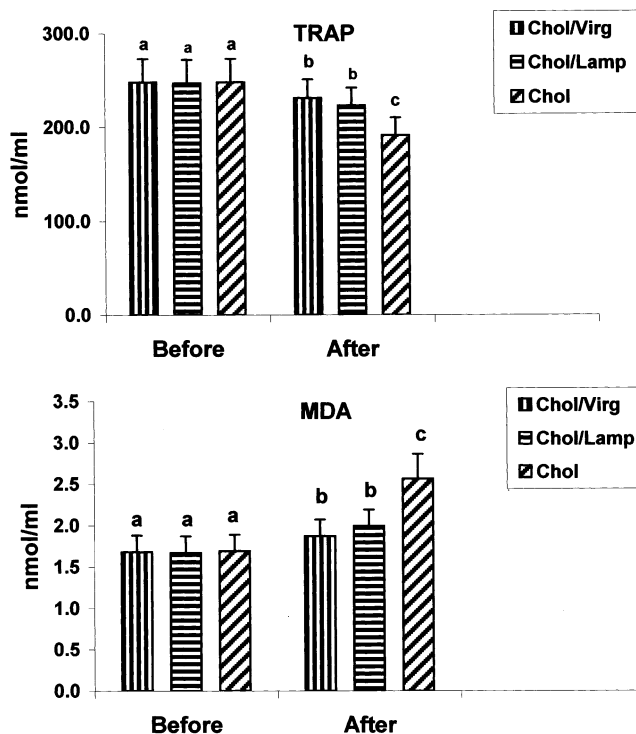
TC, LDL-C, TG, and TPH concentrations in the chol/virg and chol/Lamp diet groups were significantly lower than those in the chol diet group. These results were predictable. It was expected that the oil-supplemented diet, which contains a high concentration of antioxidant components, will positively influence lipid metabolism. It is important to emphasize that in the rats fed the chol/virgin and chol/Lamp diets, the level of HDL-PH was higher than that in the chol diet group.

It must be emphasized that the improvement in lipid metabolism was observed only in the groups of rats fed the cholesterol-containing diet. These results are consistent with those obtained by others (*4*, *5*). Since Kiryama et al., (*42*) it was proved that lipid-lowering natural products are effective only in cases of hyperlipidemia both in experiments on laboratory animals and in investigations of humans. Our previous experience is in accordance with these data (*20*, *34*, *43*).

Virgin, and to a lesser degree Lampante, oils have exerted marked antioxidant effects in both groups of rats fed cholesterol-containing and cholesterol-free diets. Also these results are in correspondence with the results of others (*6*−*8*).

Therefore, also the results of our in vivo investigation are challenging the old conception that monounsaturated fatty acids are the main bioactive factors of olive oils.

The results of this investigation are supporting our working hypothesis: Virgin oil with the highest content of total polyphenols and related high level of the total radical-trapping antioxidative potential is more efficient than Lampante oil. Therefore, the findings of Truswell and Choudhury (*13*), that vegetables oils rich in monounsaturated fatty acids do not have the same effect could be explained by their different contents of phenolics.

In the present in vitro experiment we have found that phenolic compounds, not monounsaturated oleic acid, are the main

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bioactive components in olive oils and could account for the cardioprotective effect of the Mediterranean diet. These suggestions were proved in the experiment on rats: the positive influence on plasma lipids and antioxidant activity was significantly higher in the group of rats fed virgin oil, which possesses the highest antioxidant potential. Therefore, it could be suggested that the use of olive oils with a higher antioxidant potential by patients suffering from coronary atherosclerosis would prevent development of this disease.

In conclusion, the main bioactive components of olive oils are phenolic compounds. Virgin, and to a lesser degree Lampante, olive oils positively influence lipid metabolism and antioxidant capacity in plasma of rats. This effect is more evident in groups of rats fed cholesterol-containing diets. The degree of this influence is directly connected to the content of total phenols and the related total radical-trapping antioxidative potential of the used oils. The addition of olive oil with high antioxidative potential to the generally accepted diet could be beneficial in prevention of atherosclerosis, mainly in hypercholesterolemic patients; however it has to be proved on human beings.

ABBREVIATIONS USED

HDL-C, HDL-cholesterol; HDL-PH, HDL-phospholipids; LDL-C, LDL-cholesterol; MDA, malondialdehyde lipid peroxidation test; NOC, nonoxidized cholesterol; TC, total cholesterol; TG, triglycerides; TPH, total phospholipids; TRAP, total radical-trapping antioxidative potential; VLDL-C, VLDL-cholesterol.

LITERATURE CITED

(1) Ross, R. The pathogenesis of atherosclerosis. In *Heart Diseases;* Braunwald E., Ed.; W. B. Saunders Company: Philadelphia London Toronto Montreal Sydney Tokyo, 1997; pp 1105−1121.

(2) Longeril, M.; Renaud, S.; Mamelle, N.; Salen, P.; Martin, J. L.; Monjaud, I.; Guidollet, J.; Toubolt, P.; Delaye, J. Mediterranean alpha-linolic acid-rich diet in secondary prevention of coronary heart disease. *Lancet* **1994**, *343*, 1454−1459.

(3) Partiff, V. J.; Rubba, P.; Bolton, C.; Marotta, G.; Hartog, M.; Mancini, M. A comparison of antioxidant status and free radical peroxidation of plasma lipoproteins in healthy young persons from Naples and Bristol. *Eur. Heart J.* **1994**, *15*, 71−876.

(4) Mattson, F. H. A changing role for dietary monounsaturated fatty acids. *J. Am. Diet. Assoc.* **1989**, *89*, 387−391.

(5) Spiller, G. A. Health effects of Mediterranean diets and monounsaturated fats. *Cereal Foods World* **1991**, *36*, 812−814.

(6) Scaccini, C.; Nardini, M.; D'Aquino, M.; Gentili, V.; Di Felice, M.; Tomassi, G. Effect of dietary oils on lipid peroxidation and on antioxidant parameters of rat plasma and lipoprotein fractions. *J. Lipid Res.* **1992**, *33*, 627−633.

(7) Visioli, F.; Bellomo, G.; Montedoro, G.; Galli, C. Low-density lipoprotein oxidation is inhibited in vitro by olive oil constituents. *Atherosclerosis* **1995**, *117*, 25−32.

(8) Gustafsson, I. B.; Vessby, B.; Ohrvall, M.; Nydahl, M. A diet rich in monounsaturated rapeseed oil reduces the lipoprotein cholesterol concentration and increases the relative content of n-3 fatty acids in serum in hyperlipidemic subjects. *Am. J. Clin. Nutr.* **1994**, *59*, 667−674.

(9) Topping, D. Dietary fatty acids and protection against experimental cardiac arrhythmias in rats. *Nutr. Rev.* **1993**, *51*, 271−273.

(10) McLennan, P. Relative effects of dietary saturated, monounsaturated, and polyunsaturated fatty acids on cardiac arrhythmias in rats. *Am. J. Clin. Nutr.* **1993**, *57*, 207−212.

(11) Brosins, F. C.; Roberts, W. S. Significance of coronary arterial thrombus in transmural acute myocardial infarction. A study of 54 necropsy patients. *Circulation* **1981**, *63*, 810−816.

(12) Petroni, A.; Blasevich, M.; Salami, M.; Papini, N.; Montedoro, G. F.; Galli, C. Inhibition of platelet aggregation and eicosanoid production by phenolic components of olive oil. *Thromb. Res.* **1995**, *78*, 151−160.

(13) Truswell, A. S.; Choudhury, N. Monounsaturated oils do not all have the same effect on plasma cholesterol. *Eur. J. Clin. Nutr.* **1998**, *52*, 312−315.

(14) Espin, J. C.; Soler-Rivas, C.; Wichers, H. J. Characterization of the total free radical scavenger capacity of vegetable oils and oil fractions using 2,2-Diphenyl-1-picrylhydrazyl radical. *J. Agric. Food Chem.* **2000**, *48*, 640−656.

(15) Pellegrini, N.; Visioli, F.; Buratti, S.; Brighenti, F. Direct analysis of total antioxidant activity of olive oil and studies on the influence of heating. *J. Agric. Food Chem.* **2001**, *49*, 2532−2538.

(16) Singleton, V. L.; Rossi, J. A. Colorimetry of total phenolic with phosphomolybdicphosphotungstic acid reagents. *Am. J. Enol. Vitic.* **1965**, *16*, 144−158.

(17) Aparicio, R.; Roda, L.; Albi, M. A.; Gutiérrez F. Effect of various compounds on virgin olive oil stability measured by Rancimat. *J. Agric. Food Chem.* **1999**, *47*, 4150−4155.

(18) Slavíková, H.; Lojek, A.; Hamar, J.; Dušková, M.; Kubala, L.; Vondráček, J.; Číž, M. Total antioxidant capacity of serum increased in early but not late period after intestinal ischemia in rats. *Free Radical Biol. Med.* **1998**, *25*, 9−18.

(19) Gorinstein, S.; Martin-Belloso, O.; Park, Y. S.; Haruenkit, R.; Lojek, A.; Číž, M.; Caspi, A.; Libman, I.; Trakhtenberg, S. Comparison of some biochemical characteristics of different citrus fruits. *Food Chem.* **2001**, *74*, 309−315.

(20) Gorinstein, S.; Bartnikowska, E.; Kulasek, G.; Zemser, M.; Trakhtenberg, S. Dietary persimmon improves lipid metabolism in rats fed diets containing cholesterol. *J. Nutr.* **1998**, *128*, 2023−2027.

(21) Leontowicz, M.; Gorinstein, S.; Bartnikowska, E.; Leontowicz, H.; Kulasek, G.; Trakhtenberg, S. Sugar beet pulp and apple pomace dietary fibers improve lipid metabolism in rats fed cholesterol. *Food Chem.* **2001**, *72*, 73−78.

(22) Duncan, D. B. Multiple range test. *Biometrics* **1995**, *11*, 1−42.

(23) Tsai, A. C.; Chen, N. S. C. Effect of cholesterol feeding on tissue glucose uptake, insulin degradation, serum lipids and serum lipoperoxides levels in rabbits. *J. Nutr.* **1979**, *109*, 606−612.

(24) Uysal, M. Effect of cholesterol feeding on plasma lipids, lipid peroxides levels and lecithin-cholesterol acyl transferase activity in rabbits. *Biochem. Archive* **1986**, *2*, 287−292.

(25) Kwiterovich, P. O.; Coresh, J.; Smith, H. H.; Bachoric, P. S.; Derby, C. A.; Pearson, T. A. Comparison of the plasma level of alolipoprotein B and A-1 and other risk factors in men and in women with premature coronary artery disease. *Am. J. Cardiol.* **1992**, *69*, 1015−1021.

(26) Hodis, H. N.; Mack, W. J.; Azen, S. P.; Alaupovic, P.; Pagoda, J. M.; La Bree, L.; Hemphill, L. C.; Kramsh, D. M.; Blankenhorn, D. H. Triglyceride- and cholesterol-rich lipoproteins have a different effect on mild/moderate and severe lesion progression as assessed by quantitative coronary angiography in a controlled trial of lovastin. *Circulation* **1994**, *90*, 42−49.

(27) Faggiotto, A.; Ross, R.; Harker, L. Studies of hypercholesterolemia in the nonhuman primate. I. Changes, that lead to fatty streak formation. *Atherosclerosis* **1984**, *4*, 323−340.

(28) Faggiotto, A.; Ross, R. Studies of hypercholesterolemia in the nonhuman primate. II. Fatty streak conversion to fibrous plaque. *Atherosclerosis* **1984**, *4*, 341−356.

(29) Freedman, D.; Newman, W. I.; Tracy, R.; Voors, A. E.; Srinivasan, S. R.; Webber, L. S.; Restrepo, C.; Strong J. P.; Berenson, G. S. Black-white differences in aortic fatty streaks in adolescence and early adulthood. The Bogalusa heart study. *Circulation* **1988**, *77*, 856−864.

(30) Steinberg, D.; Parthasarathy, S.; Carew, T.; Khoo, J.; Witztum, J. Beyond cholesterol: modifications of low-density lipoprotein that increases its atherogenicity. *N. Engl. J. Med.* **1989**, *320*, 915−924.

Gorinstein et al.

(31) Witztum, J. L.; Steinberg, D. Role of oxidized low-density lipoprotein in atherogenesis. *J. Clin. Invest.* **1991**, *88*, 1785–1792.

(32) Gorinstein, S.; Zemser, M.; Lichman, I.; Berebi, A.; Kleipfish, A.; Libman, I.; Trakhtenberg, S.; Caspi, A. Moderate beer consumption and the blood coagulation in patients with coronary atherosclerosis. *J. Intern. Med.* **1997**, *241*, 47–51.

(33) Gorinstein, S.; Zemser, M.; Berliner, M.; Goldstein, R.; Libman, I.; Trakhtenberg, S.; Caspi, A. Moderate beer consumption and some positive biochemical changes in patients with coronary atherosclerosis. *J. Intern. Med.* **1997**, *242*, 219–224.

(34) Gorinstein, S.; Zemser, M.; Haruenkit, R.; Chuthakorn, R.; Martin-Belloso, O.; Trakhtenberg, S. Comparative content of total polyphenols and dietary fiber in tropical fruits and persimmon. *J. Nutr. Biochem.* **1998**, *10*, 367–371.

(35) Gorinstein, S.; Kulasek, G.; Bartnikowska, E.; Leontowicz, M.; Morawiec, M.; Zemser, M.; Trakhtenberg, S. The effect of diet, supplemented with either whole persimmon or phenol-free persimmon, on rats fed cholesterol. *Food Chem.* **2000**, *70*, 303–308.

(36) Gorinstein, S.; Zachwieja, Z.; Folta, M.; Barton, H.; Piotrowicz, J.; Zemser, M.; Weisz, M.; Trakhtenberg, S.; Màrtín-Belloso, O. Comparative content of dietary fiber, total phenolics, and minerals in persimmon and apples. *J. Agric. Food Chem* **2001**, *49*, 952–957.

(37) Papadopoulos, G.; Boskou, D. Antioxidant effect of natural phenols on olive oil. *J. Am. Oil Chem. Soc.* **1991**, *68*, 669–671.

(38) Baldioli, M.; Servili, M.; Perretti, G.; Montedoro, G. Antioxidant activity of tocopherols and phenolic compounds in virgin olive oil. *J. Am. Oil Chem. Soc.* **1996**, *73*, 1589–1593.

(39) Angerosa, F. D.; Alexandro, N.; Konstantinou, P.; Di Giacinto, L. Evaluation of phenolic compounds in virgin olive oil. *J. Agric. Food Chem.* **1995**, *43*, 1802–1807.

(40) Gutierrez, F.; Arnaud T.; Garrido, A. Contribution of polyphenols to the oxidative stability of virgin olive oil. *J. Sci. Food Agric.* **2001**, *81*, 1463–1470.

(41) Visioli, F.; Galli, C. Antiatherogenic compounds of olive oils. *Curr. Atheroscler. Rep.* **2001**, *3*, 64–67.

(42) Kiryama, S.; Okazaki, J.; Joshida, A. Hypocholesterolemic effect of polysaccharides and polysaccharide-rich foodstuffs in cholesterol-fed rats. *J. Nutr.* **1969**, *97*, 382–388.

(43) Gorinstein, S.; Kulasek, G.; Bartnikowska, E.; Leontowicz, M.; Morawiec, M.; Zemser, M.; Trakhtenberg, S. The influence of Persimmon peel and Persimmon pulp on the lipid metabolism and antioxidant activity of rats fed cholesterol. *J. Nutr. Biochem.* **1998**, *9*, 223–227.