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Consumption of Juice Fortified with Oregano Extract Markedly Increases Excretion of Phenolic Acids but Lacks Short- and Long-Term Effects on Lipid Peroxidation in Healthy Nonsmoking Men

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Oregano has been shown to possess antioxidant capacity in various in vitro models and has thus been suggested to be potentially beneficial to human health, but studies in humans are lacking. The aim of this study was to investigate the bioavailability and the effects of *Origanum vulgare* extract supplementation on serum lipids and lipid peroxidation in healthy nonsmoking men. A four-week double-blinded supplementation trial was concluded in which volunteers ($n = 45$) were randomized to consume daily mango−orange juice (placebo), mango−orange juice enriched with 300 mg/d total phenolic compounds from oregano extract, or mango−orange juice enriched with 600 mg/d total phenolic compounds from oregano extract. The excretion of phenolic compounds was markedly increased in the higher phenolic group as compared to the placebo group, but no significant changes were observed in the safety parameters, serum lipids, or biomarkers of lipid peroxidation.

KEYWORDS: Antioxidants; double-blinded supplementation trial; lipid peroxidation; oregano; *Origanum vulgare*; phenolic compounds, healthy nonsmoking men.

INTRODUCTION

Although plant-derived components of our diet include fiber, vitamins, phytosterols, and carotenoids, phenolic compounds are one group of phytochemicals on which intensive research interest has focused during recent years. This is largely because diets abundant in polyphenols have been linked with a decreased risk of chronic degenerative diseases, such as cardiovascular diseases and possibly cancer (*1*−*3*). The mechanism via which flavonoids and other phenolic compounds would offer protection against these diseases is thought to relate at least partly to the antioxidant properties of these compounds (*4*, *5*). Studies carried out in vitro and in animal models provide evidence for the antioxidant properties of phenolic compounds (*5*). However, the potential health effects of polyphenols in humans are dependent on both their abundance in our diets and their bioavailability in the body; their in vivo relevance or health effects can only be finally concluded by studies carried out in humans.

The results of supplementation studies carried out in humans with polyphenols or foods rich in them have been inconsistent (*6*). Plasma or serum antioxidant capacity or resistance of plasma lipids or lipoproteins toward oxidation has been reported to be increased after consumption of red wine (*7*, *8*), green and black tea (*9*, *10*), cocoa or chocolate (*11*, *12*), fruits and berries (*13*), strawberries and spinach (*8*), black currant and apple juices (*14*), and pine phloem (*15*). On the other hand, lack of effect on antioxidant capacity or different markers of lipid/lipoprotein peroxidation have been reported after regular ingestion of green or black tea (*16*, *17*), after consumption of onions and black tea (*18*) or red wine (*19*). A rather common finding seems to be that, while monitoring several biomarkers of lipid peroxidation, some may show effect whereas others do not. Usually, for example, an increase in the plasma antioxidant capacity may be observed, but no effects on lipid peroxidation parameters can be seen (*6*, *20*). On the basis of these contradictory findings, using more than one biomarker of lipid peroxidation or antioxidant capacity in supplementation studies is important.

Commonly used culinary herbs of the Lamiaceae family, one of the most commonly known being *Origanum vulgare*, are abundant in various phenolic compounds such as phenolic acids and flavonoids with high antioxidant activity in vitro (*21*−*26*).

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As judged by the wide traditional use of Lamiaceae species and the promising results from numerous antioxidant studies on oregano and other Lamiaceae herbs in vitro, they have been suggested to be potentially beneficial to health and to offer a good source of natural phenolic substances protective against lipid peroxidation (*27*). Despite the numerous studies concerning the in vitro antioxidant potential of culinary herbs, to our knowledge no human supplementation trials on these herbs or extracts thereof have been reported. The aim of this study was to investigate the short- and long-term effects of *Origanum vulgare* extract supplementation on serum lipids and markers of antioxidant status and lipid peroxidation in healthy nonsmoking men.

MATERIALS AND METHODS

Subjects. Forty-five nonsmoking male volunteers were recruited from the Kuopio area in eastern Finland through advertisements in local papers and by e-mail lists. Potential participants were screened in an interview for the following inclusion criteria: no severe obesity (body mass index, BMI < 32 kg/m^2), no regular use of any drug or supplement with antioxidant (β-carotene, vitamins C or E) or lipid-lowering properties, no chronic diseases such as diabetes, coronary heart disease, or other major illness, and willingness to consume 375 mL of the study beverage daily for four weeks. A written informed consent was obtained from all participants. The study protocol was approved by the Research Ethics Committee, Hospital District of Northern Savo, Kuopio, Finland.

Study Design. The study was a four-week double-blinded supplementation trial. Subjects were randomly assigned to consume daily 375 mL of the study beverage, either mango−orange juice (placebo group, $n = 15$); mango−orange juice enriched with oregano extract, daily dosage of total phenolic compounds from the extract being 300 mg (low phenolic group, LP, $n = 15$); or mango−orange juice enriched with oregano extract, daily dosage of total phenolic compounds from the extract being 600 mg (high phenolic group, HP, $n = 15$). A description of the extract preparation is given later in the article. All the juices contained 30 mg/dL of ascorbic acid, added as a preservative.

The daily amount of study beverages used in this study was packed in three 125-mL tetra brick packages. The subjects were advised to drink beverages with meals and to consume one package in the morning, one in the afternoon, and one in the evening. The subjects were also advised to discontinue the use of tea, red wine, cocoa, and chocolate one week prior to the study and to avoid the use of alcohol and analgesics three days before and vigorous physical activity one day before the study visits. A four-day food recording was required before and during the last week of the intervention period to control the possible confounding factors and to check the compliance to the given instructions. The instructions for the food records were given, checked with the subjects, and analyzed by a nutritionist using the Nutrica software, version 2.5 (Social Insurance Institution, Research and Development Centre, Turku, Finland). The compliance to assess the amount of study beverages consumed was checked by a questionnaire designed for the study.

Blood samples were drawn into Venoject (Terumo, Leuven, Belgium) and Vacutainer (Becton Dickinson, Franklin Lakes, NJ) vacuum tubes after overnight fast (10 h). All measurements were done at the baseline and after the four-week supplementation period. In this study, we evaluated also the short-term effects of juice consumption. The short-term study was conducted at the study baseline (day 0) before the long-term supplementation study using the same subjects and study groups as in long-term study. Blood samples were drawn after the overnight fast and 1.5 h after the consumption of two beverage packages (250 mL) of study juices. In the short-term study, the same selection of measurements used in the long-term study was employed. Subjects collected 24-h urine samples on the preceding day of the baseline study visit and during the last 24 h of the supplementation period. The volume of the urine was determined, and aliquots were stored at −70 °C until urinary phenolic acids were determined.

Preparation of the Oregano Extract and Study Beverages. The oregano extract used as a supplement in the study was prepared from ground *Origanum vulgare* material obtained from Control-Ox Ltd (Helsinki, Finland). The preparation was carried out as described previously (*28*). By hydrodistillation extraction, the aromatic volatile components of the herb material were removed and hydrophilic phenolic compounds remained in the aqueous extract. The extraction was free of organic solvents, and the resulting water-soluble extract was mild in odor and taste, which makes it suitable to be used as a food supplement. The yield of the dry extract was 36% of the original dried plant material.

The juice base used as the placebo beverage and base for the extract-fortified study beverages was prepared in water by adding the following constituents: 2.1% orange−mango juice concentrate (Tekno-Food, Finland), 10% saccharose, 0.012% potassium sorbate, 0.030% ascorbic acid, and 0.025% citric acid. Mango−orange juice was chosen to be used as a placebo beverage and base for the fortified juices due to its very low antioxidant activity in the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH$^\bullet$) scavenging assay and low content of phenolic compounds (data not shown). On the basis of the determined total phenolic content of the oregano extract, the study beverages were prepared to give two different levels, 300 mg LP and 600 mg HP gallic acid equivalent (GAE), of oregano-derived phenolic compounds.

Screening of the Phenolic Acids and Flavonoids in the Oregano Extract. The oregano extract was screened for the presence of altogether 39 phenolic compounds using an HPLC method with coulometric electrode array detection as described earlier (*29*). The purity of the standards purchased from the usual suppliers was 98% or higher. The oregano extract was found to contain two identified phenolic main components: rosmarinic acid and protocatechuic acid. Minor amounts of the following phenolic compounds (in decreasing order of appearance) were also detected: caffeic acid, eriodictyol, *p*-coumaric acid, ferulic acid, luteolin, sinapic acid, and naringenin. The daily dosage of all these identified compounds in the LP and HP groups were 70 and 140 mg, respectively. This corresponds to 300 and 600 μmol of identified compounds, respectively (the method of determining the total phenolic content is given below).

Determination of the Total Phenolic Content of the Oregano Extract. The total phenolic content of the oregano extract was determined as GAE using the spectrophotometric Folin−Ciocalteu method (*30*) as described in detail earlier (*28*). The total phenolic determination gave a value of 156 mg GAE/g for the dry extract.

Measurement of the DPPH$^\bullet$ Radical Scavenging Capacity of the Study Beverages. The in vitro antioxidant activity of the study beverages (placebo, LP, HP) was evaluated by measuring their abilities to reduce the stable nitrogen-centered DPPH• radical in vitro. The study beverages were diluted into a range of concentrations and reacted with DPPH$^\bullet$ radical solution, after which the absorbance caused by the DPPH$^\bullet$ radical was determined at 517 nm by a UV 500 spectrophotometer (Unicam, U.K.) as described earlier in detail (*31*). The IC_{50} value (i.e., the concentration on beverage sample yielding 50% inhibition of the radical absorbance) was calculated. Radical scavenging capacity is expressed as $1/IC_{50}$ units, and the presented values are a mean of three replicates.

Screening of the Phenolic Compounds in the Urine of the Study Participants. To study the amount of urinary excreted phenolic compounds prior to and after the supplementation, the urine samples were screened for the presence of the following phenolic compounds: 3,4-dihydroxyphenylacetic acid, *m*-hydroxyphenylacetic acid, *p*-coumaric acid, *p*-hydroxybenzoic acid, caffeic acid, chlorogenic acid, ferulic acid, gallic acid, protocatechuic acid, syringic acid, and vanillic acid. The HPLC method and conditions have been described earlier (*15*).

Safety Measurements. Serum alanine aminotransferase (ALAT, ALT/GPT Cat. No. 981110, Thermo Electron, Vantaa, Finland) and creatinine (Creatinine, Cat. No. 981811, Thermo Electron) were measured with a Kone Specific clinical chemistry analyzer (Thermo Electron). The catalytic concentration of γ-glutamyltransferase (γ-GT, Cat. No. 981093, Thermo Electron) was measured using the International Federation of Clinical Chemistry method (*32*).

Serum Lipids and Lipoproteins. Serum cholesterol (Cholesterol, Cat. No. 981261, Thermo Electron) and triglycerides (Triglycerides GPO-PAP, Cat. No. 701904, Roche Diagnostics, Mannheim, Germany)

were determined with enzymatic colorimetric tests. The serum concentration of high-density lipoprotein (HDL) cholesterol was measured from supernatant after magnesium chloride dextran sulfate precipitation (*33*). The serum concentration of low-density lipoprotein (LDL) cholesterol was determined by a direct cholesterol measurement (Cat. No. 981656, Thermo Electron).

Serum Fatty Acids. Serum fatty acids (arachidonic, linoleic and linolenic adic) were extracted from 250 μL of serum with 6 mL of chloroform/methanol (2:1), and 1.5 mL of water was added. The two phases were separated after centrifugation, and the upper phase was discarded. To the chloroform phase, 1 mL of methanol/water (1:1) was added, and extraction was repeated. The chloroform phase was evaporated under nitrogen. For methylation, the remainder was treated with sulfuric acid/methanol (1:50) at + 85 °C for 2 h. The mixture was extracted with light petroleum. The fatty acids from the ether phase were determined by a model 5890 gas chromatograph (Hewlett-Packard, Avondale, PA) equipped with a flame ionization detector and NB-351 capillary column (HNU-Nordion, Helsinki, Finland).

Serum Folate and Plasma Homocysteine. Serum folate concentrations were measured by Quantaphase II radioimmunoassay (Bio-Rad, Hercules, CA). The serum total homocysteine (tHcy) concentration was analyzed by HPLC at the National Public Health Institute, Helsinki, Finland (*34*).

Plasma TRAP. The total peroxyl radical trapping potential (TRAP) was determined from plasma with a method modified from ref *35* as previously described (*36*).

Resistance of Serum Lipids to Oxidation. The resistance of serum lipids to oxidation was measured as described earlier (*36*). Briefly, serum was diluted to a concentration of 0.67% in 0.02 mol/L phosphate-buffered saline, pH 7.4. Oxidation was initiated by the addition of 100 μL of 1 mmol/L $CuCl_2$ into 2 mL of diluted, prewarmed (30 °C) serum. The formation of conjugated dienes was followed by monitoring the change in the UV absorbance at 234 nm on a Beckman DU-6401 spectrophotometer (Fullerton, CA) equipped with a six-cuvette automatic sample changer. The change in the absorbance was recorded every 5 min for 4 h. The lagtime was determined as the time required from the start of the measurement to the beginning of the propagation phase (maximum rate of oxidation).

Baseline Conjugated Dienes. The oxidation of LDL in vivo was assessed as the amount of conjugated dienes as described previously (*37*). In brief, serum LDL was isolated by precipitation with buffered heparin. The precipitate was resuspended in phosphate-buffered saline. The cholesterol concentration was determined, and the rest of the suspension was used for the measurement of conjugated dienes. Lipids were extracted from the LDL by a mixture of chloroform and methanol (3:1), dried under nitrogen, and redissolved in cyclohexane. The amount of conjugated dienes was measured spectrophotometrically at 234 and 300 nm. The absorbance at 300 nm was subtracted from that at 234 nm to reduce background interference, and the concentration of conjugated dienes in LDL was calculated per concentration of cholesterol in LDL.

Plasma F_2-Isoprostanes. The plasma-free $F_{2\alpha}$-isoprostane concentrations were determined at Oy Jurilab Ltd., Kuopio, Finland. A deuterated prostaglandin $F_{2\alpha}$ internal standard was added to plasma, and F_2-isoprostanes were extracted with C_{18} and silica minicolumns. Compounds were converted to pentafluorobenzyl ester trimethylsilyl ether derivates and analyzed by a gas chromatographic−mass spectrometric assay (*38*).

Statistical Analysis. Results are displayed as means ± standard deviations (SD). Means between study groups were compared by analysis of variance (ANOVA), and post hoc Tukey's test was used whenever a statistically significant heterogeneity between groups was shown by the ANOVA. As the total excretion of phenolic compounds and the intake of fruits and berries differed significantly between the groups at the study entry, these baseline factors were taken into account as covariates in the analysis of variance. A stepwise linear multivariate regression analysis was used to find strongest determinants of serum creatinine concentration. Differences with *p*-value of 0.05 or less were considered significant. The SPSS for Windows, software version 11.5, was used for statistical analyses.

RESULTS

All 45 recruited volunteers completed the study, and no adverse effects due to the consumption of the study juices were reported during the supplementation period. Age or other physical characteristics did not differ significantly between the study groups at the study baseline. The 24-h excretion of phenolic acids was, however, different between the study groups ($p = 0.013$) at the study baseline, being 26% higher in the placebo and 72% higher in the LP group when compared with the HP group (**Table 1**). At the beginning of the study the intake of fruits, berries, and vegetables was highest in the HP group and lowest in the placebo group.

There were no significantly different changes in the serum ALAT or γ-GT measurements between the study groups during the four-week study period ($p = 0.606$ and $p = 0.121$, respectively) (**Table 1**). Serum creatinine concentration increased 5% ($p = 0.015$) in the HP group and decreased 2% ($p = 0.123$) in the LP group. In the post hoc analysis, the increase was significantly greater in the HP group as compared with that of the LP group ($p = 0.002$), but not between the HP and placebo groups ($p = 0.144$). This significant change observed in the HP group was mostly due to >10 μmol/L increase in the concentration of serum creatinine in three study subjects (**Figure 1**). Pearson's correlation coefficient between the changes in excretion of phenolic acids and changes in serum creatinine was 0.25 ($p = 0.107$) in the whole study population. In a linear regression model, the variables with the strongest associations with increase in serum creatinine concentration were changes in serum TRAP concentration (standardized coefficient 0.44, $p = 0.001$), urinary excretion of vanillic acid (0.36, 0.009), serum oxidation resistance (−0.38, 0.004), plasma total homocysteine (0.30, 0.023), and serum γ-GT (0.34, 0.010), adjusted R^2 for model was 0.44, $P < 0.001$.

In the in vitro DPPH$^\bullet$ radical scavenging assay that was employed to study the antioxidant potential of oregano and other Lamiaceae extracts, the polyphenol enrichment of the juice with oregano extract resulted in an increased radical scavenging capacity of the beverages. The increase in the radical scavenging capacity was dose-dependent: the radical scavenging capacities of LP juice and HP juice compared to placebo juice were 31- and 54-fold, respectively.

The 24-h excretion of phenolic acids decreased 10% in the placebo group, while excretion increased 2% in the LP and 85% in the HP group, and these changes were statistically different between the study groups ($p < 0.001$). The consumption of placebo juice or juices enriched with oregano extract did not have effects on the concentrations of serum lipoproteins (triglycerides, total-, LDL-, or HDL cholesterol) or plasma tHcy concentrations (**Table 1**).

Although we used several biomarkers of lipid peroxidation, there were no significant differences in the changes in those measurements among the study groups either in the short (1.5 h) or in the long-term (four weeks) (**Table 2**). As baseline excretion of phenolic acids and intake of fruits and berries differed among study groups, we also repeated all analyses using baseline excretion of phenolic acids and intake of fruits, berries, and vegetables as covariates, but it did not affect the results. Plasma TRAP increased significantly (favorable effect) in short-term measurements within the HP group ($p = 0.017$) and LDL baseline conjugated dienes (unfavorable effect) in long-term measurement within the LP group ($p = 0.023$). No other significant changes in any study groups in either short- or long-term measurements were found.

Table 1. Baseline Characteristics and Changes in Physical and Biochemical Measurements and in Dietary Factors of Study Subjects during the Four-Week Study Period[a]

	placebo group ($n = 15$)		LP group ($n = 15$)		HP group ($n = 15$)		p for difference[b]	
	baseline	change	baseline	change	baseline	change	baseline	change
age (years)	33.0 ± 13.1		36.6 ± 11.8		33.8 ± 11.1		0.698	
body mass index (kg/m^2)	24.7 ± 2.9	–0.05 ± 0.48	25.0 ± 3.5	–0.01 ± 0.25	26.0 ± 3.3	–0.06 ± 0.62	0.538	0.956
Safety Measurements								
serum ALAT (U/L)	25.1 ± 13.5	–1.1 ± 14.3	33.8 ± 13.6	+0.7 ± 8.4	30.9 ± 15.2	+3.5 ± 13.7	0.241	0.606
serum γ-GT (U/L)	21.5 ± 7.3	–1.2 ± 3.5	34.1 ± 24.7	+1.6 ± 5.0	20.4 ± 7.1	+1.9 ± 4.6	**0.034**	0.121
serum creatinine (μmol/L)	93.5 ± 9.6	+1.1 ± 4.6	95.5 ± 11.3	–2.1 ± 5.0	93.3 ± 10.8	+4.7 ± 5.7	0.824	**0.003**
Measured Factors								
serum total cholesterol (mmol/L)	4.86 ± 0.87	–0.01 ± 0.46	5.10 ± 1.17	+0.17 ± 0.38	5.32 ± 0.86	+0.02 ± 0.59	0.446	0.542
serum LDL cholesterol (mmol/L)	2.69 ± 0.79	–0.01 ± 0.46	2.90 ± 1.03	+0.17 ± 0.38	3.02 ± 0.74	+0.02 ± 0.59	0.569	0.191
serum HDL cholesterol (mmol/L)	1.15 ± 0.21	+0.01 ± 0.06	1.17 ± 0.24	–0.03 ± 0.09	1.14 ± 0.22	–0.02 ± 0.09	0.930	0.366
serum triglycerides (mmol/L)	1.05 ± 0.43	+0.10 ± 0.42	1.36 ± 0.61	+0.15 ± 0.66	1.51 ± 0.85	–0.10 ± 0.75	0.160	0.511
serum folate (nmol/L)	8.14 ± 2.25	–0.42 ± 1.78	7.89 ± 3.46	+0.00 ± 1.38	7.51 ± 1.95	–0.09 ± 1.39	0.807	0.734
plasma total homocysteine (μmol/L)	10.2 ± 2.0	–0.42 ± 1.09	10.3 ± 4.2	+0.13 ± 2.25	10.2 ± 1.9	+0.11 ± 1.29	0.993	0.600
serum arachidonic acid (μmol/L)	538.1 ± 123.9	–7.9 ± 80.2	587.5 ± 156.0	+9.0 ± 61.7	554.8 ± 132.9	+26.2 ± 108.4	0.613	0.556
serum linoleic acid (μmol/L)	2355 ± 214	+118 ± 411	2491 ± 414	+158 ± 369	2595 ± 430	+107 ± 401	0.209	0.934
serum linolenic acid (μmol/L)	81.2 ± 17.9	+13.2 ± 43.9	105.0 ± 39.7	+15.4 ± 48.9	112.1 ± 53.1	+13.6 ± 41.2	0.094	0.990
excretion of phenolic acids (μmol/d)	206.1 ± 58.6	–19.7 ± 65.6	280.1 ± 156.3	+5.2 ± 139.2	163.2 ± 37.1	+139.2 ± 62.7	**0.013**	**<0.00 1**
Dietary Factors[c]								
total energy (kJ/d)	8863 ± 1613	–90 ± 1277	8645 ± 2136	+919 ± 1119	9034 ± 1489	+354 ± 1781	0.838	0.176
saturated fatty acids (E%)	13.9 ± 2.8	–1.5 ± 3.4	13.3 ± 2.0	–0.7 ± 2.0	12.3 ± 2.3	+0.0 ± 2.1	0.217	0.302
monounsaturated fatty acids (E%)	11.2 ± 2.76	+1.27 ± 2.11	11.04 ± 1.78	+0.00 ± 2.40	9.53 ± 2.26	–0.42 ± 2.08	0.108	0.347
polyunsaturated fatty acids (E%)	5.65 ± 1.72	–0.61 ± 1.72	5.27 ± 1.19	+0.19 ± 1.74	4.72 ± 1.10	+0.08 ± 1.31	0.190	0.104
carbohydrates (E%)	46.1 ± 8.4	+4.1 ± 5.5	47.6 ± 7.3	+1.1 ± 5.1	51.5 ± 4.0	+0.7 ± 5.8	0.205	0.096
fiber (mg/g)	22.6 ± 5.4	+0.6 ± 6.2	22.7 ± 9.6	+1.35 ± 4.2	27.6 ± 16.3	–1.5 ± 6.5	0.403	0.397
folate (μg/d)	275 ± 56	–9 ± 54	272 ± 66	–16 ± 41	305 ± 130	–28 ± 65	0.540	0.624
vitamin C (mg/d)	117 ± 79	–30 ± 66	132 ± 82	–19 ± 58	140 ± 101	–17 ± 82	0.766	0.859
vitamin E (mg/d)	9.8 ± 3.8	–0.4 ± 2.6	9.7 ± 2.7	+0.4 ± 3.1	9.9 ± 4.1	–0.4 ± 2.5	0.987	0.663
whole grain products (g/d)	137 ± 65	+19 ± 65	144 ± 76	+4 ± 48	161 ± 98	–26 ± 64	0.125	0.696
fruit and vegetables intake (g/d)	469 ± 168	–184 ± 129	485 ± 184	–229 ± 142	641 ± 386	–272 ± 220	0.171	0.393
coffee + tea intake (g/d)	249 ± 1025	5 ± 65	218 ± 89	–9 ± 62	210 ± 164	+15 ± 104	0.665	0.714

[a] Mean ± SD. LP, lower polyphenol group; HP, higher polyphenol group. [b] One-way Anova. p value for the difference between study groups in baseline values and in changes during the study period (four weeks). [c] Based on four-day food records.

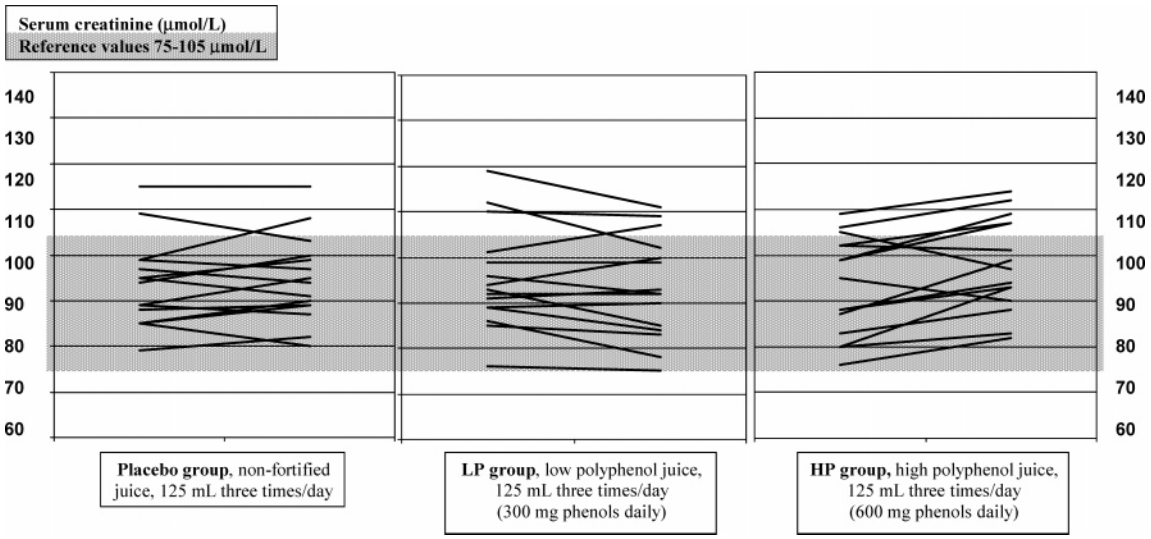

Figure 1. Changes in serum creatinine concentration during the four-week study. Lines represent the results from each subject.

DISCUSSION

Herbs used as a part of a normal diet provide humans with an abundance of various polyphenols and have thus been suggested to contribute significantly to the total intake of plant antioxidants and to offer a good source of dietary antioxidants in addition to fruits, berries, cereals, and vegetables (*27*). In our previous work, we found polyphenols from oregano (*Origanum vulgare* L.) to be absorbed from the GI tract and extensively metabolized before urinary excretion (*39*). In addition, similarly prepared *Origanum vulgare* extract has been shown to possess antioxidant potential in vitro (*28*). On the basis of these findings, we found it worthwhile also to study the effects of oregano extract on lipid peroxidation in vivo. In this study, the consumption of oregano extract was found to be safe as no negative effects were seen in the safety parameters. However, despite the fact that oregano extract was at least partly absorbed, we did not find the consumption of the extract administered in juice to have any detectable short- or long-term

Table 2. Baseline Values and Short- and Long-Term Changes in Measured Parameters of Lipid Peroxidation[a]

	placebo group ($n = 15$)		LP group ($n = 15$)		HP group ($n = 15$)		p for difference[b]	
	baseline	change	baseline	change	baseline	change	baseline	change
Short-Term (1.5 h)								
plasma TRAP (μmol/L)	989 ± 137	+42 ± 74	1030 ± 127	+23 ± 88	1046 ± 162	+68 ± 91	0.528	0.356
serum lagtime (min)	75.0 ± 6.8	+6.7 ± 15.3	72.7 ± 5.6	–2.7 ± 14.3	74.7 ± 7.4	+6.0 ± 13.0	0.588	0.146
serum LDL dienes (μmol/mmol chol)	16.15 ± 5.32	–0.58 ± 1.93	11.77 ± 4.19	–0.28 ± 1.66	11.84 ± 4.24	–0.57 ± 2.38	0.016	0.899
plasma $F_{2\alpha}$-isoprostanes (pg/mL)	33.20 ± 8.83	–2.47 ± 3.54	29.07 ± 6.04	–0.07 ± 3.67	34.20 ± 9.46	–0.40 ± 4.70	0.207	0.218
Long-Term (4 Weeks)								
plasma TRAP (μmol/L)		+59 ± 120		+28 ± 88		+24 ± 112		0. 630
serum lagtime (min)		+1.7 ± 6.7		+5.0 ± 11.3		–2.3 ± 7.0		0.078
serum LDL dienes (μmol/mmol chol)		+0.06 ± 3.52		+0.69 ± 3.11		+0.01 ± 2.63		0.803
plasma $F_{2\alpha}$-isoprostanes (pg/mL)		–1.60 ± 5.68		+1.47 ± 3.14		–0.33 ± 5.90		0.260

[a] Mean ± SD. LP, lower polyphenol group; HP, higher polyphenol group. [b] One-way ANOVA. p value for the difference between study groups in baseline values and in changes during the study period (1.5 h or 4 weeks).

effects on serum lipids or lipid peroxidation in healthy nonsmoking men.

The supplementation with the extract did not have any effects on lipid peroxidation, although it was assessed with a variety of methods: plasma TRAP, the oxidation susceptibility of serum, susceptibility of LDL to oxidation, formation of plasma $F_{2\alpha}$-isoprostanes, and LDL baseline conjugated dienes. Among these measurements, levels of plasma-free $F_{2\alpha}$-isoprostanes are considered to be the most reliable marker of lipid peroxidation in vivo (*40*, *41*). In addition, we evaluated both the short- and long-term effects of the extract. The 1.5-h time point for the short-term determination was chosen on the basis of the pharmacokinetic data obtained in our work (*39*) with another oregano extract, showing an early excretory maximum in urine for the major metabolite (*p*-hydroxybenzoic acid) of oregano extract at 2−4 h after ingestion of oregano supplement in hard gelatine capsules. In the present study, the extract was administered in juice and should thus be bioavailable at least as quickly as extract dosed in capsules. In the oregano bioavailability study, a single dose of 500 mg of oregano-derived total polyphenols was enough to increase the excretion of phenolic acids 4-fold during the first 24 h of the follow-up and 2-fold even during the second day of the follow-up (*39*). On the basis of this fact, we deduced 300 and 600 mg GAE of oregano-derived total phenolic compounds to be reasonable amounts to be supplemented and that the effects on serum lipids or markers of lipid peroxidation should be detectable. All in all, we think that our study should have been able to detect the significant short- or long-term effects of oregano extract on lipid peroxidation if such exists.

On the other hand, our study population consisted of young, healthy, and nonsmoking men, who most likely have optimally functional antioxidant systems. It has been suggested that the effects of antioxidant supplementation would be beneficial only to those who have increased state of oxidative stress, for example, as a result of some disease (*42*). It is possible that the results could be different with smokers, type II diabetics, or the elderly, and this hypothesis would be interesting to test in future studies. The number of study subjects was quite small. To minimize the variation in subjects' health status, affecting also the basal status of oxidative stress, only healthy nonsmoking men were recruited for the study. It is also possible that the effects could have been seen with a higher daily dosage of oregano extract; however, we wanted to keep the amounts of the extract and the study beverages such that they would be reasonable to consume as part of a normal diet.

Our results seem to be in line with the previous bioavailability and supplementation studies, even though direct comparison with our results is difficult because of wide variation in the types and amounts of supplemented compounds and methods used to assess the effects on lipid peroxidation. The amounts of phenolic compounds provided in supplements in different studies have varied greatly; for example, amounts of phenolic acids or flavonoids supplemented have ranged from tens of milligrams to grams (*6*, *43*). Overall, phenolic compounds seem to be at least to some extent absorbed and to be effective antioxidants in vitro. The published supplementation studies carried out in humans have shown both positive (*8*−*15*) and negative (*16*−*18*) outcomes concerning the antioxidative action of phenolic compounds in vivo. The reasons for this discrepancy may lie in the methodology but also in the distinct bioavailability and metabolism of these compounds in the human body (*6*). The polyphenols most abundant in the diet or in the supplements are not necessarily the ones best absorbed and being the most effective antioxidant in the body. Furthermore, it is possible that polyphenols and their metabolites are beneficial for health through some other mechanisms than those evaluated as straight antioxidant action (*6*, *20*); they may for example have interactions with the mucosa and content of the GI or urinary tract, participate in the enzymatic antioxidant defense system, affect the platelet aggregation or endothelial functions, affect tumor initiation or proliferation, or contribute to the blood sugar homeostasis.

Even though phenolic compounds have been shown to act as antioxidants in various in vitro models, even the results on their antioxidant activity in vitro are not unequivocal. There are differences between the methodologies, and even within the same method of assessment the sensitivity and details of practice vary from one laboratory to another. When in vitro studies are carried out, the antioxidant activity should be evaluated using several methods that are validated for use. Furthermore, the huge differences in the bioavailability of phenolic compounds as well as their interactions with other biomolecules and extensive metabolism in the body mean that finding a correlation between results obtained with the laboratory test models and results of in vivo studies is more unlikely than likely (*6*, *20*). However, the value of in vitro work should not be understated. An important issue for future research in this field is to gain more knowledge about the antioxidant properties of human metabolites of dietary polyphenols. The only type of study to finally conclude the health effects of polyphenols would be long-term double-blinded supplementation trials where the effects on diseases themselves instead of biomarkers would be used to reflect the risk of a disease. Due to obvious problematic nature of such studies (e.g., high costs and long duration), shorter-

term supplementation studies monitoring the biomarkers are and will be used.

In the safety parameters, we found that during the four-week study period serum creatinine concentration increased significantly in the HP group. The increase in the HP group was significant only when compared with the change in the LP group, where a small decrease was seen, but not with the placebo group. This lack of dose-dependent effect suggests that the finding was more likely a coincidence. However, to confirm this finding we recruited an additional 15 healthy volunteers to drink HP juice (600 mg polyphenols/day) for four weeks. The consumption of the high polyphenol juice did not significantly increase the serum creatinine concentration (increase of 0.7%, $p = 0.738$), and thus we concluded that the earlier finding was a coincidence and this juice (extract) is safe to use. We did not find any significant changes in the other safety laboratory measurements, serum ALAT, or γ-GT.

In conclusion, we found short- and long-term consumption of oregano extract (*Origanum onites* L.) to be safe as measured by basic parameters monitoring the function of liver and kidneys, but no detectable effects on serum lipids or lipid peroxidation in healthy nonsmoking men could be observed. This was despite the marked increase in the urinary excretion of phenolic metabolites after ingestion of the oregano extract.

ABBREVIATIONS USED

ALAT, alanine aminotransferase; ANOVA, analysis of variance; BMI, body mass index; DPPH$^{\bullet}$, 1,1-diphenyl-2-picrylhydrazyl radical; GAE, gallic acid equivalent; γ-GT, γ-glutamyltransferase; tHcy, total homocysteine; HDL, high-density lipoprotein; HP, high phenolic; LDL; low-density lipoprotein; LP, low phenolic; SD; standard deviation, TRAP, total peroxyl radical trapping potential; VLDL, very low-density lipoprotein.

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