DOI:10.1021/jf904360y

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Effect of Micronutrient-Enriched Sunflower Oils on Plasma Lipid Profile and Antioxidant Status in High-Fat-Fed Rats

R. Di Benedetto, L. Attorri, F. Chiarotti, A. Eusepi, A. Di Biase, and S. Salvati*

Department of Public Veterinary Health and Food Safety, Istituto Superiore di Sanità, Rome, Italy

The main objective of this study was to evaluate *in vivo* whether micronutrient-enriched high-oleic sunflower oils (optimized oils) obtained using different crushing and refining procedures could have any beneficial effect on plasma lipid profile and antioxidant status. Sprague−Dawley rats were fed a high-fat diet for 4 weeks. The lipid source consisted of 20% optimized sunflower oils with different quantities and qualities of micronutrients. The control group received traditional refined high-oleic sunflower oil. The experimental optimized oils in the diets had a hypolipidemic effect. The reduction in plasma triglyceride and total cholesterol levels was 43% and 20%, respectively, in the group fed the diet with the highest levels of micronutrients. The serum ferric antioxidant capacity, superoxide dismutase, glutathione peroxidase and reduced glutathione significantly increased and lipid peroxidation decreased in parallel with the enhancement of micronutrients. These results suggest that a regular intake of optimized sunflower oils can help to improve lipid status and reduce lipid peroxidation in plasma.

KEYWORDS: Sunflower oil; CVD; micronutrients; high-fat diet; antioxidant enzymes; rat

INTRODUCTION

The sunflower is one of the four most important oilseed crops in the world, and the nutritional quality of its edible oil makes it one of the most valuable vegetable oils in cultivation (*1*). Typically, up to 90% of the fatty acids in conventional sunflower oil are unsaturated, namely, oleic (C 18:1 n-9, 16%−19%) and linoleic (C 18:2 n-6, 68%−72%) fatty acids. The development by conventional genetic selection of new sunflower seeds producing oils enriched in oleic acid (high-oleic acid sunflower oils) at the expense of linoleic acid has made sunflower oils more stable and favorably impacts health.

High MUFA diets, such as the Mediterranean diet, are associated with reduced cardiovascular disease (CVD) mortality (*2*, *3*). The predominant dietary MUFA, oleic acid, is effective in lowering plasma LDL cholesterol (LDL-C) and triglyceride (TG) levels without affecting HDL cholesterol (HDL-C) concentration (*4*). Elevated plasma total cholesterol (TC), TG and LDL-C levels are well-known CVD risk factors (*5*). In addition to common risk factors, oxidative stress has emerged as another key risk factor for CVD (*6*, *7*).

High-oleic sunflower seeds are an excellent food both for their fatty acid composition and for their content of numerous bioactive compounds that may help to reduce the risks associated with CVD. In fact, the high level of naturally occurring antioxidants (*8*−*10*) (tocopherols and CoQ10) and hypolipidemic compounds such as phytosterols and phospholipids (PL) may offer better protection than other MUFA-rich seeds. Natural antioxidants occurring in food are able, through different mechanisms and effects, to scavenge the free radicals that lead to auto-oxidation of cellular lipids. Overall, the available evidence suggests that increased intakes of antioxidants could be used as a nutritional strategy for the management of CVD (*11*, *12*). Furthermore, epidemiological and interventional studies emphasize the need for new antioxidants and new antioxidant combinations to reduce CVD risks (*13*, *14*). However, the industrial processes currently used in the production of edible oils (extraction and refining) are not optimally suited to the satisfactory preservation of these minor nutritional compounds.

The present investigation is part of the European Union Project OPTIM'OILS ("Valorisation of healthy lipidic micronutrients by optimizing food processing of edible oils and fats"), which aims to improve the processes currently used for seed oil production in order to increase the levels of micronutrients and to develop new healthy oils to be marketed in the European Union. The aim within the EU project of the present study was to evaluate *in vivo* whether high-oleic acid sunflower oils enriched in micronutrients on account of the different crushing and soft-refining procedures adopted in their preparation would favorably affect CVD risk factors. Diets containing 20% sunflower oils as lipid source were fed to rats for 4 weeks. In rats HFD induces obesity, dyslipidemia and hypertension and decreases antioxidant capacity (*15*). The interaction among these factors plays an important role in the pathophysiology of CVD.

Enzymatic (superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX)) and nonenzymatic defense mechanisms (reduced glutathione (GSH)) as well as lipid peroxidation, lipid profiles and antioxidant capacity were all measured in rat plasma. The study aimed to investigate whether these micronutrient-enriched oils might have potential in the management of CVD.

MATERIALS AND METHODS

Optimized Oils. High-oleic (~80%) sunflower oils obtained by different technical procedures (optimized oils) were supplied by CREOL

*To whom correspondence should be addressed. Tel: + 39 06 49902574. Fax: + 39 06 49387149. E-mail: salvatis@iss.it.

 Published on Web 03/31/2010

Table 1. Micronutrients in Different Sunflower Oils

mg/kg oil	SUN REF	OPTIM SUN 1 DH-COOK-P	OPTIM SUN 3 EXT-HEX	OPTIM SUN 4 TSE-BETH
sterols	2895	2677	4702	8679
tocopherols	624	694	695	791
phospholipids	0	96	10103	0
phenols (in eq, caffeic acid)	52	41	149	475
CoQ9 + CoQ10	17.6	20.6	167.7	181.2

and ITERG (Pessac-France). Three main crushing procedures are used to obtain higher levels of micronutrients. (1) "OPTIMSUN 1/DH-COOK-P" is obtained as follows: The sunflower seeds are dehulled, cooked at 96 °C for 60 min and then expelled (the temperature of the press was 80 °C). The crude oil is directly deodorized for 2 h at 145 °C. (2) "OPTIMSUN 3/EXT-HEX" is obtained as follows: The sunflower seeds are cold pressed, then extruded in a single-screw extruder (115 °C) and finally batch extracted with hexane (55 °C). The crude oil is directly deodorized for 2 h at 170 °C. (3) "OPTIMSUN 4/TSE-BETH" is obtained as follows: The sunflower seeds are extruded in a twin-screw extruder with deoiling barrel (130 °C), dried to a water content under 2% and then extracted with azeotropic ethanol (55 °C). After evaporation of the solvent, two phases are obtained, the lower one comprising mostly gums, while the higher phase has been directly deodorized for 2 h at 190 °C. The micronutrient composition of the different oils is shown in **Table 1**. Tocopherols and sterols were determined by ANIA (Valencia, Spain); the former were quantified by high-performance liquid chromatography coupled to photodiode array detection (HPLC/DAD), using the official method NF ISO 9936, while the latter were quantified by gas chromatography using the methodology based on the EN ISO 12228:1999 standard. Phospholipids determined by HPLC according to Rombaut et al. (*17*) were performed by FUSAGx (Gembloux, Belgium), Phenols, analyzed by AgroParisTech (Massay, France), were quantified by NF ISO 9936. CoEQ was determined by reverse-phase high-performance liquid chromatography with a mass detector as described by Rodríguez-Acuña et al. (*18*).

Animals and Diets. Sprague−Dawley rats weighing 150−170 g were obtained from Harlan-Nossan (Italy). The animals were housed in stainless steel cages (2 rats per cage) at a controlled room temperature of 24 °C, under a 12 h:12 h light:dark cycle. After 1 week of acclimatization, they were divided into 5 groups (n = 10 rats per group). They were allowed *ad libitum* access to food and tap water. All groups received the same basal synthetic diet containing 18% proteins, 56% carbohydrates, 3% fiber, 20% fat. The mineral and vitamin contents in the diets (3%) were identical (AIN-76). The lipid fraction was provided by different sunflower oils. Experimental diets were administered for 4 weeks. Group 1 received the reference refined sunflower oil (SUN REF) and was the control group. Group 2 consisted of oil obtained by cooking and pressing (DH-COOK-P). Group 3 was oil obtained by hexane extraction after extrusion (EXT-HEX). Group 4 was oil obtained by ethanol extraction after extrusion (TSE-BETH). The diet was prepared under vacuum and stored in the dark at 4 °C. Animals were weighed twice each week and food intake was recorded weekly to monitor growth rates and the potential effect of diet on food consumption.

At the end of the experiment, fasting blood samples were collected in EDTA (0.2 mg/100 mL) from the left atrium of the heart of rats anesthetized by a mixture of medetomidine and ketamine (1:1 v/v). Blood was centrifuged at 1500*g* for 10 min at 4 °C, and plasma was separated, aliquoted and stored at −80 °C until analysis.

All animal experiments were performed according to European Community Council Directive 86/609/ECC and Italian legislation (DL 116/92) on animal experimentation.

Plasma Lipids. Concentrations of total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) in plasma were determined by enzymatic colorimetric methods using commercial kits (BPC- Biosed, Italy).

Fatty Acid Analysis. Lipids were extracted from plasma according to Folch et al. (*16*) and transmethylated with methanolic HCl (Supelco, Bellefonte, PA) overnight at 70 °C. Fatty acid methyl esters were extracted with hexane and analyzed by gas chromatography (Agilent, Palo Alto, CA). The gas chromatograph was equipped with a fused silica capillary column (Supelcowax, 30 m × 0.53 mm i.d. and 1.0 μm film thickness, Supelco) and a flame ionization detector. The injector (split 50:1) temperature was 260 °C, and the detector temperature was set at 280 °C. The heating program began at 220 °C, increased by 2 °C per minute and was held at 240 °C for 10 min. The carrier gas was helium at a flow rate of 13 mL/min.The fatty acid methyl esters were identified by comparison with authentic standards (Supelco) and calculated as percentages of total fatty acids.

Lipid Peroxidation. Plasma peroxidation products were measured by the formation of thiobarbituric acid reacting substances (TBARS) according to Fremont et al. (*19*). Briefly, 100 μL of 3 mol/L trichloracetic acid and 150 μL of 90 mmol/L thiobarbituric acid in 75 mmol/L NaOH were added to 50 μL of rat plasma. The tubes were incubated at 80 °C for 40 min. After cooling, the tubes were centrifuged for 10 min (1500*g*) at 4 °C. The supernatant absorbance was read at 535 nm. The concentration was calculated using an extinction coefficient of 1.56×10^5 M^{-1} cm^{-1} and expressed as nmol of TBARS/mL of plasma. In addition, a calibration curve was established with malondialdehyde produced by hydrolysis of 1,1,3,3-tetramethoxypropane in 0.1 M HCl as standard.

Enzymatic Antioxidant Activity. Plasma CAT (EC. 1.11.1.6), SOD (EC 1.15.1.1) and GPX (EC. 1.11.1.9) activities were evaluated by commercial kits following the manufacturer's instructions (Cayman, MI).

Glutathione Assay. Reduced glutathione (GSH) content was measured in plasma using a commercial kit (Cayman, MI). This method is based on a chemical reaction that involves the formation of a chromophoric thione, which has maximum absorbance at 405 nm.

Total Plasma Antioxidant Activity. Plasma antioxidant activity was analyzed by the ferric-reducing antioxidant potential (FRAP) method according to Benzie and Strain (*20*) using a DU 800 spectrophotometer (Beckman, Brea, CA) equipped with a thermostatically controlled cell-holder. The method is based on the reduction of the Fe^{3+}−2,4,6-tripyridyl-*s*-triazine (TPTZ) complex to ferrous Fe^{2+} at low pH. Briefly, 800 μL of FRAP reagent prepared daily was mixed with 50 μL of diluted sample and the absorbance was recorded at 595 nm after 0 and 30 min of incubation at 37 °C. The change in absorbance was calculated for each sample and compared with a $FeSO_4 \cdot 7H_2O$ standard solution tested in parallel. Values were expressed as μmol/L of Fe^{2+}.

Statistical Analysis. All data are presented as the mean ± SD. The data were evaluated by one-way ANOVA. Post-hoc Duncan's test was performed to evaluate differences between groups. The correlation coefficient was computed to assess the association between different CVD risk factors.

Multiple linear regression analysis (stepwise backward procedure) was performed to assess the effect of the micronutrient intake (independent variables, selected among sterols, tocopherols, phospholipids, phenols and CoQ) on oxidative stress indices (dependent variables analyzed separately). The effect of each micronutrient included in the model was computed taking into account the effect of the others. Standardized regression coefficients (SRCs) were used to measure the size of effects, with the SRC representing the quantitative difference in the oxidative stress indices, expressed in SD units, for a one SD-unit increase in the intake of the specific micronutrient. Statistical significance was set at $p < 0.05$. SPSS and Stata software was used for the statistical analyses.

RESULTS

No significant differences were observed in food intake and weight gain in rats fed diets rich in optimized oils compared with controls as shown in **Table 2**. No side effects were induced by optimized oils during the dietary treatment.

The high percentage of oleic acid (∼80%) in sunflower oils affects the composition of plasma fatty acids. As shown in **Table 3** oleic acid is one of the most representative plasma fatty acids. The values of plasma fatty acid composition are averaged since no significant differences within the different groups were observed.

Values of lipid parameters obtained after feeding rats HFDs for 4 weeks are summarized in **Table 4**. The experimental optimized oils significantly decreased the levels of TG and TC, with the largest reduction being observed in group 4, which had the highest level of micronutrients. As the LDL-C levels are not affected by optimized sunflower oils, the TC reduction mainly affects the HDL-C levels.

The FRAP values, an indicator of total antioxidant defense, and plasma TBARS levels, a biomarker of lipid peroxidation, were significantly influenced by a diet containing micronutrient-enriched oils. The experimental diets caused an increase in serum ferric antioxidant capacity with a concomitant decrease in TBARS values as shown in **Table 5**. The TBARS and FRAP values recorded the highest reduction (~32%) and increase (~21%), respectively, in group 4, and the regression coefficient test indicates a significant correlation between them ($r = -0.99$). Positive correlation was observed between TBARS and TG ($r = 0.94$).

Plasma CAT, SOD and GPX enzymatic activities are shown in **Table 6**. As for the plasma lipid profile, the enzymatic activities are affected by the composition and micronutrient levels of experimental oils. The enhancement of SOD activity in optimized sunflower groups is accompanied by a concomitant decrease in CAT activity. The regression coefficient test indicates a significant correlation between these enzymatic activities ($r = -0.96$).

On the contrary, plasma GPX activity is not affected by dietary oils except in the group fed a diet containing the highest concentration of micronutrients, namely, group 4. The plasma GSH concentration was higher in all groups fed optimized oils than in controls, and the highest increase is observed in group 4 (~58%) as shown in **Figure 1**.

Multiple regression analysis results are reported in **Table 7**. As can be seen, tocopherols have the most relevant effects on TBARS (SRC = −0.905, $p < 0.001$), FRAP (SRC = 22.647, $p < 0.001$), CAT activity (SRC = −8.235, $p < 0.001$) and GSH levels (SRC = 2.997, $p < 0.001$), while CoQ has the strongest effect on GPX (SRC = 4.529, $p < 0.001$) and SOD activities (SRC = 1.680, $p = 0.006$).

Table 2. Body Weight Gain, Food Intake and Food Efficiency Ratio of Different Optimized Sunflower Oil Groups

treatments	body wt gain (g)	food intake (g)	food efficiency ratio
SUN REF	110.00 ± 5.72	490.0 ± 52.4	0.22 ± 0.05
OPTIM SUN 1	107.11 ± 17.68	445.2 ± 57.3	0.24 ± 0.05
OPTIM SUN 3	110.44 ± 13.05	512.4 ± 59.6	0.22 ± 0.04
OPTIM SUN 4	112.22 ± 7.20	470.4 ± 58.3	0.24 ± 0.03

Table 3. Plasma Fatty Acid Composition (%) in Rats after Feeding with Optimized Sunflower Oils

fatty acids	sunflower
16:0	14.7 ± 2.2
16:1	1.0 ± 0.2
18:0	14.8 ± 0.8
18:1	26.9 ± 3.6
18:2 n-6	8.6 ± 1.4
18:3 n-6	0.2 ± 0.1
18:3 n-3	1.0 ± 0.2
20:1	0.2 ± 0.0
20:3 n-6	0.7 ± 0.0
20:4 n-6	27.8 ± 3.0
20:5 n-3	0.5 ± 0.1
22:5 n-3	0.7 ± 0.2
22:6 n-3	2.9 ± 0.5
SFA	29.5 ± 2.2
MUFA	28.1 ± 2.9
PUFA	42.4 ± 4.6
n-3/n-6	0.01

DISCUSSION

The administration of HFD to induce hyperlipidemia in rat is a frequently used protocol to study several pathologies such as CVDs since hypercholesterolemia and hypertriglyceridemia are major risk factors for their development (*21*, *22*). Yang et al. (*23*) reported that rats fed an HFD for 4 weeks show an increase in plasma TC, TG and LDL-C levels. In our study the intake of 20% high-oleic optimized sunflower oils as lipid source reduced

Table 4. Effect of Different Optimized Sunflower Oils on Various Plasma Lipid Parameters[a]

param (mg/dL)	group 1: refined oil	group 2: DH-COOK-P	group 3: EXT-HEX	group 4: TSE-BETH	*p*
TG	47.3 ± 0.7	38.1 ± 0.2 a	29.0 ± 0.1 ab	27.0 ± 0.1 abc	0.000
TC	42.6 ± 0.2	38.4 ± 0.4 a	35.9 ± 0.2 ab	34.4 ± 0.1 abc	0.000
HDL	36.1 ± 0.1	30.9 ± 0.1 a	27.9 ± 0.1 ab	25.9 ± 0.2 abc	0.000
LDL	7.3 ± 0.1	7.2 ± 0.2	7.5 ± 0.2	7.5 ± 0.3	0.044

[a] Values represent the mean ± SD of 7 samples. a = $p < 0.001$ vs group 1; b = $p < 0.001$ vs group 2; c = $p < 0.001$ vs group 3.

Table 5. Effect of Different Optimized Sunflower Oils on Antioxidant Status[a]

parameter	group 1: refined oil	group 2: DH-COOK-P	group 3: EXT-HEX	group 4: TSE-BETH	*p*
FRAP (μmol of Fe^{2+}/L)	58.30 ± 0.43	61.82 ± 0.42 a	66.72 ± 0.41 ab	70.70 ± 0.41 abc	0.000
TBARS(μmol/mL)	2.00 ± 0.01	1.86 ± 0.01 a	1.60 ± 0.16 ab	1.37 ± 0.01 abc	0.000

[a] Values represent the mean ± SD of samples. a = $p < 0.001$ vs group 1; b = $p < 0.001$ vs group 2; c = $p < 0.001$ vs group 3.

Table 6. Effect of Different Optimized Sunflower Oils on Plasma Enzymatic Activities[a]

	group 1: refined oil	group 2: DH-COOK-P	group 3: EXT-HEX	group 4: TSE-BETH	*p*
CAT (nmol/min/mL)	3.71 ± 0.10	2.32 ± 0.14 a	1.34 ± 0.07 ab	0.78 ± 0.09 abc	0.000
SOD (U/mL)	3.46 ± 0.05	3.79 ± 0.02 a	4.26 ± 0.04 ab	4.73 ± 0.04 abc	0.000
GPX nmol/min/mL)	66.0 ± 1.6	65.3 ± 1.6	67.2 ± 3.4	72.9 ± 1.9 abc	0.000

[a] Values represent the mean ± SD of 7 samples. a = $p < 0.001$ vs group 1; b = $p < 0.001$ vs group 2; c = $p < 0.001$ vs group 3.

plasma lipids, and the effects are most pronounced in group 4 in which the micronutrient content was highest.

The quantity of micronutrients in the optimized oils differs on account of the different crushing and refining procedures used. The different extraction procedures may also selectively extract different bioactive compounds within the same class of micronutrients. The hypolipidemic effect observed in group 2 rats whose diet contained total micronutrient levels similar to those of controls could be due to a different mixture of bioactive compounds. By acting together, these compounds may have different effects than they do singly, thereby modifying biological activity. It is also possible that the varied modulation of CVD risk factors by the different optimized oils may be due not only to the presence of a single compound but also to the different levels at which it is present within the same micronutrient class. It has been reported that the antioxidant activity of polyphenols is influenced not only by the subclasses of each single antioxidant compound but also by their ratio (*24*). Moreover, Franconi et al. (*25*) reported an *in vitro* study in which the antioxidant effects of olive oils, such as the prevention of human LDL oxidation, are affected by quantitative and qualitative differences in their phenolic compounds.

Recently, the importance of oil quality and of the synergic and/or additional action of sunflower micronutrients on cholesterol metabolism was highlighted by Chan et al.(*26*). The authors showed that MUFA oil rich in oleic acid, supplemented with plant sterols, provides superior protection against CVD risks and is more effective at reducing LDL-C levels than a MUFA-oil based diet (*26*). The effect of high-oleic acid sunflower oil in lowering LDL-C levels has also been reported by Allman-Farinelli et al (*27*) in human studies. The authors observed that when foods rich in saturated fat were replaced with foods rich in high-oleic acid sunflower oil, the concentration of LDL-C and TG was reduced. In our study no differences were observed in LDL-C levels between optimized groups and controls, suggesting that the interaction between sterols and fatty acid composition may be already optimized in refined oil.

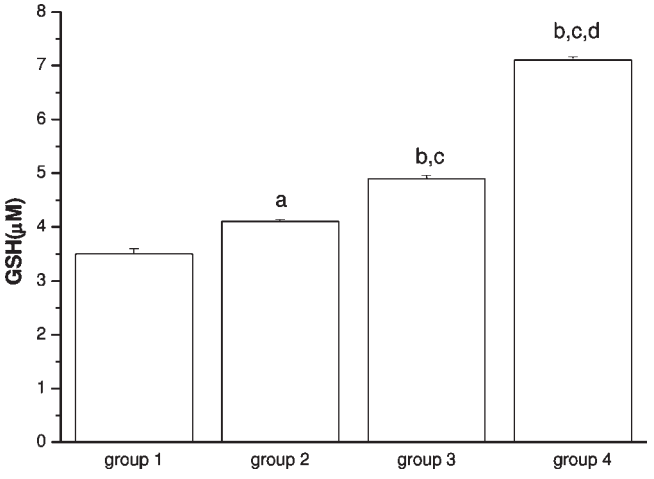

Figure 1. GSH concentration expressed in μM in plasma of rats fed with different optimized sunflower oils for 4 weeks. Values represent the mean $\pm$ SD of 7 samples. a = $p < 0.05$ vs group 1; b = $p < 0.001$ vs group 1; c = $p < 0.001$ vs group 2; d = $p < 0.001$ vs group 3.

The increase in FRAP values in the plasma of animals fed optimized oils suggests a satisfactory level of micronutrient bioavailability. Our results are supported by preliminary experiments that indicate a good absorption of oil micronutrients (personal communication). It has been reported that an enhancement of plasma total antioxidant capacity due to an enrichment of antioxidants in HFD is correlated with an increase in the lipoprotein lipase (LPL) activity that plays an important role in lipid metabolism by hydrolyzing core TG from circulating chylomicrometers and very-low-density lipoproteins (*23*). In our study the TG reduction could be due to the increase in LPL by plasma antioxidant capacity when the latter is enhanced along with the increase in micronutrients.

Oxidative stress is one of the causative factors that link hyperlipidemia with the pathogenesis of atherosclerosis (*28*) as it is thought that oxLDLs play a key role in the development of atherosclerotic lesions (*29*−*31*). Many authors (*32*, *33*) have reported that an HFD can lead to an imbalance between oxidation and antioxidation and a consequent increase in TBARS levels. The increase in lipid peroxidation can be explained by the excessive availability of substrates in the form of fatty acids contained in plasma triglycerides (*34*). Moreover, hypertriglyceridemia has been shown to increase oxidative metabolism and the release of superoxide anions by polymorphonuclear leukocytes and monocytes (*34*).

In rats fed an HFD containing optimized oils, we observed a reduction in TBARS that is positively correlated with TG values and consequently the lowest concentration was in group 4. This group also showed the highest activity values for SOD, GPX and GSH, which represent the endogenous antioxidant resources. SOD promptly converts the superoxide anions generated during oxidative stress to H_2O_2 and subsequently CAT and GPX convert H_2O_2 to H_2O. The decrease in CAT activity does not disagree with the antioxidative effect of the oils, since the increase in catalase activity indicates an excess of hydrogen peroxide when the detoxification capacity of the glutathione system is not sufficient (*35*). Taken together, our results show that optimized sunflower oils exert their effects on CVD risk factors and that SUN 4, obtained by TSE-BETH, is the most effective. Since PL are not present in group 4, the effects observed in groups given optimized oils could be due to the enrichment of oils with other bioactive compounds. However, multiple linear regression analysis indicates that tocopherols and CoQ are the micronutrients with the most relevant effect on oxidative stress

Table 7. Multiple Regression Analysis for the Relation between Micronutrients and CVD Risk Factors[a]

	TBARS		FRAP		CAT		SOD		GPX		GSH	
	SRC	p	SRC	p	SRC	p	SRC	p	SRC	p	SRC	p
sterols			2.75470	0.000							1.06148	0.000
tocopherols	−0.90460	0.000	22.64720	0.000	−8.23515	0.000	1.54835	0.000			2.99655	0.000
phospholipids	−0.04944	0.000	0.94410	0.000			−0.38761	0.023	−1.37184	0.000	0.19758	0.000
phenols	−0.11933	0.000			0.74068	0.000	−0.82847	0.022				
CoEQ					−0.98637	0.000	1.67986	0.006	4.52856	0.000		

[a] In multiple regression analysis sterols, tocopherols, phospholipids, phenols and CoQ were independent variables and oxidative stress indices were the dependent variables. We report only micronutrients significantly associated with CVD risk factors, which were selected by the stepwise backward procedure and included in the final models. SRC = standardized regression coefficient.

parameters. On the other hand, an increase in PL and phenols is not always accompanied by an improvement in oxidative stress parameters, so that, even though the effects when present are small, a supplementation of these micronutrients may be ineffective.

The present study demonstrates that improved oil refining processes produce optimized oils that are able to exert beneficial effects on CVD risk factors, enhancing the hypolipidemic effects of high-oleic acid sunflower oil and the antioxidant defense mechanisms. Even though the plasma lipid profile of rats is different from that of humans, we believe that this model contributes in evaluating the effects of bioactive compounds on CVD risk factors, as the results obtained largely reproduce others obtained in humans.

ABBREVATIONS USED

MUFA, monounsaturated fatty acids; CVD, cardiovascular disease; LDL-C, low-density-lipoprotein-cholesterol; HDL-C, high-density-lipoprotein-cholesterol; TG, triglycerides; SOD, superoxide dismutase; CAT, catalase; GPX, glutathione peroxidase; GSH, reduced glutathione; TBARS, thiobarbituric acid reacting substances; HFD, high fat diet; FRAP, ferric-reducing antioxidant potential; PL, phospholipids.

ACKNOWLEDGMENT

The authors wish to thank Mr. Antonio Di Virgilio for his assistance in animal care and management.

LITERATURE CITED

(1) Skorić, D.; Jocić, S.; Sakac, Z.; Lecić, N. Genetic possibilities for altering sunflower oil quality to obtain novel oils. *Can. J. Physiol. Pharmacol*. **2008**, *86*, 215–21.

(2) Ascherio, A. Epidemiologic studies on dietary fats and coronary heart disease. *Am. J. Med.* **2002**, *113* (S 9B), 9S–12S.

(3) Kris-Etherton, P. M.; Pearson, T. A.; Wan, Y.; Hargrove, R. L.; Moriarty, K.; Fishell, V.; et al. High-monounsaturated fatty acid diets lower both plasma cholesterol and triacylglycerol concentrations. *Am. J. Clin. Nutr*. **1999**, *70*, 1009–1015.

(4) Perez-Jimenez, F.; Lopez-Miranda, J.; Mata, P. Protective effect of dietary monounsaturated fat on arteriosclerosis: beyond cholesterol. *Atherosclerosis* **2002**, *163*, 385–398.

(5) Kannel, W. B.; Vasan, R. S. Triglycerides as vascular risk factors: new epidemiological insights. *Curr. Opin. Cardiol*. **2009**, *24*, 345–350.

(6) Witztum, J. L.; Steinberg, D. The oxidative modification hypothesis of atherosclerosis: does it hold for humans? *Trends Cardiovasc. Med.* **2001**, *11*, 93–102.

(7) Bonomini, F.; Tengattini, S.; Fabiano, A.; Bianchi, R.; Rezzani, R. Atherosclerosis and oxidative stress. *Histol. Histopathol*. **2008**, *23*, 381–390.

(8) Cabrini, L.; Barzanti, V.; Cipollone, M.; Fiorentini, D.; Grossi, G.; Tolomelli, B.; Zambonin, L.; Landi, L. Antioxidants and total peroxyl radical-trapping ability of olive and seed oils. *J. Agric. Food Chem*. **2001**, *49*, 6026–6032.

(9) Rodriguez-Acuna, R.; Brenne, E.; Lacoste, F. Determination of Coenzyme Q10 and Q9 in vegetables oils. *J. Agric. Food Chem*. **2008**, *56*, 6241–6245.

(10) Sanchez, A.; Delgado, M. M.; Bustamente, M.; Fernandez, J. Automated analysis of Vit. E isomers in vegetable oils by continuous membrane extraction and liquid chromatography eletrochemical detection. *J. Chromatogr., A* **2000**, *881*, 229–241.

(11) Sarter, B. Coenzyme Q10 and cardiovascular disease: a review. *J. Cardiovasc. Nurs*. **2002**, *16*, 9–20.

(12) Blomhoff, R. Dietary antioxidants and cardiovascular disease. *Curr. Opin. Lipidol*. **2005**, *16*, 47–54.

(13) Barbaste, M.; Berke, B.; Dumas, M.; Soulet, S.; Delaunay, J. C.; Castagnino, C.; Arnaudinaud, V.; Cheze, C.; Vercauteren, J. Dietary antioxidants, peroxidation and cardiovascular risks. *J. Nutr. Health Aging* **2002**, *6*, 209–223.

(14) Heart Protection Study Collaborative GroupMRC/BHF heart protection study of antioxidant vitamin supplementation in 20536 high-risk individuals: a randomised placebo controlled trial. *Lancet* **2002**,*360*, 23−33.

(15) Nascimento, A. F.; Sugizaki, M. M.; Leopoldo, A. S.; Lima-Leopold, A. P.; Luvizotto, R. A. M.; Nogueira, C. R.; Cicogna, A. C. A Hypercaloric pellet-diet cycle induces obesity and co-morbidities in Wistar rats. *Arq. Bras. Endocrinol. Metab*. **2008**, *52*, 968–74.

(16) Folch, J.; Less, M.; Sloane, G.; Stanley, H. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem*. **1957**, *226*, 497–509.

(17) Rombaut, R; Camp, J. V.; Dewettinck, K. Analysis of Phospho- and Sphingolipids in Dairy Products by a new HPLC method. *J. Dairy Sci*. **2005**, *88*, 482–488.

(18) Rodriguez- Acuña, R; Brenne, E; Lacoste, F. Determination of coenzyme Q10 and Q9 in vegetable oils. *J. Agric. Food Chem*. **2008**, *56*, 6241–5.

(19) Frémont, L.; Gozzélino, M. T.; Franchi, M. P.; Linard, A. Dietary flavonoids reduce lipid peroxidation in rats fed polyunsaturated or monounsaturated fat diets. *J. Nutr*. **1998**, *128*, 1495–502.

(20) Benzie, I. F.; Strains, J. J. The ferric reducing ability of plasma (FRAP) as a measure of "Antioxidant Power": the FRAP assay. *Anal. Biochem*. **1996**, *239*, 70–76.

(21) McBride, P. Triglycerides and risk for coronary artery disease. *Curr. Atheroscler. Rep*. **2008**, *10*, 386–390.

(22) Tajima, N; Kurata, H.; Nakaya, N.; Mizuno, K.; Ohashi, Y.; Kushiro, T.; Teramoto, T.; Uchiyama, S.; Nakamura, H. Pravastatin reduces the risk for cardiovascular disease in Japanese hypercholesterolemic patients with impaired fasting glucose or diabetes: diabetes subanalysis of the Management of Elevated Cholesterol in the Primary Prevention Group of Adult Japanese (MEGA) Study. *Atherosclerosis* **2008**, *199*, 455–462.

(23) Yang, R.; Le, G.; Li, A.; Zheng, J.; Shi, Y. Effect of antioxidant capacity on blood lipid metabolism and lipoprotein lipase activity of rats fed a high-fat diet. *Nutrition* **2006**, *22*, 1185–1191.

(24) Romani, A.; Coinu, R.; Carta, S.; Pinelli, P.; Galardi, C.; Vincieri, F. F.; Franconi, F. Evaluation of antioxidant effect of different extracts of Myrtus communis L. *Free Radical Res*. **2004**, *38*, 97–103.

(25) Franconi, F.; Coinu, R.; Carta, S.; Urgeghe, P. P.; Ieri, F.; Mulinacci, N.; Romani, A. Antioxidant effect of two virgin olive oils depends on the concentration and composition of minor polar compounds. *J. Agric. Food Chem*. **2006**, *54*, 3121–3124.

(26) Chan, Y. M.; Demonty, I.; Pelled, D.; Jones, P. J. H. Olive oil containing olive oil fatty acid esters of plant sterols and dietary diacylglycerol reduces low-density lipoprotein cholesterol and decreases the tendency for peroxidation in hypercholesterolaemic subjects. *Br. J. Nutr*. **2007**, *98*, 563–570.

(27) Allman-Farinelli, M. A.; Gomes, K; Favaloro, E. J.; Petocz, P. A diet rich in high-oleic- acid sunflower oil favorably alters low-density lipoprotein cholesterol, triglicerides, and factor vii coagulant activity. *J. Am. Diet. Assoc*. **2005**, *105*, 1071–1079.

(28) Young, I. S.; McEnemy, J. Lipoprotein oxidation and atherosclerosis. *Biochem. Soc. Trans*. **2001**, *29*, 358.

(29) Ehara, S.; Ueda, M.; Naruko, T.; Haze, K.; Itoh, A.; Otsuka, M.; Komatsu, R.; Matsuo, T.; Itabe, H.; Takano, T.; Tsukamoto, Y.; Yoshiyama, M.; Takeuchi, K.; Yoshikawa, J.; Becker, A. E. Elevated Levels of Oxidized Low Density Lipoprotein Show a Positive Relationship With the Severity of Acute Coronary Syndromes. *Circulation* **2001**, *103*, 1955–1960.

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

(31) Lapointe, A.; Couillard, C.; Lemieux, S. Effects of dietary factors on oxidation of low-density lipoprotein particles. *J. Nutr. Biochem*. **2006**, *17*, 645–658.

(32) Yazdanparast, R.; Bahramikia, S.; Ardestani, A. Nasturtium officinale reduces oxidative stress and enhances antioxidant capacity

in hypercholesterolaemic rats. *Chem.-Biol. Interact*. **2008**, *172*, 176–184.

(33) Hsu, C. L.; Wu, C. H.; Huang, S. L.; Yen, G. C. Phenolic compounds rutin and o-coumaric acid ameliorate obesity induced by high-fat diet in rats. *J. Agric. Food Chem*. **2009**, *57*, 425–431.

(34) Beltowski, J.; Wójcika, G.; Górny, D.; Marciniak, A. The effect of dietary-induced obesity on lipid peroxidation, antioxidant enzymes and total plasma antioxidant capacity. *J. Physiol. Pharmacol*. **2000**, *51*, 883–896.

(35) Ozansoy, G.; Akin, B.; Aktan, F.; Karasu, C. Short-term gemfibrozil treatment reverses lipid profile and peroxidation but does not alter blood glucose and tissue antioxidant enzymes in chronically diabetic rats. *Mol. Cell. Biochem*. **2001**, *216*, 59–63.

Received for review December 9, 2009. Revised manuscript received March 23, 2010. Accepted March 25, 2010. OPTIM'OILS "Valorisation of healthy lipidic micro-nutrients by optimising food processing of edible oils and fats" is a Specific Targeted Research Project supported by the thematic priority "Food Quality and Safety" of the European Commission 6th Framework Programme, Contract No. FOOD-CT-2006-36318, www.optimoils.com. Contact: a.rossignol-castera@iterg.com.