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Basic nutritional investigation

Dietary lipid-induced changes in enzymes of hepatic lipid metabolism

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ABSTRACT

Objective: To investigate the effect of different dietary oils on the main hepatic enzymes involved in metabolism and their impact on oxidative stress status.
Methods: Twenty-four male Wistar rats were fed for 60 d on the same basal diet plus different lipid sources from commercial oils: soybean (S), olive (O), coconut (C), and grape seed (G). After sacrifice, the liver lipid fatty acid composition, enzymatic and non-enzymatic components of the antioxidant defense system, and the activity of enzymes involved in lipid metabolism were determined. The concentration of Ca^{2+} in plasma and liver homogenates was also measured.
Results: The diets produced significant changes in the total and polar lipid fatty acid compositions and alterations in key enzyme activities involved in lipid metabolism. The S and G groups showed significantly increased oxidative stress biomarkers. The enzymatic and non-enzymatic components of the antioxidant defense system were increased in the O and C groups. The highest levels of nitrite plus nitrate were observed in the S and G groups compared with the O and C groups in plasma and in liver homogenates. These were directly correlated with the Ca^{2+} concentration. The most beneficial effects were obtained with olive oil. However, it is necessary to study in more detail appropriate mixtures of olive and soybean oils to provide an adequate balance between ω-3 and ω-6 fatty acids.
Conclusion: Different dietary oils modify the lipid composition of the plasma and liver, local and systemic antioxidant statuses, and the activity of the key enzymes of lipid metabolism. The interrelation between Ca^{2+} and nitrite plus nitrate could be the causal factor underlying the observed changes.

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Introduction

Lipids play relevant roles in the human body, not only as structural components of cell membranes but also in particular as signaling intracellular molecules. Several laboratories have documented that the composition of plasma and tissue lipids in humans and animals is a reflection on the type and amount of dietary lipids consumed [1,2]. Moreover, the fatty acid (FA) composition of the diet is an important factor that can modulate liver lipid metabolism [3–5], and it plays an important role in the physiology of the whole body. Disorders in lipid metabolism have been associated with vascular-related diseases [6,7], insulin resistance [8], and neurodegenerative illnesses such as Alzheimer's disease [9] and Parkinson' disease [10], among others. In addition, lipids have been closely associated with the intracellular generation of reactive oxygen and nitrogen species (ROS and RNS, respectively) [11] as substrates for oxidative attack and a source of free radical generation and/or chain propagator reactants.

Many studies have shown that the long-chain ω-3 polyunsaturated FAs (PUFAs) present in fish oil may decrease the risk of cardiovascular diseases and other chronic illnesses [12]. These acids can rapidly be oxidized to lipid peroxides because of their high concentration of unsaturated FAs. Lipid peroxidation is considered a pivotal mechanism of cell membrane destruction and cell damage, and it has been suggested to be associated with several pathologic conditions, especially with the initiation and progression of atherosclerosis [13]. Other investigators have documented that supplementation of the animal diet with ω-3 PUFAs produces hypocholesterolemic and hypotriglyceridemic effects [14]. Further, it has been shown that plasma and liver triacylglycerol concentrations are controlled mostly by the intake of dietary fat through changes in the hepatic enzyme activities involved in the metabolism of FAs and lipids [3]. The

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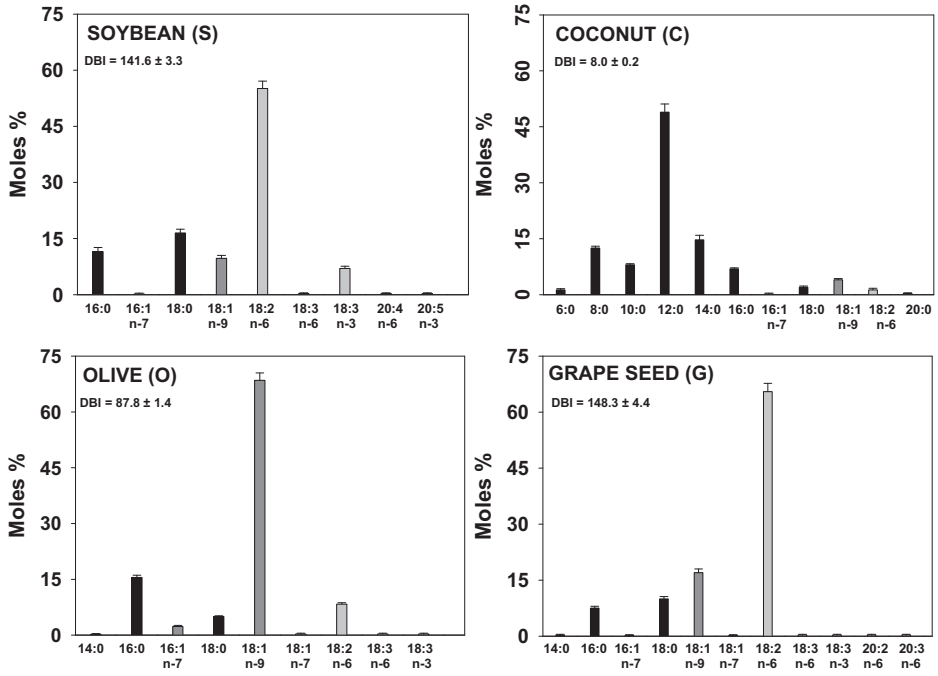

Fig. 1. Fatty acid composition of the diets. Capillary gas–liquid chromatography of fatty acid methyl esters was performed as described in MATERIALS AND METHODS. Results were expressed as mole percentage (mean ± 1 SD; $n = 6$). Saturated, monounsaturated, and di-/polyunsaturated fatty acids are represented as black, dark gray, and light gray bars, respectively. DBI, double bound index.

use of commercial oils by the population depends on many factors (social, economic, cultural, or even geographic). Moreover, apparently similar oils such as grape seed and soybean oils are very different from a physiologic/nutritional point of view. Soybean oil has α-linolenic acid (an essential fatty acid that may produce anti-inflammatory actions) and minor amounts of eicosapentaenoic acid (a precursor of neuroprotectin D1 with important scavenger actions), whereas the content of these acids in grape seed oil is negligible.

The intimate mechanisms involved in the regulatory processes exerted by different oils on lipid metabolism remain unknown, especially those aspects concerning the interrelation between the peroxidability of fatty acyl chains and the impact of key enzymes of lipid metabolism. Therefore, the aim of this study was to evaluate the effect of diet supplementation, using four commercial oils used worldwide, on lipid composition, the main hepatic enzymes involved in FA metabolism, and their effects on the antioxidant defense system.

Materials and methods

Chemicals

Solvents (high-performance liquid chromatographic grade) were provided by Carlo Erba (Milan, Italy). Other chemicals (analytical grade) were from Sigma Chemical Co. (St. Louis, MO, USA), Merck (Darmstadt, Germany), or local sources. Unlabeled FAs were from Nu-Check-Prep (Elysian, MN, USA). Labeled FAs (98–99% pure, 50–60 mCi/mmol) were from Amersham Biosciences (Buckinghamshire, UK). Dietary commercial oils were from Molinos Río de La Plata SAIC and Platafarm SA (La Plata, Argentina).

Animal treatment

Twenty-four male Wistar pups (48 ± 3 g/animal) were used. The animals were maintained in a temperature-controlled room with a 12-h light/12-h dark cycle. They were divided in four groups of six animals each and fed ad libitum on the specific diets for 60 d. Four diets were used, and each was supplemented with a different oil as the lipid source: soybean (S), olive (O), coconut (C), or grape seed (G). During the feeding period, clinical examinations, body weights, foot intakes, and water consumption were controlled daily [15]. All isocaloric diets were prepared in an identical manner with the addition of one commercial oil (70 g/kg of diet), as detailed previously [15]. Figure 1 shows the FA composition of the diets. The rats were fed according to the American Institute of Nutrition [16]. All procedures were approved by the local laboratory animal committee of the Facultad de Ciencias Médicas, UNLP, Argentina.

Experimental design

To avoid individual differences among animals, on day 59 all rats were fasted for 24 h, re-fed with the corresponding diet for 2 h, and euthanized by decapitation after the refeeding period. The livers were excised and placed in ice-cold homogenizing medium [17]. The homogenates were processed individually at 2°C and microsomes and cytosols (soluble fractions) were prepared by differential centrifugation at 110 000 × g [17]. Microsomal pellets were resuspended at a final protein concentration of 30 mg/mL. Mitochondrial suspensions from liver homogenates were obtained [18] and diluted to a final protein concentration of 1 mg/mL. Blood samples were fractionated by centrifugation. Plasmas, whole-liver homogenates, mitochondrial, and postmitochondrial fractions were processed for calcium determination [19]. The protein content of each biological material was measured using the method of Bradford [20]. These data were used for the analytical assay procedures and for the calculation of the specific enzyme activities.

Oxidative stress biomarkers

Nitrite plus nitrate [NOx] and lipid hydroperoxide levels were determined as described previously [21]. Lipid peroxidation was assayed as thiobarbituric acid-reactive substances, as previously reported [22]. Catalase activity, the activity of enzymes involved in glutathione metabolism (glutathione peroxidase, glutathione transferase, and glutathione reductase), the ferric-reducing activity of plasma assay, and vitamin E (α-tocopherol) content were determined as previously reported [22]. Cytosolic (Cu,Zn) and mitochondrial (Mn) superoxide dismutase (SOD) activities and oxidized glutathione (GSSG) and reduced glutathione (GSH) were also assayed [21].

Lipid analysis

Total lipids (TLs) were extracted with the reactive method described by Folch et al. [23]. Polar lipids were separated by microcolumn chromatographic and/or thin-layer chromatographic methods, as described previously [15]. The analysis

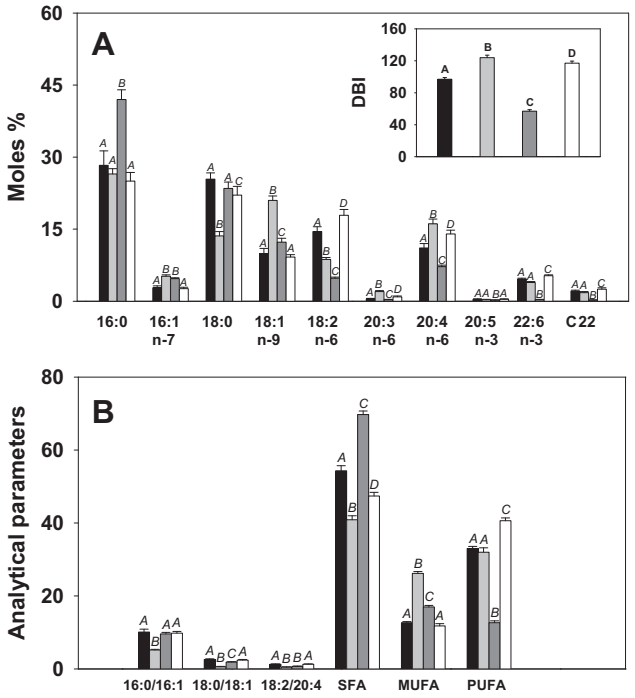

Fig. 2. (A) Effect of dietary lipids on total lipid fatty acid composition in the liver. Capillary gas–liquid chromatography of fatty acid methyl esters was performed as described in MATERIALS AND METHODS. Results were expressed as mole percentage (mean ± 1 SD; $n = 6$). The black, light gray, dark gray, and white bars, represent soybean, olive, coconut, and grape seed oils, respectively. (B) Main fatty-acyl ratios in total lipids of the liver from rats fed on the different diets. The *y* axis indicates the values of the ratios and the sum of the indicated fatty acids (mole percentage). Owing to the multiple comparisons than can be made, statistical differences (analysis of variance + Tukey test, $P < 0.01$) within data from the same group are indicated with letters (results with different letters are statistically significant). C22, sum of 22:4 ω-3 + 22:4 ω-6 + 22:5 ω-6 + 22:5 ω-3; DBI, double bound index; MUFA, monounsaturated fatty acid; PUFA, disaturated/polyunsaturated fatty acid; SFA, saturated fatty acid.

of FA methyl esters was performed by capillary gas–liquid chromatography and quantified as reported previously [15].

Enzyme activities of lipid metabolism

Phospholipase A2 (PL-A2) was measured using [2-^{14}C]20:4 phosphatidyl choline (PC; 24.0 mCi/mmol, 99% pure) as a substrate. The FA desaturase activities were determined in microsomal suspensions using substrates [1-^{14}C]16:0 or [1-^{14}C]18:0 for Δ9-desaturase, [1-^{14}C]18:2 ω-6 or [1-^{14}C]18:3 ω-3 for Δ6, and [1-^{14}C]20:3 ω-6 for Δ5. The labeled FAs were diluted with the respective unlabeled pure FA to a specific activity of 0.20 μCi/mol. After incubations, the reactions were stopped by adding the Folch reactive agent, and samples were processed for the radio-chromatographic analysis of FA methyl esters. Acyl-coenzyme A synthetase, FA synthetase, and carnitine palmitoyl transferase activities were also measured. The rate of β-oxidation was determined as the production of acid-soluble metabolites derived from labeled palmitic acid. All enzymatic assays were performed as described previously [21].

Statistical analyses

The experimental data represent the mean ± standard deviation of six independent determinations assayed in duplicate. The data were analyzed by Student's *t* test or by analysis of variance plus the Tukey test. The correlation, regression, and data plotting were analyzed using SPSS 12.0 for Windows (SPSS, Inc., Chicago, IL, USA), Graphing 8.0 (Sigma Chemical Co.), and/or GB-STAT Professional Statistics Program 4.0 (Dynamic Microsystems, Inc., Silver Spring, MD, USA).

Results

FA composition of different diets

Each diet was characterized by its particular FA composition (Fig. 1). The C diet was rich in saturated FAs (SFAs); the O diet had increased proportions of monounsaturated FAs (mainly 18:1); and the S and G diets had high levels of PUFAs (mainly linoleic acid). α-Linolenic acid occurred in small percentages in all but the S diet. The double bound index depends on the FA composition of a diet and decreased in the following order: G > S > O > C.

FA composition of rat liver lipids

Each diet profoundly modified the relative distribution of FAs in TLs from the liver, PC, and phosphatidylethanolamine (Figs. 2, 3, and 4, respectively). The SFAs (mainly palmitic and stearic acids) were higher in the TL, PC, and phosphatidylethanolamine of rats fed on the C diet. Increased levels of PUFAs (mainly linoleic and α-linolenic acids) were also observed in the TLs and phospholipids in the S, O, and G-groups compared with the C group. Monounsaturated FAs were almost exclusively oleic acid, and the O group showed the highest level. The addition of soybean oil to the diet produced a significant increase in the content of PUFAs of the ω-6 and ω-3 series in phospholipids. The highest levels of arachidonic acid (AA) corresponded to the PC level in the S and O groups, with the level of docosahexaenoic acid in the S group being the highest among the four groups. The double bound index was markedly high in the S, O, and G groups of all lipid subclasses analyzed.

Dietary lipid effects on oxidative stress biomarkers

Various antioxidants and oxidative stress biomarkers were determined in the plasma and liver homogenates from rats fed on the different diets (Table 1). Both tissues exhibited a similar pattern. The levels of [NOx], lipid hydroperoxides, thiobarbituric acid-reactive substances, and GSSG were higher in the S and G groups than in the other groups. Levels of α- + β-tocopherol and GSH were higher in the O and C groups than in the S and G groups. The GSH/GSSG ratio and the ferric-reducing activity of plasma were significantly increased in the O and C groups compared with the S and G groups. Also, the level of vitamin E, determined by high-performance liquid chromatography, showed no significant differences among the S, C, and G oils (approximately 9.1 ± 0.2 mg/kg); the O oil had a level of 19.8 ± 0.3 mg/kg.

To provide more realistic information about the effect of the dietary oils on the antioxidant defense system in the liver, various antioxidant enzyme activities were evaluated (Table 2). The activity of total SOD and its isoforms were increased in the S and G groups compared with the O and C groups. Catalase activity and the enzymes involved in glutathione metabolism (glutathione peroxidase, glutathione transferase, and glutathione reductase) were also higher in those diets enriched in PUFAs (groups S and G) compared with groups O and C.

Activity of enzymes involved in liver lipid metabolism

The two forms (soluble and particulate) of PL-A2 activity increased in the S and G groups compared with the O and C groups (Table 3). Conversely, the activity of carnitine-palmitoyl-transferase was lower in the S and G groups than in the O and C groups. This pattern of activity among the groups was also observed for acyl-coenzyme A synthetase, FA synthetase, and the palmitate β-oxidation rate. The Δ9, Δ6, and Δ5 FA desaturase activities were also measured in microsomal fractions of the liver homogenates. All desaturase activities were higher in the O group than in the other groups (Table 3). Among the groups, desaturase activities decreased as follows:

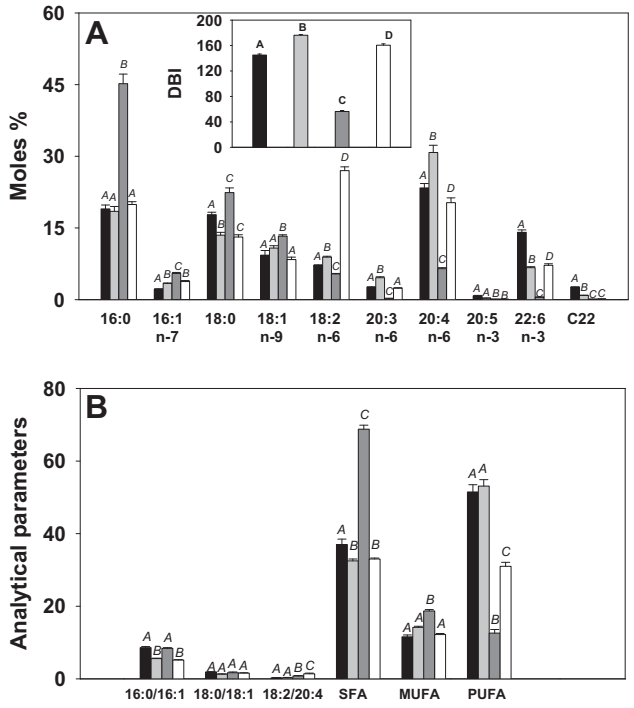

Fig. 3. (A) Effect of dietary lipids on the fatty acid composition of liver phosphatidylcholine. Capillary gas–liquid chromatography of phosphatidylcholine fatty acid methyl esters was performed as described in MATERIALS AND METHODS. Values represent mole percentage (mean ± 1 SD; $n = 6$). The black, light gray, dark gray, and white bars represent soybean, olive, coconut, and grape seed oils, respectively. (B) Main fatty-acyl ratios in phosphatidylcholine lipids from rat fed on different diets. The *y* axis indicates the values of the ratios and the sum of the indicated fatty acids (mole percentage). Owing to the multiple comparisons than can be made, statistical differences (analysis of variance + Tukey test, $P < 0.01$) within data from the same group are indicated with letters (results with different letters are statistically significant). C22, sum of 22:4 ω-3 + 22:4 ω-6 + 22:5 ω-6 + 22:5 ω-3; DBI, double bound index; MUFA, monounsaturated fatty acid; PUFA, disaturated/polyunsaturated fatty acid; SFA, saturated fatty acid.

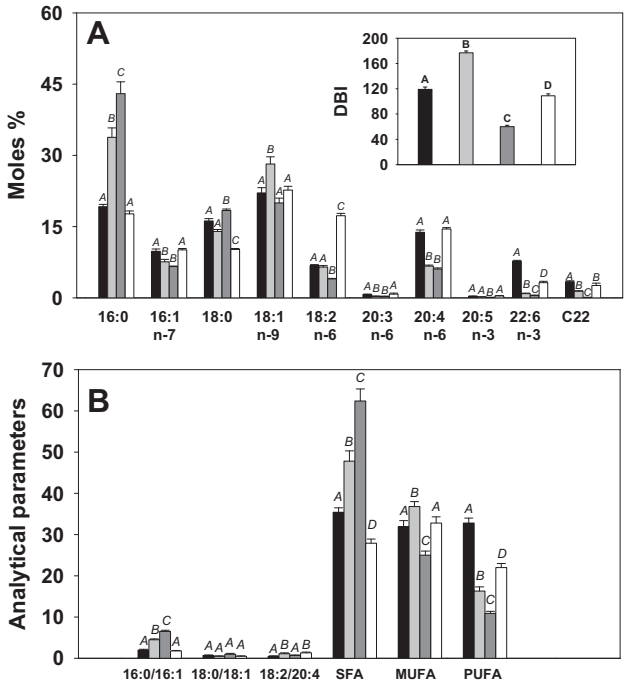

Fig. 4. (A) Effect of dietary lipids on fatty acid composition of liver phosphatidylethanolamine. Capillary gas–liquid chromatography of phosphatidylethanolamine fatty acid methyl esters was performed as described in MATERIALS AND METHODS. Values represent mole percentage (mean ± 1 SD; $n = 6$). The black, light gray, dark gray, and white bars represent soybean, olive, coconut and grape seed oils, respectively. (B) Main fatty-acyl ratios in phosphatidylethanolamine lipids from rats fed the different diets. The *y* axis indicates the values of the ratios and the sum of the indicated fatty acids (mole percentage). Owing to the multiple comparisons than can be made, statistical differences (analysis of variance + Tukey test, $P < 0.01$) within data from the same group are indicated with letters (results with different letters are statistically significant). C22, sum of 22:4 ω-3 + 22:4 ω-6 + 22:5 ω-6 + 22:5 ω-3; DBI, double bound index; MUFA, monounsaturated fatty acid; PUFA, disaturated/polyunsaturated fatty acid; SFA, saturated fatty acid.

$$\Delta 6\ (18:2\ \omega-6 \text{ or } 18:3\ \omega-3) \rightarrow (18:3\ \omega-6 \text{ or } 18:4\ \omega-3) : O > S > G > C$$
$$\Delta 5\ (20:3\ \omega-6) \rightarrow (20:4\ \omega-6) : O > G > S > C$$
$$\Delta 9\ (16:0 \text{ or } 18:0) \rightarrow (16:1 \text{ or } 18:1) :\ O > C > S = G$$

Calcium levels

The Ca^{2+} levels were higher in the mitochondrial and post-mitochondrial subfractions isolated from the livers of rats fed diets rich in PUFAs (groups S and G) compared with those fed the O or C diet. The plasma levels were different in each diet; however, the differences were not statistically significant (Fig. 5).

Discussion

We studied the relation between the lipid composition of the diets and the behavior of the main hepatic enzymes involved in FA metabolism and their impact on the oxidative stress status.

The proportion of the different FAs in the diets was reflected in the general distribution of FAs in the liver lipid subclasses. The C supplementation provided a diet rich in SFAs with a low level of linoleic acid and an absence of α-linolenic acid. Because of the accepted link between the excessive consumption of saturated fats and coronary disease, several investigators have suggested that coconut oil should not be used on a regular basis by adults [24]. The grape seed and soybean oils showed large proportions of linoleic acid accompanied by a considerable amount of α-linolenic acid in the soybean oil. These two classes of essential FAs are metabolically and functionally distinct, and they often have important opposing physiologic functions. The balance of essential FAs is crucial for good health and normal development [25]. In the G diet, the PUFAs are provided mostly by linoleic acid through its conversion into AA. The eicosanoids from AA (specifically prostaglandins, thromboxanes, leukotrienes, hydroxyl Fas, and lipoxins) are biologically active in very small amounts, but, if formed in large amounts, can contribute to thrombus and atheroma formation, allergic and inflammatory disorders, and cell proliferation. Consequently, supplementation of the diet with grape seed oil could change the physiologic condition to a prothrombotic, proconstrictive, and proinflammatory state [25]. For the S diet, the presence of α-linolenic acid may result in a more favorable cardiovascular risk profile [26]. However, we have to take in account another important factor of the diets, which is their antioxidant content [27].

Lipids have been closely associated with the intracellular generation of ROS and RNS [11], which are recognized by their dual role, because they can be harmful or beneficial to living systems [28]. The beneficial effects of ROS occur at low to moderate concentrations and involve physiologic roles in the cellular response to noxa, for example, in the defense against infectious agents and in some cellular signaling systems. In contrast, the overproduction of ROS and/or RNS can lead to

Table 1
Biomarkers of oxidative damage in plasma and liver homogenates from rats fed oil-supplemented diets

Biomarkers	Plasma				Liver			
	S	O	C	G	S	O	C	G
[NOx] (pmol/mg protein)	5.6 ± 0.1^{a}	2.1 ± 0.1^{b}	2.3 ± 0.2^{b}	7.7 ± 0.2^{c}	155.0 ± 11^{a}	101.0 ± 9^{b}	96.0 ± 7^{b}	166.0 ± 14^{c}
ROOH (pmol/mg protein)	68.0 ± 4^{a}	33.0 ± 7^{b}	31.0 ± 5^{b}	83.0 ± 10^{a}	161.0 ± 5^{a}	122.0 ± 7^{b}	114.0 ± 8^{b}	185.0 ± 11^{c}
TBARS (pmol/mg protein)	28.0 ± 5^{a}	16.0 ± 2^{b}	12.0 ± 4^{b}	31.0 ± 6^{a}	88.0 ± 6^{a}	43.0 ± 7^{b}	36.0 ± 4^{c}	99.0 ± 8^{a}
(α + β)Toc (nmol/mg protein)	0.9 ± 0.04^{a}	1.7 ± 0.05^{b}	1.3 ± 0.1^{c}	0.8 ± 0.02^{a}	3.2 ± 0.1^{a}	5.1 ± 0.04^{b}	3.8 ± 0.02^{c}	2.7 ± 0.05^{d}
GSH (nmol/mg protein)	794.0 ± 29^{a}	919.0 ± 17^{b}	885.0 ± 23^{b}	705.0 ± 41^{a}	1022.0 ± 28^{a}	1461.0 ± 33^{b}	1393.0 ± 41^{b}	972.0 ± 19^{c}
GSSG (nmol/mg protein)	77.0 ± 5^{a}	58.0 ± 3^{b}	67.0 ± 5^{c}	96.0 ± 6^{d}	81.0 ± 3^{a}	53.0 ± 4^{b}	60.0 ± 7^{c}	104.0 ± 8^{d}
GSH/GSSG	10.3 ± 0.1^{a}	15.8 ± 1.7^{b}	13.2 ± 1.9^{b}	7.3 ± 0.1^{a}	12.6 ± 0.3^{a}	27.6 ± 1.2^{b}	23.2 ± 1.3^{b}	9.3 ± 0.1^{c}
FRAP*	553.0 ± 22^{a}	754.0 ± 13^{b}	706.0 ± 25^{b}	537.0 ± 14^{a}	227.0 ± 8^{a}	362.0 ± 16^{b}	384.0 ± 14^{b}	211.0 ± 9^{a}

(α + β)Toc, α- + β-tocopherol; C, coconut oil; FRAP, ferric-reducing ability in plasma; G, grape seed oil; GSH, reduced glutathione; GSSG, oxidized glutathione; [NOx], nitrite plus nitrate; O, olive oil; ROOH, lipid hydroperoxide; S, soybean oil; TBARS, thiobarbituric acid-reactive substances
Assays were performed as described in MATERIALS AND METHODS. Results are presented as mean ± SD ($n = 6$). Different superscript letters within each group of samples indicate significant differences ($P < 0.01$)
* Expressed as micromoles per liter of an equivalent concentration of Trolox (plasma) or micromoles of Trolox per milligram of protein (liver).

harmful biological effects in many tissues. These occur when there is an inefficient neutralizing action of the antioxidant enzymes and/or water- or lipid-soluble antioxidant deficiencies (leading to oxidative and/or nitrative stress) [29]. Therefore, the precise balance between the beneficial and harmful effects of free radicals is a crucial aspect in living systems [29].

The special FA compositions of the commercial oils (and the antioxidant properties of their non-saponifiable fractions) were reflected in the values of several biomarkers of oxidative damage in the plasma and liver homogenates. The rats fed the S or G diets showed the highest values of oxidative biomarkers in the plasma and liver. Specifically, the S and G groups showed increased values of [NOx], lipid hydroperoxides, and GSSG concentrations. In these diets, the content of docosahexaenoic acid in the TLs from the liver was increased. Previous experimental evidence has shown that this acid is extremely important in the central nervous physiology and that they are very susceptible to lipid peroxidation even in the presence of dietary antioxidants [30].

Concomitantly, the concentrations of antioxidants α- + β-tocopherol and GSH and the GSH/GSSG ratio showed the lowest values in the S and G groups, whereas the ferric-reducing activity of plasma was lower in the S and G groups compared with the O and C groups.

The supplementation of diets with soybean and grape seed oils (rich in PUFAs) increased the antioxidant enzyme activities in the plasma and liver homogenates. SOD is one of the key enzymes for controlling excessive superoxide production in the subcellular compartments (mitochondrial matrix and cytosol) through the activities of their specific isoforms (Table 2). Previous evidence has shown that SOD [21] and catalase [22] are induced by oxidative stress and by increased nitric oxide levels [31]. In addition, glutathione peroxidase, glutathione transferase, and glutathione reductase increased in agreement with the decrease observed for the GSH/GSSG ratio. All these effects were more pronounced in the G than in the S group, probably due to the high concentration of ω-3 FAs in soybean oil, which can suppress, at least in part, the formation of lipid peroxidation products derived from AA oxidation [32,33]. In contrast, we observed that rats fed olive oil exhibited the lowest values of several biomarkers of oxidative damage (in plasma and liver homogenates) and in antioxidant enzyme activities (in liver homogenates) and the highest values of α- + β-tocopherol, GSH, and the GSH/GSSG ratio. Because SFAs and monounsaturated FAs are not substrates for lipid radical propagation, whereas PUFAs are effective generators of ROS, a decrease in unsaturated fat content would explain those effects. Epidemiologic studies have largely suggested that the consumption of high amounts of saturated fats and cholesterol-rich diets produce undesirable effects rather than benefits [34]. In contrast, the inclusion of olive oil in the diet is significantly associated to a decrease in cardiovascular risk and many other benefic effects. This association has been attributed not only to the FA composition of this oil [35] but also to the presence of polyphenolic compounds that exhibit strong antioxidant properties [36,37]. In agreement with this notion, our results showed a marked improvement of the antioxidant status in the O group. Thus, regular consumption of olive oil may provide a constant supply of natural antioxidants that decrease reduce oxidative stress through the inhibition of lipid peroxidation, a condition that is currently linked to scavenging free radicals [38] and disease prevention [39]. Vitamin E is the most important lipid-soluble antioxidant present in oils. However, the total amount of this vitamin that was taken daily by the rats is too low to be significant from a physiologic point of view (most probably, the amount required to stabilize the oils). We believe that the content of vitamin E and the composition of fatty-acyl chains in olive oil are not responsible for the protective effect observed for this oil. Probably, the beneficial effect may be due to the presence of many polyphenolic compounds [36,37].

We found that oxidative damage per se and the high values of [NOx] observed when the diet was supplemented with soybean or grape seed oil correlated with the simultaneous increase of Ca^{2+} (Fig. 6). This finding may be produced indirectly through

Table 2
Antioxidant enzyme activities in liver homogenates from rats fed different experimental diets

Enzymes	Diets			
	S	O	C	G
SOD (U/mg protein)				
Total	29.2 ± 0.6^{a}	20.2 ± 0.5^{b}	22.8 ± 0.8^{b}	35.6 ± 0.8^{c}
Mitochondrial (Mn-SOD)	5.9 ± 0.1^{a}	3.2 ± 0.1^{b}	2.9 ± 0.3^{b}	7.1 ± 0.3^{a}
Cytosolic (Cu,Zn-SOD)	23.3 ± 0.3^{a}	17.0 ± 0.5^{b}	20.1 ± 0.7^{b}	28.5 ± 0.7^{c}
CAT (k/mg protein)	1.9 ± 0.1^{a}	0.4 ± 0.1^{b}	0.5 ± 0.1^{b}	2.6 ± 0.2^{c}
GPx (U/mg protein)	6.0 ± 0.2^{a}	3.2 ± 0.1^{b}	2.2 ± 0.3^{c}	7.1 ± 0.2^{d}
GTr (U/mg protein)	15.2 ± 0.2^{a}	10.1 ± 0.2^{b}	11.1 ± 0.4^{b}	18.5 ± 0.3^{c}
GRd (U/mg protein)	1.2 ± 0.03^{a}	0.3 ± 0.01^{b}	0.3 ± 0.03^{b}	2.2 ± 0.05^{c}

C, coconut oil; CAT, catalase; G, grape seed oil; GPx, glutathione peroxidase; GRd, glutathione reductase; GTr glutathione transferase; O, olive oil; S, soybean oil; SOD, superoxide dismutase; k, velocity constant for CAT activity measurement; U, enzyme units
Enzyme activities were determined as described in MATERIALS AND METHODS. Values are presented as mean ± 1 SD ($n = 6$). Different superscript letters within each group of samples indicate significant differences ($P < 0.01$)

Table 3
Enzyme activities of lipid metabolism in microsomal, mitochondrial, and soluble fractions from rats fed experimental diets

Specific enzyme activities	Diets			
	S	O	C	G
PL-A2 (dpm · mg^{-1} protein)				
Soluble	551 ± 31[a]	258 ± 20[b]	302 ± 39[c]	693 ± 49[d]
Particulate	4919 ± 67[a]	3389 ± 101[b]	3001 ± 88[c]	5012 ± 97[d]
CPT (nmol · min^{-1} · mg^{-1} protein)	3.01 ± 0.12[a]	4.61 ± 0.09[b]	4.54 ± 0.08[d]	2.15 ± 0.14[d]
Fatty acid β-oxidation rate (nmol acetate · min^{-1} · mg^{-1} protein)	2.11 ± 0.10[a]	3.87 ± 0.10[b]	3.91 ± 0.05[b]	2.13 ± 0.14[a]
ACS synthetase (nmol · min^{-1} · mg^{-1} protein)	110 ± 18[a]	177 ± 13[b]	159 ± 15[c]	98 ± 7[a]
Fatty acid synthetase (ODU · min^{-1} · mg^{-1} protein)	0.29 ± 0.01[a]	0.41 ± 0.05[b]	0.38 ± 0.03[b]	0.26 ± 0.02[a]
Fatty acid desaturases (nmol · min^{-1} · mg^{-1} protein)				
Δ9 (16:0 → 16:1)	39.5 ± 4.2[a]	111.2 ± 21.3[b]	83.5 ± 5.1[c]	37.7 ± 2.2[a]
Δ9 (18:0 →18:1)	26.4 ± 2.5[a]	92.0 ± 17.1[b]	62.6 ± 3.4[c]	30.4 ± 2.9[a]
Δ6 (18:2 ω-6 → 18:3 ω-6)	83.0 ± 4.1[a]	98.6 ± 3.2[b]	56.1 ± 2.7[c]	63.2 ± 4.1[d]
Δ6 (18:3 ω-3 → 18:4 ω-3)	44.5 ± 3.1[a]	71.2 ± 4.4[b]	30.3 ± 2.8[c]	36.7 ± 3.0[d]
Δ5 (20:3 ω-6 → 20:4 ω-6)	156.1 ± 12.2[a]	229.8 ± 14.3[b]	116.7 ± 11.1[c]	214.4 ± 13.6[b]

ACS, acyl-coenzyme A synthetase; C, coconut oil; CPT, carnitine-palmitoyl-transferase; G, grape seed oil; O, olive oil; ODU, optical density unit; PL-A2, phospholipase A2; S, soybean oil; dpm, disintegrations per minute
Enzyme activities were determined as described in MATERIALS AND METHODS. Values are presented as mean ± 1 SD ($n = 6$). Different superscript letters within each group of samples indicate significant differences ($P < 0.01$)

alterations in superoxide and/or peroxynitrite concentrations, as suggested previously [40,41]. Moreover, the key enzymes of lipid metabolism were modified in a calcium- and [NOx]-dependent way. Those diets that produced oxidative damage and increased Ca^{2+} availability (such as S and G) provoked decreases in the β-oxidation rate, fatty-acyl synthetase, and acyl-coenzyme A synthetase activities with a simultaneous increase in PL-A2 activity. These results are in agreement with our previous work [21], where we studied the mechanism by which changes in nitric oxide production affect biochemical pathways involved in FAs and complex lipid metabolism. We previously reported that the decreased Ca^{2+} concentration led to an inhibition of PL-A2 and, conversely, increased the levels of nitric oxide and Ca^{2+} stimulated by this enzyme [21]. This question is crucially important from a physiologic point of view because the release of PUFAs (especially arachidonate) from phospholipid stores plays a key role in different pathologic conditions associated with inflammation and the modification of local and/or systemic nitric oxide concentrations [42,43].

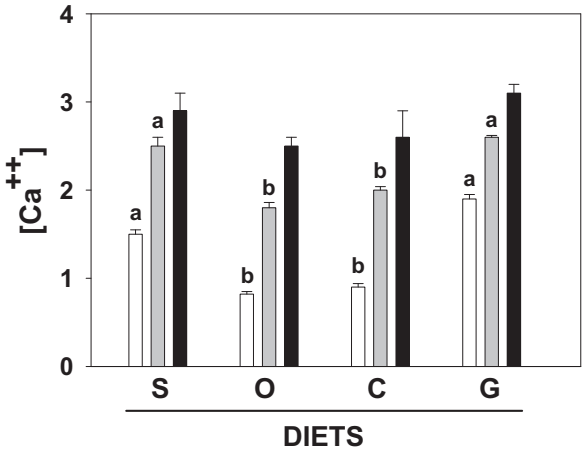

Fig. 5. Calcium levels in postmitochondrial (white bars) and mitochondrial (gray bars) suspensions from liver homogenates and plasma (black bars) from rats fed the experimental diets. Calcium content was analyzed as described in MATERIALS AND METHODS. Results were expressed as nanomoles per milligram of protein (liver) or millimoles per liter (plasma) and represent mean ± 1 SD ($n = 6$). Different letters indicate statistical significance ($P < 0.01$) for the same bar among the four diets (bars with different letters are statistically significant). C, coconut oil; G, grape seed oil; O, olive oil; S, soybean oil.

Concerning FA desaturase activities, the decreased conversion of palmitic or stearic acid to palmitoleic or oleic acid, respectively, in the S and G groups was reflected in the 16:0/16:1 and 18:0/18:1 ratios of TLs and PC fatty-acyl composition. It is well known that PC is the predominant phospholipid in microsomes. The highest values of Δ6 and Δ5 desaturation activity were obtained after supplementation of the diet with olive oil. In accord with similar results observed previously [44,45], we can assume that a high oleic acid content and a proportional decrease in linoleic acid intake would stimulate the expression and/or activities of these enzymes, leading to a greater conversion of linoleic or α-linolenic acid into PUFAs.

In view of these findings, we propose a mechanism by which dietary lipids could modify the lipid FA composition and enzyme activities involved in lipid metabolism (Fig. 7). The different degrees of lipid peroxidation induced by the particular FA composition of each commercial oil (and its non-saponifiable fractions) trigger subsequent alterations in the antioxidant defense system and determine the availability of nitric oxide and peroxide ($O_2^{\cdot -}$), which in turn lead to an increase in the $ONOO^{\cdot -}$ concentration. Oxidative damage per se and the increase of $ONOO^{\cdot -}$ significantly increased Ca^{2+} availability and provoked a decrease in the β-oxidation rate, FA synthetase, and acyl-

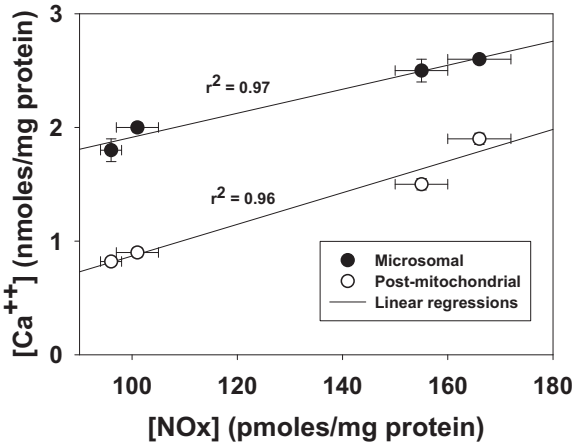

Fig. 6. Linear correlation coefficient (95% confidence interval) between the Ca^{2+} concentration for mitochondrial (black circles) or postmitochondrial (white circles) fractions and the [NOx] concentration in liver homogenates from rats fed the experimental diets. [NOx], nitrite plus nitrate.

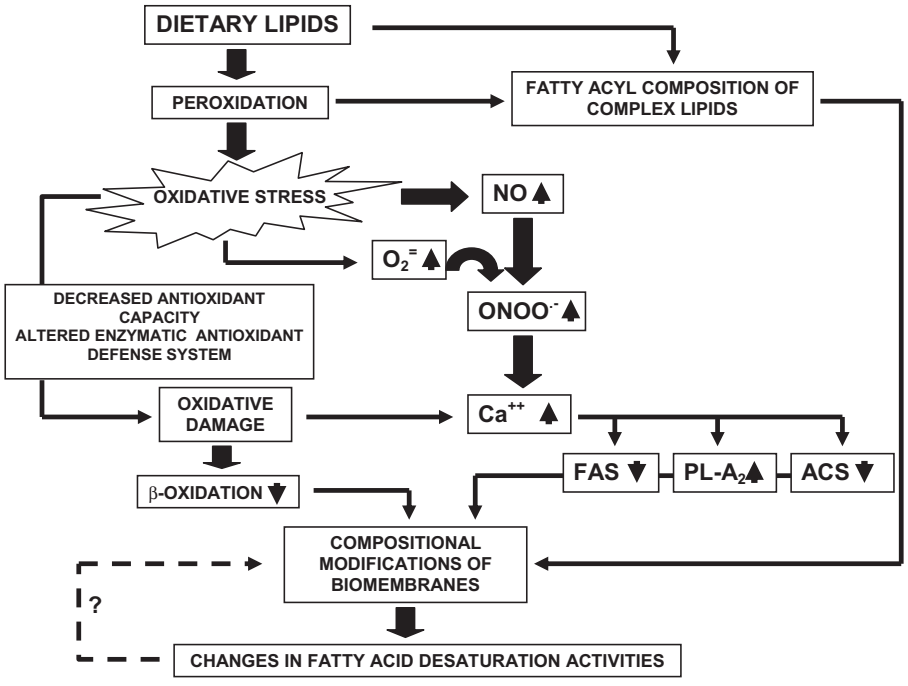

Fig. 7. General scheme showing the main changes evoked by dietary lipids on the liver fatty acid composition of complex biomembrane lipids, oxidative stress status, antioxidant defense system components, and the relation between NO and Ca^{2+} levels with the activity of key enzymes involved in liver lipid metabolism (FAS, ACS, and fatty acid desaturases) and β-oxidation. ACS, acyl-coenzyme A synthetase; FAS, fatty acid synthetase; NO, nitric oxide; $O_2^{=}$, peroxide; ONOO, peroxynitrite; PL-A_2, phospholipase A2.

coenzyme A synthetase activities with a simultaneous stimulation of PL-A2 activity. The direct and indirect influences of these effects were reflected in the compositional changes of complex biomembrane lipids and ultimately in the FA desaturation rates. However, whether these changes in desaturation activities were the final consequence or the partial cause of this mechanism of action remains to be elucidated.

From a physiologic point of view, the most beneficial effects were observed with olive oil. However, considering the low levels of essential FAs provided by this oil, an appropriate mixture of olive and soybean oils could be a healthy recommendation. Further studies dealing with different proportions of oils are needed to assess accurately their potential beneficial effect(s) on human health.

Conclusions

Dietary oils significantly modify the lipid composition, antioxidant status, and enzyme activity of liver lipid metabolism. These effects would be produced through an interrelation between Ca^{2+} and [NOx] (Fig. 7).

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