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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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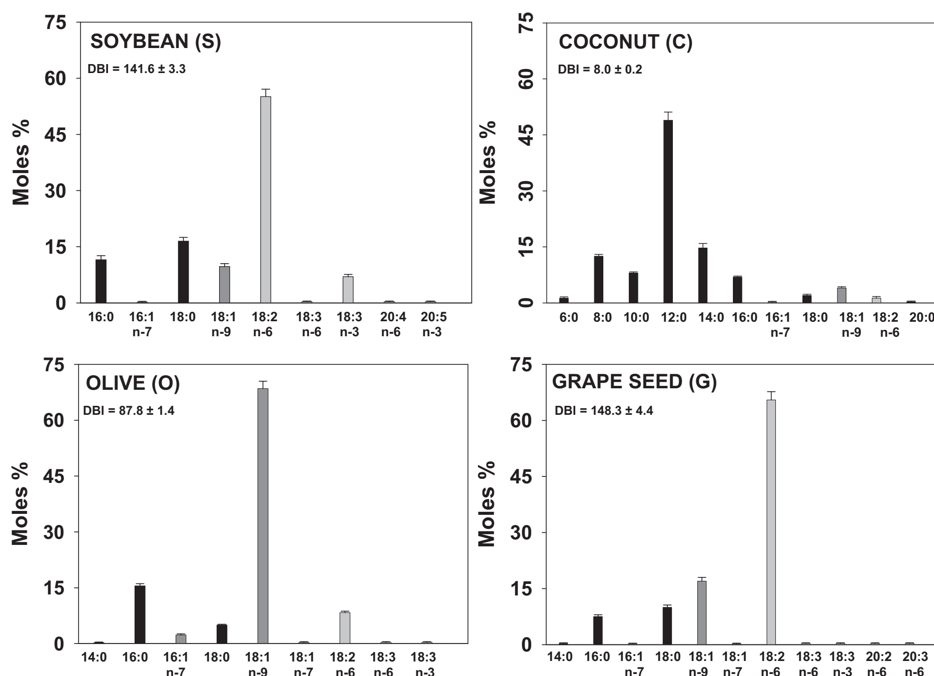

Fig. 1. Fatty acid composition of the diets. Capillary gas–liquid chromatography of fatty acid methyl esters was performed as described in MATERIALS AND METHODS. Results were expressed as mole percentage (mean ± 1 SD; $n = 6$). Saturated, monounsaturated, and di-/polyunsaturated fatty acids are represented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

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