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Basic nutritional investigation

Effect of dietary argan oil on fatty acid composition, proliferation, and phospholipase D activity of rat thymocytes

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Abstract

Objective: Argan oil is receiving increasing attention due to its potential health benefits in the prevention of cardiovascular risk, but no information to date is available about its possible effect on immune cells and functions.

Methods: To address this issue male rats were fed one of five diets that contained fish oil, argan oil, olive oil, coconut oil, or sunflower oil for 4 wk. The fatty acid composition of plasma and thymocyte lipids was then analyzed in relation to the mitogen-induced proliferation and phospholipase D (PLD) activity of thymocytes.

Results: The 18:2ω-6 proportion in thymocyte phospholipids from rats fed argan oil was significantly lower than that observed in phospholipids from rats fed sunflower oil and fish oil but higher than that found in the olive oil and coconut oil groups. Further, a significant positive linear relation was found between thymocyte proliferation and the 18:2ω-6 proportion in thymocyte phospholipids, whatever the diet. The proliferation response of thymocytes to mitogenic activation was also inversely correlated to PLD activity measured in intact thymocytes. Subsequent western blotting experiments indicated that the diet-induced variations in PLD activity mainly reflected variations in the expression of PLD2 protein.

Conclusions: On the whole, the present study shows that the effects of argan oil on immune cells are very similar to those of olive oil, and that, as a consequence, argan oil can be used as a balanced dietary supply without marked adverse effects on immune cell function.

Keywords: Dietary lipids; Oleic and linoleic acids; 18:2ω-6 proportion and thymocyte proliferation relation; Phospholipase D activity and thymocyte proliferation relation

Introduction

Argan oil obtained from the argan fruit of *Argania spinosa* represents about 25% of dietary fats consumed by the southwestern Moroccan population. It is characterized by its unique fatty acid composition [1,2]. This oil is particularly rich in oleic (45% to 48%) and linoleic (32% to 35%) acids and quite devoid of polyunsaturated fatty acids of the ω-3 family. It is currently used in traditional medicine for its cosmetic, bactericide, and fungicide properties. Several recent dietary studies have pointed out hypolipidemic, hypocholesterolemic, and antihypertensive effects in the rat [3,4]. Further, a clinical study conducted with southwest Moroccan subjects showed that a mean daily intake of 15 g of argan oil significantly decreases low-density lipoprotein cholesterol compared with non-consumers [5]. Dietary lipids may influence several aspects of lymphocyte function such as lymphocyte proliferation, lymphocyte-derived cytokine production, and cell-mediated immunity [6,7]. To date, the potential effect of argan oil consumption on lym-

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phocyte function has never been reported. Thus, we investigated whether feeding rats a diet enriched with argan oil would influence thymocyte proliferation. Because oleic and linoleic acids are the two main unsaturated fatty acids of argan oil, we also investigated the effect of olive and sunflower oils. Coconut oil, which is rich in saturated fatty acids, and sardine oil were used as sources of saturated and ω-3 polyunsaturated fatty acids, respectively, for comparison.

Dietary lipids modify the fatty acid composition of cell membrane and, more specifically, the composition of lymphocyte rafts, which are involved in the formation of an immunologic synapse [7,8]. These alterations may in turn influence a large variety of signaling pathways initiated by mitogenic activation. We previously reported that incorporation of docosahexaenoic acid (22:6ω-3) or 12-hydroxyeicosatetraenoic acid, in phospholipids of human lymphocytes markedly stimulates their phospholipase D (PLD) activity [9,10], and that this was accompanied by an inhibition of their proliferative response to mitogens [10,11]. PLD, which hydrolyzes the distal phosphodiester bound of phosphatidylcholine, has been described as a key regulator of cell proliferation in several cell models [12,13]. Two mammalian PLDs have been cloned from various human and murine sources. They are differently regulated by small G-proteins and protein kinase-C, but the activity of both enzymes is highly dependent on phosphatidylinositol bisphosphate [13]. Whereas PLD activation seems to favor cell proliferation in most cell types, this is not the case in human lymphocytes, where we observed opposite effects [9,10]. In addition, activation of a phosphatidylcholine-specific PLD has been shown to inhibit the proliferation of B lymphocytes [14]. Thus, in addition to their effects on thymocyte proliferation, we also investigated whether the different diets could influence PLD activity of rat thymocytes

Materials and methods

Animals and diets

All experimental procedures complied with the *Guide for the Care and Use of Laboratory Animals*. Male Sprague-Dawley rats weighing 140 to 200 g (mean 160.2 ± 2.7, n = 70) were housed individually at 22°C, with a 12-h light/dark cycle. They had free access to food and water. After 1 wk of acclimatization, animals were randomly assigned to one of five groups that differed only in the type of oil added to the fat-free semipurified diet (Usine d'Alimentation Rationnelle, Villemoisson-sur-Orge, France). Because fish and coconut oils must be supplemented with a minimal amount of linoleic acid to prevent essential fatty acid deficiency, 2 g of sunflower oil per 100 of g diet was added to each diet to standardize preparations. All diets were isoenergetic and provided the same amount of fat (10 g of oil/100 g of diet.).

Table 1
Composition of experimental diets*

Ingredient (g/100 g diet)	FO diet	AO diet	OO diet	CO diet	SO diet
Delipidated casein†	20.25	20.25	20.25	20.25	20.25
Cornstarch + glucose	57.0	57.0	57.0	57.0	57.0
Cellulose	5.50	5.50	5.50	5.50	5.50
Mineral mix‡	6.25	6.25	6.25	6.25	6.25
Vitamin mix§	1.0	1.0	1.0	1.0	1.0
FO	8	0	0	0	0
AO	0	8	0	0	0
OO	0	0	8	0	0
CO	0	0	0	8	0
SO	2	2	2	2	10

AO, argan oil; CO, coconut oil; FO, fish oil; OO, olive oil; SO, sunflower oil

* Diets were isoenergetic and provided 14.6 MJ/kg of diet.

† Supplied the following amino acids (mg/g diet): arginine, 8.5; cystine, 3; lysine, 17.4; methionine, 7.1; tryptophane, 5.0; glycine, 1.0.

‡ Provided (g/kg diet): $CaHPO_4$ $2H_2O$, 30; KCl, 7; MgO, 3; $MgSO_4$ $7H_2O$, 3.5; Fe_2O_3, 0.2; $FeSO_4$ $7H_2O$, 0.35; $MnSO_4$ H_2O, 0.17; $CuSO_4$ $5H_2O$, 0.03; $ZnSO_4$ $7H_2O$, 0.14; $CoSO_4$ $7H_2O$, 0.0003; stabilized KI, 0.0006.

§ Provided (mg/kg diet): retinyl acetate, 6.93; cholecalciferol, 0.0025; thiamine HCl, 20; riboflavin, 15; *dl*-calcium pantothenate, 70; pyridoxine HCl, 10; meso-inositol, 150; cyanocobalamin, 0.05; all-*rac*-α-tocopheryl acetate, 170; menadione sodium bisulfite, 40; nicotinic acid, 100; choline, 1360; folic acid, 5; *p*-aminobenzoic acid, 50; biotin, 0.3.

The various oil combinations used are presented in Table 1. Concentrate of sardine oil (Radi Holding, Casablanca, Morocco) constituted the ω-3 fatty acid diet (FO diet). Argan oil (Targanine Association, Tamanar, Morocco) constituted the ω-6/ω-9 diet (AO diet). Olive oil (local oil factory, Fes, Morocco) served as the ω-9 diet (OO diet). Coconut oil (Coopération pharmaceutique française, Rhône-Poulenc Rorer, Lyon, France) was used as the saturated fat diet (CO diet). Commercial edible sunflower oil was used as a source of ω-6 fatty acids (SO diet). The diets were thoroughly mixed after the addition of oil combinations and daily amounts were transferred to capped plastic tubes, flushed with nitrogen, and stored at −20°C in the dark. Under these conditions, no oxidation of dietary fat was noticed, as indicated by the absence of thiobarbituric acid-reactive materials. Fresh diets were prepared every 3 d and were fed to the rats daily after uneaten food was discarded. The fatty acid composition of the different diets is presented in Table 2.

Tissue preparation

After 4 wk, rats were anesthetized by diethyl ether and decapitated. Blood was collected immediately on heparin (50 U/mL), and 50 μM butylated hydroxytoluene was added to recovered plasma before freezing at −80°C until further analysis. Thymus glands were removed immediately and thymic lymphocytes were separated on a density gradient as previously described [15]. After washing, cells were suspended at a concentration of 4×10^7cells/mL and incubated for 60 min at 37°C before starting the experiments.