ELSEVIER

Nutrition 24 (2008) 717–726

NUTRITION

www.elsevier.com/locate/nut

Basic nutritional investigation

Dietary lipids modify redox homeostasis and steroidogenic status in rat testis

Graciela E. Hurtado de Catalfo, Ph.D., María J. T. de Alaniz, Ph.D., and Carlos A. Marra, Ph.D.*

Instituto de Investigaciones Bioquímicas de La Plata, CONICET-UNLP, Cátedra de Bioquímica, Facultad de Ciencias Médicas, Universidad Nacional de La Plata, La Plata, Argentina

Manuscript received October 2, 2007; accepted March 7, 2008.

Abstract

Objective: The present study explored the effect of dietary oils on lipid composition, antioxidant status, and the activity of the main steroidogenic enzymes in the testis.

Methods: Forty Wistar rats were randomly assigned to one of four groups ($n = 10$) fed for 60 d on the same basal diet plus different lipid sources as commercial oils: soybean, olive, coconut, or grapeseed. After sacrifice, testicular lipids and fatty acid composition, free radical biomarkers, antioxidant levels, hormones, and steroidogenic enzymes were determined.

Results: The lipid composition of diets produced significant changes in neutral/phospholipids, free/esterified cholesterol, and plasmalogen proportion. Fatty acid patterns of these lipids were also strongly modified, influencing the double bond index. We also found a close correlation between the type of diet and the generation of free radicals. The oxidative stress in testes was higher with the grapeseed oil–supplemented diet and decreased with the other diets in this order: soybean oil > olive oil > coconut oil. Animals fed with the olive oil and coconut oil diets showed the highest testicular levels of antioxidants in addition to significantly high levels of testosterone and 3β- or 17β-hydroxysteroid dehydrogenase enzymes.

Conclusion: Different oils in the diets strongly modified the homeostasis of the testicular antioxidant defense system and, in consequence, affected steroidogenic function, showing a clear correlation with the damage induced. According to our results, an appropriate mixture of olive and soybean oils could be a healthy recommendation.

Keywords: Dietary lipids; Oxidative stress; Steroidogenic enzymes; Hormonal levels; Lipid composition; Rat

Introduction

During previous decades great interest was focused on the relation between lipid metabolism and the atherogenic condition as a cause of different vascular-related diseases [1]. However, much less attention was given to the role played by lipid metabolism in other human pathologies, especially those involved in reproductive function. Disorders associated with male infertility have exhibited an increased incidence during the past few years, due to many concomitant factors not fully understood or even recognized [2].

It is well known that testicular lipids strongly influence the histology and physiology of this tissue [3–5]. Essential fatty acid deficiency [6,7] or alterations in fatty acid metabolism in diabetic patients [7] have been associated with testicular malfunction. Moreover, it was previously demonstrated that polyunsaturated fatty acid (PUFA) supplementation to diets produced significant changes in testicular lipid composition, modifying the metabolism of C_{20-22} fatty acids with a strong impact on the physiology of germinal and steroidogenic cells [3,5]. In addition, lipids have been closely associated with intracellular generation of reactive oxygen species [8] as substrates for oxidative attack and a source for free radical generation and/or chain propagator reactions. This “two-face” characteristic of lipids depends on multiple factors; one of them is the quantity and quality of the fatty acyl chains acylated to complex cellular lipids

This study was supported in part by grants from CIC and CONICET, Argentina.

* Corresponding author. Tel.: +54-221-482-4894; fax: +54-221-425-8988.
E-mail address: camarra@atlas.med.unlp.edu.ar (C. A. Marra).

0899-9007/08/$ – see front matter
doi:10.1016/j.nut.2008.03.008

[8–10]. Several reports demonstrated that under oxidative stress conditions testes are especially susceptible to develop damage derived from the redox imbalance [11–17]. This fact is considered one of the most important features in the etiology of human reproductive illnesses of high incidence such as varicocele [2,18].

However, strong differences in the lipid composition of diets across the world have been poorly studied from the point of view of their influence on the relation between free radical generation and testicular steroidogenesis. Thus, the lipid composition of testes (modifiable by the diet), the regulation of reactive oxygen species (and other reactive species), and the testicular integrity and function are closely related factors.

In this study we investigated the effect of diet-induced modification of testicular lipids on the generation of reactive oxygen species and the activity of the main steroidogenic enzymes (3β- and 17β-hydroxysteroid dehydrogenases [3β-HSD and 17β-HSD]). Results obtained may contribute to the understanding of the role played by dietary lipid sources on testicular function and its relation to redox homeostasis.

Materials and methods

Chemicals

Solvents (high-performance liquid chromatographic grade) were provided by Carlo Erba (Milano, Italy). Other chemicals were from Sigma Chemical Co. and Fluka Chemie AG (Buenos Aires, Argentina).

Lipids used as standards were from Serdary Research Laboratory (London, ON, Canada) or Nu-Check-Prep (Elysian, MN, USA). Commercial oils added to diets were from Molinos Río de La Plata SAIC and Platafarm SA (La Plata, Argentina).

Diets and animal treatment

Female Wistar rats weighing 180 ± 10 g were bred and maintained on a control diet (Cargill type "C", Rosario, Argentina) throughout gestation and lactation. The dams were housed individually and kept at 22 ± 1°C with a 12-h light/12-h dark cycle. After weaning, 40 male pups (weighing 47 ± 4 g/animal) were randomized to one of four groups of 10 animals each and fed ad libitum on one of four different diets. Diets were prepared in an identical manner (Table 1), except for the lipid source added as commercial oils: soybean (S), olive (O), coconut (C), or grapeseed (G; 70 g/kg of diet). The final fatty acid composition of each diet is presented in Table 2. Rats were fed according the American Institute of Nutrition [19]. Animal maintenance and handling were as recommended by guidelines from the National Institutes of Health [20]. All procedures were approved by the local laboratory animal committee of Facultad de Ciencias Médicas, UNLP, Argentina.

Table 1
Composition of basal AIN-93 diet

Ingredients	g/kg
Casein, high protein	250.0
Sucrose	100.0
Corn starch	397.4
Cellulose	50.0
Commercial oil	70.0
Mineral mixture*	35.0
Sodium phosphate, monobasic	8.9
Potassium phosphate, monobasic	8.8
Calcium carbonate	12.5
Calcium phosphate-2H_2O	6.3
Tert-butyl hydroquinone	0.014
Vitamin mixture†	10.0
Choline bitartrate	2.5
L-Cystine	3.0

* Contained (grams per kilogram of mixture): NaCl, 184; K_2SO_4, 136; $C_6H_5O_7(K)_3$576; MgO, 63; $MnCO_3$, 9.2; ferric citrate, 17; $ZnCO_3$, 4.2; $CuCO_3$, 1; ammonium molybdate, 0.03; Na_2SeO_3-5H_2O, 0.03; $CrK(SO_4)$-12H_2O, 1.0; KIO_4, 0.05; delipidated casein, 8.09.

† Contained (grams per kilogram of mixture): thiamine hydrochloride, 0.5; riboflavin hydrochloride, 0.5; niacin, 0.5; pyridoxine, 0.5; Ca-pantothenate, 2.6; biotin, 0.12; choline hydrochloride, 50; folic acid, 0.1; nicotinamide, 1.26; *p*-aminobenzoic acid, 0.5; inositol, 50; vitamin B12, 0.006; vitamin A, 0.06; D-calciferol, 0.01; α-tocopherol, 1.5; menadione, 2.5; ascorbate, 6.0; L-methionine, 2.0; delipidated casein, 986.7.

Experimental design

After feeding for 60 d, rats were sacrificed. 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 killed by decapitation 12 h after the refeeding period. Food intake, water consumption, and body weights were individually recorded along the feeding period. Testes were removed, decapsulated, and homogenized in ice-cold buffered solution, pH 7.4, containing 0.25 M sucrose, 62 mM phosphate buffer, 0.15 M KCl, 5 mM $MgCl_2$, and 100 μM ethylene-diaminetetra-acetic acid [21]. Blood was also collected into heparinized tubes. Plasma was removed after centrifugation and stored at −70°C.

Steroidogenic enzyme activities

Testicular homogenates were centrifuged (10 000 × *g*, 15 min, 1–2°C). Aliquots of supernatants were employed for determination of 3β-HSD and 17β-HSD [22].

Lipid analysis

Total lipids were extracted from plasma and testicular homogenates by the procedure of Folch et al. [23]. Analyses of neutral lipids (NLs) and phospholipids (PLs; previously isolated from the total lipid extract by silicic acid microchromatography [24]) were performed using commercial silica gel G-60 thin-layer chromatographic plates (Merck, Darmstadt, Germany) as detailed elsewhere [25]. The total