ELSEVIER

NUTRITION

Nutrition 25 (2009) 828–838

www.nutritionjrnl.com

Basic nutritional investigation

Effect of nutritional vitamin A deficiency on lipid metabolism in the rat heart: Its relation to PPAR gene expression

Verónica Analía Vega, M.D., Ana Cecilia Anzulovich, Ph.D.†, Silvia Mabel Varas, Ph.D., Mirtha Ruth Bonomi, M.D., María Sofía Giménez, Ph.D.†, and Liliana Beatriz Oliveros, Ph.D.*

Department of Biochemistry and Biological Sciences, Faculty of Chemistry, Biochemistry and Pharmacy, National University of San Luis, and IMIBIO-SL (CONICET), San Luis, Argentina

Manuscript received September 29, 2008; accepted January 5, 2009.

Abstract

Objective: We studied the effect of dietary vitamin A deprivation on lipid composition and mRNA expression of regulatory enzymes involved in rat heart energetic lipid metabolism and its relation to the expression of peroxisome proliferator-activated receptor (PPAR) and retinoid X receptor (RXR) genes.

Methods: Male Wistar 21-d-old rats were fed for 3 mo with a vitamin A–free diet (vitamin A–deficient group) and the same diet plus 8 mg of retinol palmitate per kilogram of diet (control group). One group of deficient animals received the control diet 15 d before sacrifice (vitamin A–refed group). Heart ventricular and mitochondrial lipid contents were determined. Lipid synthesis was measured using radioactive precursors and acetyl-coenzyme A carboxylase and mitochondrial carnitine palmitoyltransferase-I (CPT-I) activities using radioactive substrates. Fatty acid composition of mitochondrial phospholipids was analyzed by gas–liquid chromatography. Heart expression of acetyl-coenzyme A carboxylase, CPT-I, PPAR-α, PPAR-β, RXR-α, and RXR-β was assessed by reverse transcriptase polymerase chain reaction, and CPT-I expression was also measured by real-time polymerase chain reaction.

Results: Vitamin A deficiency induced changes in heart ventricular lipid content and synthesis. Mitochondrial cardiolipin decreased and the proportion of phospholipids/saturated fatty acids increased. Heart activity and mRNA levels of CPT-I and expression of PPAR-α and PPAR-β genes were enhanced, whereas acetyl-coenzyme A carboxylase activity diminished. Furthermore, vitamin A deficiency decreased heart mRNA levels of RXRs. Vitamin A refeeding reverted most of the observed changes.

Conclusion: Lipid metabolism is significantly modified in hearts of vitamin A–deficient rats. Alteration of mitochondrial energetic processes by modifying the activity and gene expressions of the regulatory enzymes is associated with a high PPAR expression induced by vitamin A deprivation. © 2009 Published by Elsevier Inc.

Keywords: Vitamin A; Fatty acid oxidation; Peroxisome proliferator-activated receptors; Carnitine palmitoyltransferase; Retinoic X receptors; Acetyl-coenzyme A carboxylase; Mitochondria

Introduction

Vitamin A plays a vital role in the development and homeostasis of almost every vertebrate tissue by regulating embryogenesis, cell differentiation, proliferation, metabolism, and apoptosis [1,2]. Vitamin A (retinol) is obtained in the diet in the form of retinyl esters or through the ingestion of β-carotene, which is converted to two molecules of retinol. The carboxylic acid form of vitamin A (all-*trans*-retinoic acid) has important effects on the development of the cardiovascular system [2].

Epidemiologic evidence has suggested that vitamin A is an important dietary factor for decreasing the incidence of heart disease [3]. It has been shown that even moderate maternal vitamin A deficiency in the rat changes the expression of key

This work was supported by the National Investigations Council of Science and Technology and the Secretary of Science and Technology of San Luis University.

* Corresponding author. Tel.: +54-2652-424689; fax: +54-2652-430224.

E-mail address: lolive@unsl.edu.ar (L. B. Oliveros).

† Members of the National Investigations Council of Science and Technology (CONICET), Argentina.

0899-9007/09/$ – see front matter © 2009 Published by Elsevier Inc.
doi:10.1016/j.nut.2009.01.008

developmental genes in the fetal heart and affects perinatal organ growth and development [4]. In addition, vitamin A deficiency increases resting heart rate [5] and induces a rapid loss of mitochondrial membrane potential [6]. We previously reported that vitamin A deficiency modifies antioxidant defenses and induces lipid peroxidation in the heart of adult rats [7] and alters the aorta lipid metabolism [8].

The molecular mechanism of retinoic acid action mainly involves the binding and activation of specific nuclear receptors, retinoid X receptors (RXR-α, RXR-β, and RXR-γ) and retinoic acid receptors (RAR-α, RAR-β, and RAR-γ) that modulate gene expression [9]. During vitamin A deficiency a decreased signaling through the RAR/RXR complex leading to decreased RAR-RXR/retinoic acid response element–directed transcription has been observed [10]. However, RXRs heterodimerize with several other receptors such as peroxisome proliferator-activated receptor (PPAR)-α, PPAR-β/δ, and PPAR-γ, which mediate the expression of several genes involved in the regulation of heart lipid metabolism [11].

The high-energy demand of the heart is primarily met by the β-oxidation of fatty acids in the mitochondria. Definitive evidence for PPAR-α as a key regulator of cardiac energy metabolism has been provided by the PPAR-α "knockout" (PPAR-$\alpha^{-/-}$) mouse studies [12]. Also, the PPAR-β–selective ligands have been shown to induce expression of mitochondrial fatty acid oxidation enzymes in neonatal and adult cardiac myocytes as effectively as PPAR-α–selective ligands [13]. We previously reported that vitamin A deficiency increases the activity of liver carnitine palmitoyltransferase-I (CPT-I) [14]. This enzyme catalyzes the initial reaction in the mitochondrial import of long-chain fatty acids/coenzyme A (CoA). Its muscle-type (M-CPT-I or CPT-Iβ) is highly expressed in the heart and skeletal muscle and seems to be inhibited by malonyl-CoA [15], whose levels around heart mitochondria are controlled by acetyl-CoA carboxylase-β (ACC-β or ACC-2). Two promotors, P-I and P-II, control the transcription of the AAC-β gene. The P-I promoter has been found to be activated by RXRs and regulatory factors in a synergistic manner [16].

Considering that vitamin A deficiency is a major problem in the developing world and that vitamin A seems to be an important dietary factor for decreasing the incidence of cardiovascular disease, we examined, in vivo, the effects of nutritional vitamin A deficiency on the lipid composition and mRNA expression of regulatory enzymes involved in the energetic lipid metabolism of the rat heart and its relations to the expression of PPAR and RXR genes. In addition, the effect of vitamin A restitution to the diet of vitamin A–deficient rats on heart lipid metabolism was analyzed.

Materials and methods

Chemicals

We purchased [^{14}C]-$NaHCO_3^-$ (266.4 MBq/mmol), acetic acid, sodium salt [1-^{14}C] (2.00 mCi/mmol), choline chloride [methyl-^{14}C]choline (54.00 mCi/mmol), and [*N*-Me-^{14}C]L-carnitine from Dupont New England Company (Boston, MA, USA). Retinyl-palmitate, all-*trans*-retinol, and lipid standards were acquired from Sigma Chemical Co. (St. Louis, MO, USA).

Diet and experimental design

Male Wistar rats were bred in our animal facilities (National University of San Luis, San Luis, Argentina) and maintained in a 21–23°C controlled environment with a 12-h light/12-h dark cycle. They were weaned at 21 d of age and immediately assigned randomly to the experimental diet, devoid of vitamin A (vitamin A–deficient group), or the same diet with 4000 IU of vitamin A (8 mg of retinol as retinyl palmitate) per kilogram of diet (control group). Feeding the animals with a vitamin A–free diet for 3 mo guarantees a subclinical plasma retinol concentration and depletes retinol stores in the liver [7]. At the end of the third month of treatment, half of the vitamin A–deficient rats were fed the complete diet for 15 d to induce repletion of vitamin A (vitamin A–refed group). Rats were given free access to food and water throughout the entire 3 mo of the experimental period. All experiments were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23) and the experimental protocol was approved by the committee for the use of experimental animal of the National University of San Luis. Diets were prepared according to AIN-93 for laboratory rodents [17]. Vitamin A–deficient and control diets had the following composition (grams per kilogram): 397.5 cornstarch, 100 sucrose, 132 dextrinized cornstarch, 200 vitamin-free casein, 70 soybean oil, 50 cellulose fiber, 35 AIN-93 mineral mix, 10 AIN-93 vitamin mix (devoid of vitamin A for the vitamin A–deficient diet), 3 L-cystine, 2.5 choline bitartrate, and 0.014 *tert*-butyl hydroquinone.

Plasma and tissue retinol analyses

Rats were fasted overnight before sacrifice. Rats were killed by cervical dislocation at 09:00 h. Blood samples were collected in tubes coated with ethylene-diaminetetra-acetic acid. The liver and heart were quickly excised, washed several times with ice-cold isotonic saline, and blotted on paper to remove excess blood. The hearts were trimmed from the surrounding tissue and atria on an ice-chilled plate and immediately placed in liquid nitrogen. Analyses were carried out within 1–3 wk of obtaining the samples. Plasma and tissue retinol concentrations were determined by high-performance liquid chromatography as described previously [7]. As an internal standard, retinyl acetate was used. Chromatography was performed on a Nucleosil 125 C-18 high-performance liquid chromatographic column with methanol:water (95:5, v/v) as the mobile phase. Retinol was detected by ultraviolet absorbance at 325 nm (model 440, Waters Associates) and peak

Download English Version:

https://daneshyari.com/en/article/3277263

Download Persian Version:

https://daneshyari.com/article/3277263

Daneshyari.com