LWT - Food Science and Technology 59 (2014) 1298–1303

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Short-term dietary magnesium restriction lowers spleen iron concentrations in growing rats fed a high-fat diet

Eduardo De Carli, Alexandre R. Lobo, Cristiane H. Sales, Pryscila D.S. Teixeira, Ana Lina de Carvalho C. Sales, Célia Colli*

Department of Food and Experimental Nutrition, Faculty of Pharmaceutical Sciences, University of São Paulo, São Paulo, Brazil

ARTICLE INFO

Article history:
Received 2 February 2014
Received in revised form
11 April 2014
Accepted 13 April 2014
Available online 25 April 2014

Keywords:
Magnesium
Iron
Fat
Diet
Hepcidin

ABSTRACT

Lipotoxicity and dietary inadequacies have been associated with dysregulation of Fe homeostasis in obesity. In this study, we tested the hypothesis that dietary Mg restriction in rats fed a high-fat diet affects Fe concentrations in liver and spleen, tissues related with Fe storage and recycling, respectively. Weanling male Wistar rats ($n = 48$) were fed either control (*ad libitum* or pair-fed) or high-fat (Mg-adequate or Mg-restricted; 500 and 150 mg Mg/kg, respectively) diets for 4 weeks. Epididymal and retroperitoneal fat pad weights were used to determine the adiposity index. Serum (colorimetry) and tissue Fe (atomic absorption spectrophotometry) were analyzed. Liver hepcidin was determined by qPCR and ferroportin-1 in liver and spleen was measured by immunoblotting. Dietary fat overload increased adiposity irrespective of dietary Mg levels. Rats fed the high-fat diet, but not those fed the high-fat Mg-restricted diet, had higher spleen Fe concentrations than controls. Weak but significant associations between body adiposity and serum ($r = -0.31$, $P = 0.04$) and spleen Fe ($r = 0.29$, $P = 0.04$) concentrations were obtained. The unaltered ferroportin-1 expression with low Fe levels suggests increased Fe recycling from spleen to bone marrow due to dietary Mg restriction. A longer study could clarify the consequences of these findings on erythropoiesis and other conditions related to diet-induced obesity.

1. Introduction

Low physical activity, increased body mass index and nutritional inadequacies are recognizably risk factors associated with the development of chronic diseases such as diabetes, osteoporosis and obesity (Karalis et al., 2009; Tsai, Wen, Chiang, Tsai, & Cheng, 2008). Although matter of some controversies (Simmons, Joshi, & Shaw, 2010), dietary inadequacy as a result of low intake of Mg food sources could be linked to impairment of some pre-disease states, such as pre-diabetes (or impaired fasting glucose), overweight and borderline dyslipidemia (Elin, 2011; Hruby et al., 2013; Lima et al., 2009; Sales, Pedrosa, Lima, Lemos, & Colli, 2011). In rats, Mg deficiency results in weakened insulin signal transduction, which promotes a decrease in glucose uptake and utilization in insulin target tissues (Belin & He, 2007; Sales, dos Santos, Cintra, & Colli, 2013; Suárez et al., 1995). In addition, low Mg intake or hypomagnesemia promote inflammation, oxidative stress and dyslipidemia, all known risk factors for obesity and atherosclerosis (Belin & He, 2007).

For many years, Mg deficiency has also been related to changes in Fe metabolism in experimental rodent models, particularly by affecting the red blood cell (RBC) homeostasis (Kimura & Yokoi, 1996; Piomelli, Jansen, & Dancis, 1973; Schumann, Lebeau, Gresser, Gunther, & Vormann, 1997). Iron decompartmentalization featured by increased tissue Fe levels and RBC fragility and destruction is frequently described after short- or long-term severe dietary Mg restriction. These alterations are followed by increased intestinal Fe absorption due to the greater Fe demand for erythropoiesis (erythroid regulator) (Sanchez-Morito, Planells, Aranda, & Llopis, 2000; Schumann et al., 1997). However, these alterations seem to be dependent on the degree and duration of dietary Mg restriction and the consequent change in the mineral status (Schumann et al., 1997; Vormann, Günther, Höllriegl, & Schumann, 1995).

Abbreviations: RBC, red blood cell; HF, high-fat; AIN, American Institute of Nutrition; CT, control; PF, pair fed (feeding); HAMP, hepcidin; FPN-1, ferroportin-1; BMP, bone morphogenetic protein.

* Corresponding author. Department of Food and Experimental Nutrition, Faculty of Pharmaceutical Sciences, University of São Paulo, Av. Prof. Lineu Prestes, 580, Bloco 14, 05508 900 São Paulo, SP, Brazil. Tel.: +55 11 3091 3651; fax: +55 11 3815 4410.

E-mail addresses: edecarli@usp.br (E. De Carli), arlobo@usp.br (A.R. Lobo), cristianehermes@yahoo.com.br (C.H. Sales), pdsteixeira@usp.br (P.D.S. Teixeira), ana.lina123@gmail.com (A.L.C.C. Sales), cecolli@usp.br, collilaboratorio@gmail.com (C. Colli).

http://dx.doi.org/10.1016/j.lwt.2014.04.026
0023-6438/

Changes in Fe status described in obese individuals are attributed either to dietary Fe inadequacy or more frequently to increased liver hepcidin production in response to low-grade chronic inflammation associated with obesity (Yanoff et al., 2007; Zimmermann et al., 2008). In turn, these changes are reflected in decreases in Fe intestinal absorption and serum Fe levels, and increases in Fe stores in these individuals (Zimmermann et al., 2008). Considering that obese individuals are subject to present dietary micronutrient inadequacies, it is reasonable to assume that the expected changes in their Fe status could be due, in part, to a dietary Mg deficiency. In fact, Mg dietary inadequacy has been reported more often in obese than non-obese individuals from different populations (Cahill et al., 2013; Jarvandi, Gougeon, Bader, & Dasgupta, 2011; Moizé, Deulofeu, Torres, Osaba, & Vidal, 2011). Hence, the aim of this study was to determine the short-term effects of dietary Mg restriction in some parameters of Fe status in growing rats fed a high-fat diet. Our results indicate that the combination of these two nutritional challenges (Mg restriction and fat overload) lowers spleen Fe levels which were increased by HF feeding. These findings suggest that Mg restriction in HF animals may have contributed to Fe recycling to bone marrow to meet a higher demand of iron for erythropoiesis.

2. Material and methods

The experimental protocol was approved by the Commission on the Ethics of Animal Experiments of the Faculty of Pharmaceutical Sciences of the University of São Paulo (CEEA Number: 221/2009 FCF-USP), according to the guidelines of the Brazilian College on Animal Experimentation. All surgery was performed under anesthesia, and all efforts were made to minimize suffering.

2.1. Animals and experimental diets

Weanling male Wistar rats (*Rattus novergicus*, var. *albinus;* $n = 48$; initial body weight ~52 g) were obtained from a colony at the Faculty of Pharmaceutical Sciences of the University of São Paulo. The rats were maintained in individual stainless-steel wire-mesh (to limit coprophagy) metabolic cages under controlled temperature (22 ± 2 °C) and relative humidity (55 ± 10%), with a 12-h dark/light cycle maintained by artificial lighting (lights on from 7 AM to 7 PM).

The semipurified and pelleted AIN-93G-based diets (Reeves, Nielsen, & Fahey Jr., 1993) were purchased from Harlan Teklad Laboratories (Madison, WI, USA). The control (CT) diet (TD.110294) was prepared with 7% lipids (soybean oil; 13% energy from fat). High-fat (HF) diets were prepared with 32% lipids (a mix of soybean and lard; 52% energy from fat). These HF diets were formulated in order to provide 500 (HF[Mg500]; TD.110297) or 150 (HF[Mg150]; TD.110299; 34% of recommendations for rodents [Reeves et al., 1993]) mg Mg/kg diet as Mg oxide (MgO). All diets contained 35 mg Fe/kg diet as ferrous sulphate ($FeSO_4$). The additional amounts of lipids present in the HF diets were added at the expense of starch. The energy values of the diets were 3.7 and 5.1 kcal/g (15.4 and 21.3 kJ/g) in the CT and HF diets, respectively. Chemical composition analyses of the experimental diets have been previously reported (Sales et al., 2013). Dietary Mg concentrations were 496, 475 and 171 mg Mg/kg diet for CT, HF[Mg500] and HF[Mg150] diets, respectively. There were no significant differences in the Fe concentrations among diets (42 mg Fe/kg in the CT diet).

2.2. Experimental design

Initially, animals were fed, during one week, the same CT diet to acclimatize to the environmental conditions. Thereafter they were randomly assigned to CT ($n = 8$) or HF diets (HF[Mg500] or HF[Mg150]; $n = 12$/group) for 4 weeks. The food intake was determined daily and body weight recorded every 2 days. Food and demineralized water were offered *ad libitum*. However, since animals consuming HF diets tend to have a reduction in diet consumption (Hariri & Thibault, 2010), additional two groups received the CT diet in the equivalent amount of diet consumed by their respective HF groups (pair-fed HF[Mg500] and HF[Mg150] groups; PF(HF[Mg500]) and PF(HF[Mg150]), respectively; $n = 8$ rats/group).

At the end of experimental period, the rats were fasted for 12–14 h, weighed and anesthetized through an intraperitoneal route with a 1:2 (v/v) mixture of ketamine (10 mg/kg body weight; Vetaset, Fort Dodge, Iowa, USA) and xylazine (25 mg/kg body weight; Virbaxil 2%, Virbac, Sao Paulo, Brazil). The animals were then euthanized by exsanguinations and the liver, spleen, epididymal and retroperitoneal fat pads were then removed and weighed. The adiposity index was calculated as 100 times the quotient of the sum of the epididymal and retroperitoneal fat pads (g) and the final body weight of the animal (g). Liver and spleen samples were stored at −20 °C for mineral analyses or snap-frozen in liquid nitrogen and subjected to protein and RNA extraction. Blood samples obtained without anticoagulant were collected in demineralized containers for analysis of serum Fe concentrations (Labtest Diagnostic S/A, Lagoa Santa, Minas Gerais, Brazil), according to the manufacturer's instructions.

2.2.1. Tissue Fe analyses

Iron concentrations from the liver left lateral lobe and spleen were analyzed by atomic absorption spectrophotometry (AAnalyst 100, Perkin Elmer, Norwalk, CT, USA) employing a hollow cathode lamp ($\lambda = 283.4$ nm, slit = 0.2 nm) in samples previously digested with a 5:1 (v/v) mixture of HNO_3:H_2O_2. The working standard solutions were prepared with $FeCl_3$ (Titrisol, Merck, Darmstadt, Germany). A certificated reference material (lyophilized bovine liver, SRM1577c, National Institute of Standards & Technology, Gaithersburg, MD, USA) was used to check the Fe recovery (Fe value = 188 ± 14 μg/g, mean ± standard deviation of 6 determinations; certified value 197 ± 6 μg/g). Mineral analyses in the tissues were not corrected for mineral content in the residual blood.

2.2.2. Immunoblotting

Nitrogen-frozen samples of liver and spleen were homogenized (Homogenizer Workcenter, IKA Works, Staufen, Germany) in T-PER™ Tissue Protein Extraction Reagent (Pierce Technology, Rockford, IL, USA) containing proteases and phosphatases inhibitors (Pierce Technology, Rockford, IL, USA). The homogenates were centrifuged at 10,000 *g* for 5 min and the total protein concentration of supernatants was determined using the BCA Assay (Pierce Technology, Rockford, IL, USA). Equal amounts of total protein (50 μg) were denatured by boiling for 5 min in 4× SDS Laemmli buffer, separated by 12.5% SDS-PAGE (Fisher Scientific, Fair Lawn, NJ, USA) and then transferred onto nitrocellulose membranes (Whatman, Boston, MA, USA). The blots were pre-incubated in Blocker™ Casein Buffer (Pierce Technology) for 2 h at room temperature and then incubated with 1:500 (v/v) diluted rabbit polyclonal anti-FPN1 (Abcam Antibodies, Cambridge, MA, USA) for 14 h at 4 °C. After being washed 3 times in 0.1% PBS-T, the blots were incubated with 1:50,000 (v/v) diluted peroxidase-labeled anti-rabbit secondary antibodies (Sigma Chemical Co., St. Louis, MO, USA) and the signals of immunoreactive bands were visualized by an enhanced chemiluminescence kit (GE HealthCare, Little Chalfont, UK). ImageQuant 400 equipment (GE Healthcare, Little Chalfont, UK) and the Quantity One™ Basic software (BioRad Laboratories, Hercules, CA, USA) were used for scanning the photo-images and for densitometry analysis, respectively. Each membrane