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Resveratrol attenuates oxidative stress and prevents steatosis and hypertension in obese rats programmed by early weaning

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Abstract

We hypothesized that resveratrol, a natural phytoalexin found in grapes, can prevent oxidative stress, obesity and its related disturbances in obese rats programmed by early weaning. Lactating Wistar rats were separated into two groups: early weaning (EW) — dams who were wrapped with a bandage to interrupt the lactation in the last 3 days of lactation; control — dams whose pups had free access to milk during all lactation. At the 150th day, EW offspring were randomly subdivided into EW+resveratrol (EW+Res) — resveratrol (30 mg/kg/day); EW+vehicle (EW) — rats that received 0.5% (w/v) aqueous methylcellulose. The control group received vehicle. Rats were treated by gavage daily for 30 days. EW offspring developed hyperphagia, higher body weight, visceral obesity, higher systolic (SBP) and diastolic blood pressure (DBP) (+15% and +20%, respectively; $P<.05$) and higher serum triglycerides (TG) and low-density lipoprotein but lower high-density lipoprotein (+55%, +33% and −13%, respectively; $P<.05$). Resveratrol normalized food intake, SBP and DBP and prevented obesity and dyslipidemia in EW+Res. EW rats had higher plasma and liver thiobarbituric-acid-reactive substances (TBARS) and lower plasma superoxide dismutase (SOD) and liver glutathione peroxidase activities (+51%, +18%, −58%, −31%, respectively; $P<.05$), and resveratrol normalized both plasma and liver TBARS and increased the activity of SOD and catalase in plasma. EW rats presented liver steatosis and higher liver TG, and resveratrol prevented these hepatic alterations. In conclusion, this study demonstrated a potential therapeutic use of resveratrol in preventing obesity and oxidative stress and reducing the risk of hypertension, dyslipidemia and steatosis in adult rats programmed by early weaning.

Keywords: Early weaning; Resveratrol; Programming; Oxidative stress

1. Introduction

Adverse conditions in critical periods (gestation and lactation) such as malnutrition could affect permanently the progeny in both humans and animals [1,2]. This association is termed metabolic programming and was proposed by Barker who suggested an association between low birth weight of the newborn and an increased risk of type 2 diabetes, hypertension and cardiovascular disease at adulthood [3]. More recently, the term programming has been reviewed and renamed as developmental plasticity, which suggest a more probabilistic than deterministic phenomenon [4].

Obesity is increasing in an alarming rate throughout the world. Today, it is estimated that there are more than 1.5 billion overweight adults and nearly 43 million overweight children under 5 years old worldwide [5]. Studies have focused on identifying obesity early determinants, especially during infancy and childhood when central and peripheral systems that regulate energy balance could be programmed [6,7]. Breast-feeding duration and exclusivity have been associated with obesity prevention in humans [8,9]. The World Health Organization defines exclusive breast-feeding by the consumption of breast milk until 6 months without any other type of food intake, juice or even water [10]. However, only 35% of children worldwide are exclusively breast-fed during the first postnatal 4 months [11]. We previously showed that early weaning in rats caused by prolactin blocking with bromocriptine [12,13] as well as through a nonpharmacological model [14,15] caused neonatal malnutrition for a short period and programmed the adult offspring for higher adiposity, insulin resistance, dyslipidemia and central leptin resistance.

Growing evidence suggests that increased oxidative stress is involved in the pathogenesis of cardiovascular disease in metabolic syndrome [16]. Oxidative stress, which may occur as a consequence of the imbalance between free radical production and the capacity of cellular antioxidant systems, induces cell damage and the deregulated production of adipocytokines that contribute to obesity-associated insulin resistance, hypertension, dyslipidemia and liver steatosis [17].

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Therefore, in the programming model of early weaning, the higher adiposity and metabolic disturbances [14,15,12,13] could be at least in part due to a higher oxidative stress. Thus, we hypothesized that treating oxidative stress is an important target to prevent and treat obesity and its associated comorbidities in these animals.

The use of bioactive food compounds at pharmacological doses is a recent therapeutic approach to prevent obesity-related chronic diseases [18–20]. Resveratrol (*trans*-3,40,5-trihydroxystilbene) is a natural phytoalexin mainly found in grapes, red wine, peanuts and other plants [21,22] that has been recognized to have beneficial properties including anti-inflammatory, antioxidant, antitumor actions and antiaging effects [23,24]. Previous studies of our laboratory showed that resveratrol decreased oxidative stress in a model of programming by protein restriction during lactation [25]. In this present study, we evaluated the oxidative stress in adult rats programmed by early weaning without maternal separation or pharmacological approach as well as the effects of resveratrol in the treatment of metabolic disturbances developed as a consequence of obesity in this experimental model.

2. Methods and materials

2.1. Experimental model of early weaning

Wistar rats were kept in a temperature-controlled room (25°C±1°C) with artificial dark–light cycles (lights on 07:00 h, lights off 19:00 h). Virgin female rats, 3 months old, were caged with male rats (3:1), and after mating, each female was placed in an individual cage with free access to food and water until delivery. Our experimental design was approved by the Animal Care and Use Committee of the Biology Institute of the State University of Rio de Janeiro (CEA/017/2009) according to the Brazilian Law issued on November 8, 2008, which concerns the rearing and use of animals in teaching and research activities in Brazil [26].

At birth, 20 lactating rats were randomly assigned to each one of the groups: early weaning (EW, n=10) — dams who were lightly anesthetized with thiopental (0.06 mg/ml per 100 g) and wrapped with a bandage to interrupt the lactation in the last 3 days of lactation; control (C, n=10) — dams whose pups had free access to milk throughout lactation (21 days). All litters were adjusted to six males to each dam at birth to maximize lactation performance.

The EW and C groups received food directly into the cage and had free access to drinking water. During lactation, offspring body weight (BW) was monitored daily. Two pups from each litter were randomly chosen and followed during the experimental period. From postnatal day (PN) 21 to PN 180, BW and food intake (g) of offspring were monitored every 4 days.

2.2. Oral treatment with resveratrol

On PN 150, two male rats from each EW litter were randomly assigned to receive resveratrol or vehicle solution. Both treatments were performed by gavage daily during 30 days. The EW+resveratrol (EW+Res, n=10) group received resveratrol at a dose of 30 mg/kg/day, and the EW+vehicle (EW, n=10) group received 0.5% (w/v) aqueous methylcellulose. The control group received vehicle solution. Because of its low solubility in water, resveratrol was suspended into carboxymethylcellulose solution [27,25], and this suspension was prepared daily.

In PN 180, animals were euthanized with a nonlethal dose of thiopental (0.06 g/kg BW) to collect blood, carcass, visceral fat mass and liver samples. The blood was collected by cardiac puncture and poured in a tube containing heparin. Plasma and tissue samples were frozen at −80°C until analysis.

2.3. Body composition evaluation

Visceral fat pads from three different regions (epididymal, mesenteric and retroperitoneal) were removed and weighed. Total body fat and protein contents were determined by carcass analysis. The offspring eviscerated carcass was weighed, autoclaved for 1 h and homogenized in distilled water (1:1). Fat content was measured in the homogenates by a gravimetric method described previously [14], and protein content was determined by the Lowry method [28].

2.4. Blood pressure

Systolic (SBP) and diastolic blood pressure (DBP) was measured in conscious rats on PN 178 by use of tail-cuff plethysmography (LE 5000, LETICA Scientific Instruments, Barcelona, Spain). The first measurement of SBP and DBP was discarded, and the mean of three subsequent measurements was recorded.

2.5. Lipid profile

Total cholesterol (TC), triglycerides (TG) and high-density lipoprotein (HDL-c) plasma levels were analyzed using Biosystem (Barcelona, Spain) commercial test kits. Low-density lipoprotein (LDL-c) and very low density lipoprotein (VLDL-c) were obtained using Friedewald calculations:

$$\text{LDL} - \text{c}(\text{mg}/\text{dl}) = \text{totalcholesterol} - (\text{triglycerides}/5) - \text{HDL} - \text{c} \quad (1)$$

$$\text{VLDL} - \text{c}(\text{mg}/\text{dl}) = \text{triglycerides}/5 \quad (2)$$

2.6. Determination of antioxidant enzyme activities

Plasma and liver homogenates were used to determine superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities. Liver samples were homogenized in 0.1 M sodium phosphate buffer (pH 7.40) at the proportion of 1:10. Homogenates were centrifuged for 10 min at 4°C to remove insoluble material and then stored at −80°C until analysis. SOD activity was evaluated by measuring the inhibition of adrenaline autooxidation as absorbance at 480 nm [29]. CAT activity was assayed by the rate of decrease in H_2O_2 at 240 nm [30]. GPx activity was evaluated by measuring the oxidation of NADPH at 340 nm in the presence of H_2O_2 [31]. The total protein content in each sample was determined by the Bradford method [32].

2.7. Thiobarbituric-acid-reactive substances (TBARS)

Lipid peroxidation was measured by malondialdehyde (MDA) concentration using the TBARS method as previously described [33,25]. Briefly, plasma and liver homogenates were mixed with 1 ml of 10% trichloroacetic acid and 1 ml of 0.67% thiobarbituric acid (Sigma Chemical Co., St. Louis, MO, USA) and heated in a boiling water bath for 30 min. The absorbance of the organic phase containing the pink chromogen was measured in a spectrophotometer at 532 nm. MDA equivalents were expressed in nmol/mg protein.

2.8. Western blotting analysis

Liver samples were homogenized in cold lysis buffer (50 mM Hepes, pH 6.4, 1 mM $MgCl_2$, 10 mM EDTA and 1% Triton X-100) containing Complete Protease Inhibitor Cocktail Tablets (Roche, Basel, Switzerland) using a Ultra-Turrax homogenizer (IKA Werke GmbH & Co. KG, Staufen, Germany). The total protein content was determined by the BCA protein assay kit (Pierce, Rockford, IL, USA).

Samples (30 μg total protein) were electrophoresed in 12% Tris-glycine sodium dodecyl sulfate polyacrylamide gels. Proteins were transferred for polyvinylidene fluoride membranes (Hybond ECL; Amersham Pharmacia Biotech, London, UK), blocked in 2% bovine albumin (Sigma-Aldrich Co., St. Louis, MO, USA) in T-TBS (0.02 M Tris/0.15 M NaCl, pH 7.5, containing 0.1% Tween 20) at room temperature for 1 h, washed 3× with T-TBS and incubated with the primary antibodies (CuZnSOD, CAT and GPx at 1:500 concentration) overnight at 4°C. CuZnSOD and GPx antibodies were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA), and CAT was from Sigma-Aldrich Co. (Sigma-Aldrich Co., St. Louis, MO, USA). After washing 3× with T-TBS, blots were incubated with corresponding secondary antibodies at 1:5000 concentration (Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 1 h and then incubated with streptavidin (Zymed, San Francisco, CA, USA) in the same dilution of the secondary antibody for 1 h. Blots were developed with enhanced chemiluminescence (ECL; Amersham Biosciences Inc., Piscataway, NJ, USA).

2.9. Liver histology

Liver samples were fixed in formalin (freshly prepared 1.27 mol/L formaldehyde, 0.1 M phosphate-buffered saline, pH 7.2) and embedded in paraffin to nonserial sections of 5 μm. Sections were placed in glass slides to stain in hematoxylin/eosin. The morphological study was performed using digital images acquired at random (TIFF format, 36-bit color, 1360×1024 pixels) with an Olympus DP71 camera and an Olympus BX40 epifluorescence microscope (Olympus, Tokyo, Japan).

2.10. Liver TG content

Total lipids were extracted from liver according the method described by Folch et al. [34]. Liver samples (50 mg) were homogenized in 1 ml isopropanol and centrifuged for 10 min at 4°C. TG content was measured by using commercial kits (Quibasa, Minas Gerais, Brazil).

2.11. Statistical analysis

Results are reported as mean±S.E.M. Differences among the groups were analyzed by one-way analysis of variance followed by Newman–Keuls posttest. Differences were considered significant at P<.05.