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Original article

Ethanol in lactation promotes oxidative stress in different phases of rat offspring

Maiara Brusco de Freitas [a], Emilia Addison Machado Moreira [b,*], Danilo Wilhelm Filho [c], Gerson Luis Faccin [d], Erasmo Benício Santos de Moraes Trindade [b], Sonia Maria de Medeiros Batista [b], Regina Lúcia Martins Fagundes [b]

[a] Programa de Pós-Graduação em Nutrição, Universidade Federal de Santa Catarina, Brazil
[b] Departamento de Nutrição, Programa de Pós-Graduação em Nutrição, Universidade Federal de Santa Catarina, Brazil
[c] Departamento de Ecologia e Zoologia, Programa de Pós-Graduação em Farmácia, Universidade Federal de Santa Catarina, Brazil
[d] Departamento de Nutrição, Universidade Federal de Santa Catarina, Brazil

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SUMMARY

Backgroud & aims: To evaluate the effect of ethanol on body weight and biomarkers of oxidative stress such as the activities of catalase (CAT), superoxide dismutase (SOD), glutathione S-transferase (GST), glutathione peroxidase (GPx), and levels of lipoperoxidation (TBARS) in blood and liver of rat offspring.
Methods: Offspring ($n = 36$) of adult Wistar rats in a Control Group (CG; $n = 3$) and in an Experimental Group (EG; $n = 3$) were randomized in phases of lactation, young and adult ($n = 6$ rats/group). Controls received water *ad libitum* while EG received water with 20% ethanol during 12 days of lactation.
Results: Comparing EG to CG, there was lower weight gain in lactation and young ($p < 0.001$), in young and adults decreases of SOD ($p = 0.018$, $p < 0.001$, respectively), of GST in blood of young ($p < 0.001$), GST, GPx and CAT activities in liver of young ($p = 0.008$, $p = 0.008$, $p = 0.004$, respectively) and adults ($p < 0.001$, $p = 0.017$, $p = 0.053$, respectively) were found.
Conclusions: The maternal consumption of ethanol during lactation caused lower body weight gain in lactation and young offspring groups, normalizing in adults, as well as decreases in the activity of SOD in blood and in GST, GPx and CAT in liver of young and adults.

1. Introduction

Lactation is considered a critical phase of development of the offspring because functions and structures of the organs and systems are still in development, which make them vulnerable to many insults.[1] Therefore, changes in the diet or promoted by consumption of ethanol cause oxidative stress and several related diseases.[2]

In liver ethanol is metabolized in the cytosol of hepatocytes to acetaldehyde, mainly by the enzyme alcohol dehydrogenase (ADH).[3] Beside this, the catalase (CAT) route in peroxisomes and in mitochondria, also oxidizes ethanol into acetaldehyde in the presence of hydrogen peroxide (H_2O_2).[4] When ethanol is chronically consumed, such metabolism occurs *via* the microsomal oxidation system of ethanol (MEOS), being the cytochrome enzyme P4502E1 (CYP2E1) responsible for ethanol transformation into acetaldehyde.[5]

There is enough evidence that MEOS generates reactive oxygen species (ROS), including superoxide anion ($O^{\bullet}_{2}^{-}$) and hydrogen peroxide (H_2O_2), together with a depletion of reduced glutathione (GSH) and enhanced lipid peroxidation, resulting in an increased oxidative stress.[2,6] Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione S-transferase (GST) are among the main of the enzymatic antioxidants.[7]

The evaluation of water consumption with 10% of ethanol for 12 days of lactation showed a slower growth of the offspring compared to the control group.[8] Similarly, the water consumption with 20% of ethanol by rats in the corresponding phases of induction (prior phase to reproduction), gestation and lactation resulted in a decrease of hepatic activity of GPx, which was attributable to the entry of cytosolic GSH within the mitochondria.[9] Beside this, other studies have shown that ethanol consumption can cause depletion of antioxidants, which increase the susceptibility to oxidative stress by increased lipid peroxidation, and also neuronal changes in the transition of mitochondrial permeability.[9,10]

* Corresponding author. Departamento de Nutrição, Centro de Ciências da Saúde, Universidade Federal de Santa Catarina, Campus Universitário, Trindade, 88.040-970 Florianópolis, Brazil. Tel.: +55 48 37219784; fax: +55 48 37219542.
E-mail address: emilia.moreira@ufsc.br (E.A.M. Moreira).

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The consequences of ethanol consumption during lactation phases[11] in young and adult offspring have been little studied. Thus, this study evaluated the effect of ethanol consumption by lactating rats on oxidative stress in the offspring phases. To our knowledge, this is the first study that evaluated the effect of ethanol consumption during lactation until the adulthood.

2. Materials and methods

2.1. Experimental design and animals

Controlled experimental study developed at the Laboratory of Experimental Nutrition of the Department of Nutrition at the Federal University of Santa Catarina, with female rats of *Wistar* strain, coming from the Central Laboratory of this university. The study protocol was approved by the Ethics Committee on Animal Use of Federal University of Santa Catarina.

The rats (adults, female, 3 months of age and initially weighing between 150–200 g) were divided into 2 groups: Control Group (CG) ($n = 3$) and Experimental Group (EG) ($n = 3$). The offspring were randomized using the model of "incomplete counterbalanced blocks"[12] in three groups: at the lactation phase (12 days): the Lactation Control Group (LCG, $n = 6$) and the Lactation Experimental Group (LEG, $n = 6$). At the young phase (30 days): the Young Control Group (YCG, $n = 6$) and the Young Experimental Group (YEG, $n = 6$); and at the adult phase (90 days): the Adult Control Group (ACG, $n = 6$) and the Adult Experimental Group (AEG, $n = 6$) (Fig. 1). During the experiment, animals were kept in individual metabolic cages with constant temperature of approximately 22 ± 2 °C and light/dark cycle of 12/12 hours.

2.2. Diet

During the pregnancy the rats were maintained with water and a balanced commercial diet (Nuvilab CR-1®, Colombo, PR, Brazil) *ad libitum*.

In the CG group, the rats and offspring (lactation, young and adult) phase, received commercial diet and water *ad libitum* until the sacrifice. Rats of the EG ($n = 3$) received a commercial diet and water with 20% of ethanol (EMSURE® ACS, ISO, Reag. Ph Eur MERCK Brazil Jacarepaguá, RJ, Brazil) *ad libitum* as the only source of liquid[13] until the 12th day of lactation, after the ethanol was removed from water. The offspring at the lactation phase the both groups (CG and EG) received only milk during the 21 days of lactation.

2.3. Effect of ethanol on offspring development

The effect of ethanol on the development of offspring was evaluated by body weight gain. The animals were weighed in a Weighing Balance PW 3015 with capacity of 1500 g and a sensitivity of 0.1 g (Coleman, Santo André, SP, Brazil). During the lactation phase weight was measured by a pool of pups on days 0, 4 and 8. Animals were also weighed on the 12th day at the end of the young and adult phases, as well as before sacrifice by cervical dislocation with the rats in fasting state and anesthetized with ethyl ether.

2.4. Markers of oxidative stress

Blood was collected by cardiac puncture, using heparinised syringes as previously described.[14] After plasma removal, red cells were washed three times in an ice-cold saline solution. The hemolysates were obtained after addition of three volumes of 20 mM Tris-HCl, pH 8.0, and centrifuged at 3000 *g* for 5 min, and then were diluted five times.[14] The analyzes of the activity of catalase (CAT),[14] superoxide dismutase (SOD),[15,16] glutathione S-transferase (GST)[14] and glutathione peroxidase (GPx)[14] were performed both in hemolysates and in liver homogenates. After sacrifice the liver was quickly removed, kept on ice, and perfuse with ice saline (0.9% NaCl) solution for 5 min. A portion of 100 mg of liver was removed, placed in a buffer solution 0.1% Triton X-100, 0.12 M NaCl, 30 mM Na_2PO_4, pH 7.4 and homogenised for 1–2 min (1:9 w/v) at 4 °C in a tissue tearor (DREMEL®, Biospec Products, INC. Bartlesville, Oklahoma, USA). The homogenate was then centrifuged at 5000 *g* for 10 min and the supernatants were stored in liquid nitrogen until analysis. For determination of thiobarbituric acid reactive substances (TBARS), plasma and liver homogenates were used. It was added trichloroacetic acid 12% (1:4 v/v) to the plasma or liver portions being subsequently centrifuged at 5000 *g* for 3 min. It was added the Tris-HCl 50 mM, pH 7.0 buffer to the supernatants, vortexed for 20 s and added 0.67% of thiobarbituric acid, kept in boiling water for 60 min, and finally at 5 °C for 30 min, and subsequently analyzed at 535 nm.[14] For the readings, it was

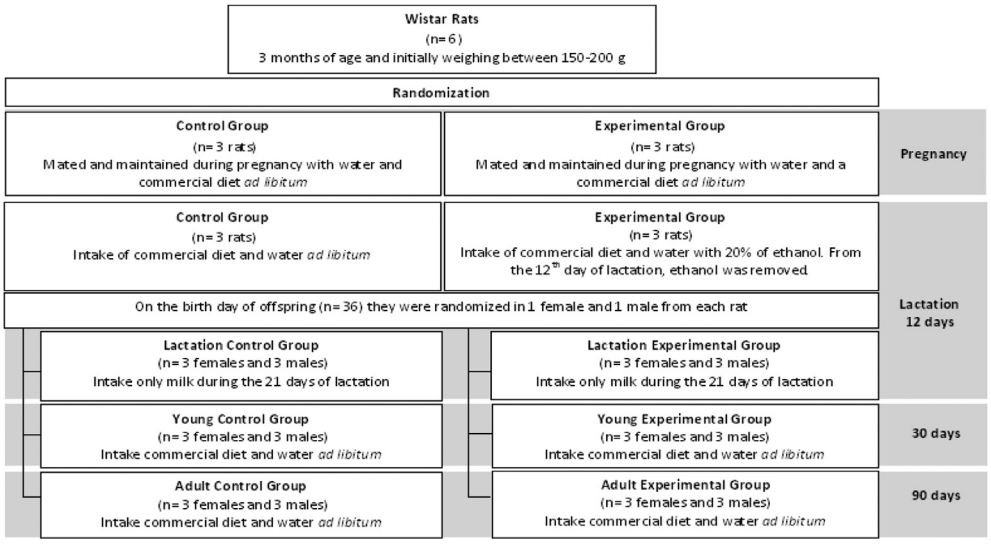

Fig. 1. Randomization of rats and offspring.