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Does bromocriptine play a role in decreasing oxidative stress for early weaned programmed obesity?

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ABSTRACT

Aims: Studies have demonstrated that early weaning can promote metabolic syndrome during adulthood and that obesity increases oxidative stress. Thus, we aimed to evaluate redox status in a pharmacological early weaning rodent model programmed for metabolic syndrome at adulthood.
Main methods: Lactating dams were randomly assigned into 2 groups: the early weaning group (BRO), which was treated intraperitoneally with bromocriptine (1 mg/day) to inhibit prolactin secretion for the last 3 days of lactation, and the control group (C), which received the BRO diluent for the same time period. The offspring were killed at 90 (PN90) and 180 (PN180) days after birth.
Key findings: Early weaning induced greater visceral adiposity and dyslipidemia. At PN90, the BRO offspring showed glucose intolerance with normoinsulinemia and increased plasma and liver superoxide dismutase, and liver glutathione peroxidase activities, which reduced the liver malondialdehyde but not the increased plasma malondialdehyde levels. However, the BRO offspring showed insulin resistance at PN180 and increased plasma glutathione peroxidase, liver superoxide dismutase, and catalase activities. These changes reduced the plasma and liver malondialdehyde levels, which aided in hepatocyte architecture preservation. Additionally, we observed that sirtuin 1 was overexpressed in the BRO group at PN90, but the increased expression was not maintained through PN180, which suggests unfavorable metabolic conditions in the older offspring.
Significance: Despite the observed obesity and glucose homeostasis dysfunction, our data suggest that the early weaning programming induced by bromocriptine can improve the offspring's redox status and may prevent liver damage during adulthood.

Introduction

Breastfeeding is associated with reduced obesity risk because maternal milk causes hypoinsulinemia, which decreases fat storage and prevents excessive early adipogenesis (Oddy, 2012). In fact, early weaning is related to childhood obesity, which demonstrates that the timing for introducing solid food to an infant's diet affects weight gain (Sloan et al., 2008).

Studies were performed to understand the mechanisms involved in the later effects triggered by early weaning. When pups were weaned through maternal separation, the offspring showed a preference for high-calorie food during adulthood, which led to obesity and metabolic changes (dos Santos et al., 2011). Additional alterations were observed during adulthood after early weaning from lactation by applying a bandage on the mother's body to interrupt lactation without maternal separation. The adult offspring exhibited obesity, dyslipidemia, and hyperglycemia, which are characteristics of metabolic syndrome, hyperleptinemia, central leptin resistance, and higher catecholamine (Lima Nda et al., 2011; Lima et al., 2013). Further, milk production can be suppressed in a pharmacological model for early weaning using bromocriptine, which is a type 2 dopaminergic receptor agonist that inhibits prolactin. Offspring with mothers treated with bromocriptine exhibited neonatal malnutrition and developed obesity, hyperleptinemia, leptin and insulin resistance, dyslipidemia, hypothyroidism, and higher adrenal hormones during adulthood (Bonomo et al., 2007, 2008; de Moura et al., 2009).

Long-lasting dysfunction induced by early weaning may lead to liver changes; the liver is a central metabolic organ that regulates energy homeostasis. In nonalcoholic fatty liver disease, metabolic syndrome is manifested; in insulin resistance, oxidative stress and inflammatory cascades can be important for hepatic disease pathogenesis and progression (Colak et al., 2011). Thus, oxidative stress is an important mechanism that links obesity and its comorbidities because both a

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suppressed antioxidant system and an enhanced reactive oxygen species production are related to central adiposity in obese individuals (Savini et al., 2013).

Recently, a non-pharmacological early weaning model was programmed for increased oxidative stress and steatosis in the liver, which was reversed through resveratrol treatment. Resveratrol is a natural phytoalexin in grapes, which produces beneficial effects because it is an antioxidant agent (Franco et al., 2013) that activates histone deacetylases, sirtuin 1 (SIRT1), and aids in treating or preventing obesity.

Notably, (i) lactation is critical for development, and (ii) a pharmacological early weaning model induces several disruptions in adult progeny that are similar to the disruptions observed in a non-pharmacological early weaning model, such as metabolic syndrome. Therefore, the study herein was designed to evaluate glucose homeostasis, redox status, and hepatic morphological alterations at 2 different time points in adult offspring whose mothers received bromocriptine at the end of lactation. For insight into the programming mechanisms, liver SIRT1 expression was measured in our study. SIRT1 is important for glucose and lipid metabolism (Guarente, 2006), and higher SIRT1 activity has been related to lower adiposity and protection against diet-induced metabolic disorders (Pfluger et al., 2008); therefore, we reasoned that SIRT1 expression may be decreased by early weaning.

Material and methods

Ethical approval

The experiment was designed and performed in accordance with the principles adopted and promulgated by Brazilian law no. 11.794/2008, and each procedure was approved by the Animal Care and Use Committee at the Biology Institute in the State University of Rio de Janeiro (CEUA/186/2007, CEUA/017/2009). The experiment was performed to minimize the number of animals used and the suffering from the procedures following the three 'R's' of ethical doctrine: reduction, refinement, and replacement (Drummond, 2009).

Animals

The animals were maintained in a temperature-controlled room (23/24 °C) with artificial dark–light cycles (lights on for 7 h and lights off for 19 h). Three-month-old virgin female Wistar rats were mated with male breeders at a 2:1 ratio. The pregnant rats were maintained in individual cages with free access to water and standard chow until delivery and during lactation. To avoid the influence of litter size on the programming effect, only the mothers with 10 pups were used. After spontaneous delivery, the litters were adjusted to 6 male pups by the mother (anogenital distance was used to determine the gender). Manipulating the litter standardized the food supply and maximized the lactation performance. Beginning at birth, the body mass (BM) and naso-anal length (NAL) were measured in the male pups.

The lactating dams were randomly assigned to 2 groups: (BRO, n = 5), which was administered 2 doses of 0.5 mg bromocriptine (Novartis, SP, Brazil) per day (8:00 AM and 8:00 PM) diluted in 200 μL of methanol–saline (1:1 v/v) and injected intraperitoneally for the last 3 days of lactation, and the control group (C, n = 5), which received only a methanol–saline treatment for the same time period.

After weaning, 1 pup was randomly selected from each litter (5 animals per group) and analyzed 90 days after birth (PN90). The analyses at 180 days after birth (PN180) were performed using the average between 2 randomly selected pups from each litter, also totaling 5 animals per group.

Biometric parameters and food intake

During lactation, the BM and NAL were measured every 3 days; after weaning, the absolute values for the parameters were measured weekly with the food intake. The food consumption was measured as the difference between the amount of food provided and remaining 7 days later divided by the number of animals in the cage.

Oral glucose tolerance test

An oral glucose tolerance test (OGTT) was performed at PN90 and PN180. After a 12 hour fasting period, 50% glucose was administered in sterile saline (0.9% NaCl) through an oral gavage at 2 g/kg BM. Blood was drawn from the tip tail of each animal to measure the plasma glucose concentration, which was assessed using a glucometer (Accu-Chek Advantage; Roche Diagnostics, Mannheim, Germany) before the glucose was administered and 15, 30, 60, and 120 min after the gavage (Peixoto-Silva et al., 2011).

Euthanasia

After fasting for 12 h, the adult rats were sacrificed by quick decapitation to collect the blood, visceral fat mass ([VFM], retroperitoneal, epididymal, and mesenteric fat) and liver. The blood was previously collected in a heparinized tube and centrifuged (2500 rpm, 25 min, 4 °C). The plasma was collected and stored individually at −20 °C until it was used for the different measurements. The hydrostatic liver weight/volume was measured using the Scherle method and normalized to the right tibia length in accordance with previous studies (Conceição et al., 2013a,b). The tissues were fractionated and stored using multiple procedures (freezing at −80 °C or fixative solution).

Plasma insulin and biochemical analyses

The insulin concentration was determined using an RIA kit (ICN Pharmaceuticals, Inc., Orangeburg, NY, USA) with the assay sensitivity 0.1 ng/mL and a 4.1% intra-assay variation. The measurements were performed using a single assay. To determine the adult animals' insulin sensitivity, we used the homeostatic model assessment of insulin resistance (HOMA-IR): (fasting glycemia [mmol/L] × fasting insulinemia [μU/mL] / 22.5). We analyzed the aspartate aminotransferase (AST), alanine aminotransferase (ALT), and triglyceride (TAG) plasma levels using Biosystem commercial test kits and a spectrophotometer (Biosystems S.A., Barcelona, Spain).

Histological processing and adipocyte morphometry

Fragments from the retroperitoneal fat and liver were collected from each lobe and fixed in a freshly prepared fixative (1.27 M—formaldehyde,

Table 1
Biometry and adiposity of rats whose were early weaned.

	C	BRO
BM (g)		
Birth	6.125 ± 0.08	5.955 ± 0.09
18 days old	38.43 ± 0.61	38.05 ± 0.79
19 days old	41.07 ± 0.69	39.91 ± 0.79
20 days old	43.89 ± 0.62	41.52 ± 0.87*
21 days old	46.62 ± 0.61	42.97 ± 0.98*
PN90	323.8 ± 9.08	331.6 ± 20.24
PN180	398.2 ± 14.03	421.0 ± 15.99
VFM (g)		
PN90 (g)	7.82 ± 0.33	11.40 ± 1.51*
PN180 (g)	9.44 ± 0.96	13.87 ± 1.64*

Values are mean and SEM. $P < 0.05$; n = 5 per group. C —control offspring; BRO —early-weaned offspring; BM —body mass; VFM —visceral fat mass.
* significantly different from control offspring.