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Food deprivation in F0 generation and hypercaloric diet in F1 generation reduce F2 generation astrogliosis in several brain areas after immune challenge

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ABSTRACT

Aims: The effects of maternal food restriction during gestation in F0 generation followed by hypercaloric diet (HD) during puberty in F1 generation (F1HD) were investigated on astrocyte behavior of F2 generation. Also, the astrocyte behavior, after an immune challenge, was examined by the immunohistochemical expression of glial fibrillary acidic protein (GFAP) in several brain areas.

Methods: The body weight gain (BW) during development and in postnatal day (PND) 90–95, the retroperitoneal fat weight (RPF), and the size of larger and smaller adipocytes in the F1 generation were assessed to observe the effects of HD in female rats. The BW, RPF weight and size of smaller and larger adipocytes was also measured to evaluate the transgenerational effects of F0 and F1 diets on F2 generation, treated or not with lipopolysaccharide (LPS).

Key findings: The F1HD group exhibited a higher BW gain than the F1 treated with normocaloric diet (ND, group F1ND), from weaning to PND65. In the frontal/parietal cortex, nucleus accumbens, hypothalamic arcuate/periventricular nuclei, molecular/granular layers of the cerebellum areas, excepting the pons, GFAP expression was greater in F1HD group relative to F1ND group. A reduced GFAP expression was observed in both groups born from F1 generation fed with HD (groups F2HDS and F2HDLPS) in relation to F2 generation born from dams fed with ND (groups F2NDS and F2NDLPS), independently of LPS challenge.

Significance: These data show an attenuation of LPS effect on GFAP expression, probably by a transgenerational effect of both maternal food deprivation in F0 generation and HD in F1 generation.

1. Introduction

Mammalian embryos have evolved to adjust their organ and tissue development in response to an adverse environment. This adaptation is called phenotypic plasticity and allows the organism to adapt to the environment where the fetus develops (Parlee and MacDougald, 2014). The thrifty phenotype hypothesis proposes that poor nutrition in early life results in poor fetal growth and an increased susceptibility to type 2 diabetes and metabolic syndrome (Hales and Barker, 2001). Adult individuals whose mothers were undernourished early in pregnancy exhibit higher rates of intra-abdominal adiposity associated with a higher risk of metabolic disease (Yang and Huffman, 2013) and heightened energy balance dysfunction in adulthood (Hales and Barker, 2001; Hales and Barker, 1992), undernutrition that causes obesity in the offspring can result in peripheral (Hotamisligil et al., 1995) and central (Velloso et al., 2009) inflammation, particularly in the hypothalamus. Several hypothalamic nuclei regulate food intake through peptides and neural actions. Impairment in the hypothalamic control of feeding can lead to lipid accumulation and bodyweight gain (García-Cáceres et al., 2013).

Astrocytes are dynamic cells that respond to changes in the central nervous system (CNS) by undergoing morphological and functional alterations that affect neuronal activity (García-Cáceres et al., 2013). In response to CNS insults, astrocytes develop a hypertrophic or reactive

Abbreviations: F0 generation, the parental generation; F1 generation, daughters of the F0 generation; F2 generation, grandchildren of the F0 generation; ND, normal diet; HD, high fat diet; GFAP, glial fibrillary acidic protein; BW, body weight; RPF, retroperitoneal fat weight; PND, postnatal day; LPS, lipopolysaccharide; HAs, hypodermal adipocytes

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phenotype termed astrogliosis (Levine et al., 1999), which is characterized by the upregulation of specific structural proteins, such as glial fibrillary acidic protein (GFAP) and vimentin (Ridet et al., 1996). Several studies have shown that glial cells are intimately involved in hypothalamic inflammation that results from high-fat diet-induced obesity, which is linked to an increase in insulin resistance (Thaler et al., 2012 Argente-Arizón et al., 2015) and other pathological processes associated with excessive weight gain (Chen et al., 2008; Horvath et al., 2010). Hypothalamic astrogliosis has been widely associated with high-fat diet-induced obesity (Buckman et al., 2013 García-Cáceres et al., 2013).

The objective of the present study was to evaluate the transgenerational effects of a combination of food imbalances: maternal food restriction in F0 generation during pregnancy followed by HD in F1 generation during puberty on astrocyte behavior of F2 generation, with or without the challenge of LPS. The astrocyte behavior was examined by assessing the immunohistochemical expression of GFAP in astrocytes of several brain areas, including the frontal and the parietal cortex, the nucleus accumbens, the arcuate and periventricular nuclei of the hypothalamus, the pons and the molecular and the granular layers of the cerebellum, in order to investigate possible morphological alterations induced by food imbalances in astrocytes from brain areas related to executive, reproductive and motivational functions.

First, the BW gain during development and in PND90-95, the RPF weight and the area of larger and smaller adipocytes in F1 generation were assessed to observe the effects of HD in F1 female rats from dams of F0 generation deprived of food during gestation. In addition, F2 generation rats were evaluated for the BW, the RPF weight and the size of smaller and larger adipocytes, to show evidence of the possible transgenerational effects of F0 and F1 diets in F2 metabolic functions. Furthermore, half of rats of F2 generation received an immune challenge with LPS, a non-replicating component of the cell walls of Gram-negative bacteria, to observe the grade of neuroinflammation. Overweight/obesity increases the expression of the GFAP, inducing astrogliosis, a defensive transformation in response to injury and inflammation as previously observed in the hypothalamus by Joaquim et al. (2016a,b).

2. Material and methods

2.1. Ethics statement

The animal procedures were performed in accordance with the guidelines of the Committee on Care and Use of Laboratory Animal Resources and Brazilian Institutional Ethics Committee guidelines, Universidade Paulista (Protocol no. 130/12, CEUA/ICS/UNIP, 28/11/2012). All efforts were made to minimize animal suffering.

2.2. Animals

Twenty-eight adult female Wistar rats, 11–12 weeks of age and weighing 200–250 g (F0 generation) and 14 sexually experienced male rats (12–14 weeks of age) from the School of Veterinary Medicine (University of São Paulo, São Paulo, Brazil) were initially used. Upon arrival in our laboratory, rats were housed in microisolator cages (two females per cage, five males per cage) under controlled temperature (22–26 °C) and humidity (50–65%) conditions in artificially lit rooms on a 12-h light–dark cycle (lights on at 07:00 h) and food ad libitum. Ten days after arrival in our laboratory, females of the F0 generation were mated with the naïve male rats to obtain the F1 generation (two females per male). On postnatal day (PND) 90–95, female rats of the F1 generation were mated with new sexually experienced males without any treatment (two females/male, n = 7), to obtain the F2 generation. All dams were allowed to give birth normally and nurture their offspring. The day of birth was recorded as PND 1. No handling was performed on PND1 to avoid cannibalism (DeSantis and Schmaltz, 1984). On PND2, eight offspring (four males and four females) were randomly selected. This procedure was performed early (PND2) to avoid differences in the amount of maternal milk that was given to the pups, which could affect the weight gain of the pups (Morag et al., 1975). No cross-fostering procedures were used (Chiavegatto and Bernardi, 1991). The eight randomly selected pups remained with their dam until weaning on PND21. On PND21, the littermates were separated and co-housed by sex under the same conditions as their parents.

2.3. Hypercaloric diet

Rats in the HD group (F1 generation) were given free access to the HD (the liquid diet is the high fat diet −Ensure®; Abbot Brasil, São Paulo, Brazil; total of 1 kcal mL^{-1} in addition to laboratory chow (Nuvilab; Sogorb Indústria e Comércio, São Paulo, Brazil); values per 100 g solid food item: 4.2 kcal g^{-1}, 56% carbohydrate, 19% protein, 4.5% cellulose, 5% vitamins and 3.5% g total fat). Ensure, is a highly palatable liquid diet supplement and each 231-kcal bottle contained 1.7 g polyunsaturated fat, 3.59 g monounsaturated fat and 2.2 g saturated fat. It did not contain any trans-fat. It was presented in a graduated cylinder with a stopper, with 600 mL per bottle. Rats in the HD and normocaloric diet (ND, nutritionally balanced food) groups were housed four per cage and Ensure and laboratory chow were made available to each cage. The standard chow was used to avoid lack of food (supplement diet) because rats of all cages of HD group consumed 600 mL of ensure/day. The consumption of each cage of standard chow was no more than 10 g/each cage. The consumption of both diets was measured daily. Both diets were changed daily.

2.4. Groups and experimental design

Twenty-eight pregnant female rats (PND90-95) of the F0 generation were food restricted (40%) from gestation day (GD) 5–18. For this, the control group, received food *ad libitum* and the pregnant females food restricted received 40% of the total amount eaten by the control individuals (Ricci et al., 2014). Half of F1 females (one female of each litter of 14 litters) were divided in two groups: one received from PND23 to 65 the HD (F1HD group, n = 6) and the second group (F1ND, n = 8) received the ND. BW during development and in adulthood (PND90-95), RPF weight, hypodermal adipocytes (HAs), and GFAP expression in the frontal and parietal cortex, nucleus accumbens, periventricular and arcuate nuclei of the hypothalamus, molecular and granular cell layers of the cerebellum and pons were examined in half the F1 female rats in each group. The other half of the F1 female rats (14 females) was mated with sexually experienced males to obtain the F2 generation. On PND50, RPF weight, HAs and GFAP expression in the same areas were evaluated in male pups of the F2 generation treated or not with LPS. In the F1 generation, only females were used (one pup per litter) in the experiments. In the F2 generation, only male pups were used (two male pups of 14 litters) in the experiments. Males of the F1 generation and females of the F2 generation were used in a different study, the results of which will be published elsewhere.

Thus, the group configuration in the present study was as follows: F0 (female rats food restricted); F1ND (F1 female rats born from F0 dams fed with normocaloric diet), F1HD (F1 female rats born from F0 dams fed with hypercaloric diet); F2NDS (F2 male rats born from F1ND dams treated with saline solution, i.p.); F2NDLPS (F2 male rats born from F1ND dams treated with LPS 100 μg/kg, i.p.); F2HDS (F2 male rats born from F1HD dams treated with saline solution, i.p.); F2HDLPS (F2 male rats born from F1HD dams treated with LPS 100 μg/kg, i.p.). Briefly, the number of animals per group was: in *F1 generation, ND group, n = 8; HD group, n = 6; in F2 generation, NDS group, n = 8; NDLPS group, n = 8; HDS group, n = 6; HDLPS group, n = 6.*

The experimental design is shown in Fig. 1.