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Supplementation of the maternal diet with Brazil nut (*Bertholletia excelsa* H.B.K.) prevents cognitive impairment in the offspring of obese mothers

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Abstract

Maternal obesity may trigger long-term neurodevelopmental disorders in offspring. Considering the benefits of the Brazil nut (*Bertholletia excelsa* H.B.K.), a rich source of nutrients such as selenium, this study aimed to evaluate its effect on the behavior of obese rat offspring and its relationship with oxidative stress. From 60 days of age until weaning, female Wistar rats were fed a high-fat diet (mHF) or an HF diet supplemented with 5% Brazil nut (mHF/BN), while control mothers (mCTL) were fed a standard diet or a standard diet supplemented with 5% Brazil nut (mBN). Male pups received a standard diet throughout life and, at 30 and 90 days old, were subjected to behavioral tasks to evaluate anxiety and cognition. Biochemical evaluations were performed at 90 days of age. No alterations were observed in the anxiety behavior of the offspring. However, the offspring of the mHF group (oHF) exhibited impaired short-term memory at 30 and 90 days of age and impaired long-term memory at 30 days. Short-term memory impairment was prevented by Brazil nuts in young rats (30 days). While the serum selenium concentration was reduced in the oHF group, the serum catalase concentration was reduced in all groups, without changes in lipid peroxidation or protein carbonylation. Brazil nut maternal diet supplementation prevented short- and long-term cognitive impairment in the offspring, which may be related to the selenium levels.

Original Article

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Introduction

Environmental stimuli, including changes in maternal nutrition and metabolic hormones during critical periods of life development, can program neuronal connections, tissue development, and maturation in neonates, predisposing offspring to long-term metabolic impairment, as can type 2 diabetes mellitus, obesity, and cardiovascular and behavioral disorders in adulthood.[1–3] In the developmental origins of health and disease (DOHaD) paradigm, changes in nutrient availability or hormone signaling can shape plasticity mechanisms adapting organisms to survive in the new environment, which contributes to the risk of nongenomic inherited long-term disease.[4] Although the mechanisms underlying the programming of metabolic disorders remain poorly defined, it has become increasingly clear that obesity and its comorbidities are associated with inflammation and oxidative stress, with the presence of reactive oxygen species (ROS) influencing the redox status of the fetal environment and its development.[5–7]

In this context, nutritional intervention has emerged as a promising therapeutic target for mitigating complications in fetal development induced by maternal obesity, given the expense and potential long-term risks of pharmacological therapies.[1,8,9] It is widely known that the Amazon region has numerous native oleaginous plant species with promising potential in the food industry, such as the Brazil nut (*Bertholletia excelsa* H.B.K.) and Lecythidaceae families.[10,11] The Brazil nut is recognized for its high selenium content as well as for the substantial bioavailability of this micronutrient.[11,12] A variation of 2.07 mg/kg to 68.15 mg/kg in Brazil nut

Table 1. Macronutrient content estimation of the diets

Diets	Protein/100 g	Lipid/100 g	Carbohydrate/100 g
Standard diet (3.44 kcal/g)	22.0 g (25.6% kcal)	4.0 g (10.5% kcal)	55.0 g (63.9% kcal)
Brazil nut diet	21.6 g (23.9% kcal)	7.0 g (17.5% kcal)	52.9 g (58.6% kcal)
High-fat diet (4.02 kcal/g)	18.7 g (18.5% kcal)	13.4 g (30% kcal)	51.8 g (51.5% kcal)
High-fat/Brazil nut diet	18.3 g (17.4% kcal)	16.4 g (35.2% kcal)	49.7 g (47.4% kcal)

samples from the Amazonia region was observed, with acidity and the total concentration of selenium in the soil playing important roles in selenium absorption by Brazil nuts.[13]

The composition of unsaturated fatty acids, high selenium content, phenolic compounds, sterols, and tocopherols of Brazil nut plants has been associated with anti-inflammatory and antioxidant benefits and a risk of chronic diseases;[11,14] moreover, reduced levels of selenium have been implicated in reduced synaptic plasticity in the hippocampus as well as impaired learning ability.[15,16] Thus, adequate selenium intake, as well as normal selenoprotein expression, is essential for neuronal functions such as learning and memory.[16–21] In this vein, maternal obesity leads to cognitive dysfunction, which is related, among other causes, to increased oxidative stress in offspring.[6,7]

Given that, we hypothesize that a selenium-rich diet offered to obese mothers over the critical stages of their offspring development can attenuate or prevent negative obesity programming to maintain long-term redox imbalance and cognitive dysfunction. In this regard, we aimed to assess the effect of a maternal diet supplemented with Brazil nut extract during pregnancy and lactation on the cognition of obese rat offspring and to investigate the likely mechanism of antioxidant action.

Methods

Ethical and animal care

The female and male Wistar rats (50 g–100 g) were provided by the Animal House of the Universidade Federal de Mato Grosso (UFMT) and kept in controlled conditions, including temperature (23 ± 2°C), humidity (55 ± 5%), and light (12-h light cycle; lights were switched from 06:00 h to 18:00 h) throughout the experimental period, and water and rodent chow were provided *ad libitum* (Nuvilab CR-1, Nuvital®, Curitiba, PR, Brazil).

The experimental protocols were carried out in accordance with current guidelines for the care of laboratory animals and ethical guidelines for investigations of experiments in animals, according to the National Council for Animal Experimentation Control (Resolução Normativa number 12/2013 and number 22/2015). All protocols employed were approved by the Animal Ethics Committee (process number 23,108.017929/2019-93).

Chemical characterization and analysis of aflatoxins in Brazil nuts

To determine the centesimal composition, all analyses were performed in duplicate. The moisture content was determined in a greenhouse with forced air circulation at 105 ± 3°C.[22] The protein, lipid, and ash contents were analyzed according to the physical–chemical methods for food analysis at the Adolfo Lutz Institute.[23] The total carbohydrate content was calculated by the difference between 100 and the sum of the percentages of water, proteins, lipids, and ashes.

The determination of selenium in the Brazil nut samples was performed in triplicate by atomic absorption spectrometry with hydride generation (HG-AAS, PerkinElmer/PinAAcle 900F).[24] The digestion process was carried out in a CEM microwave (model MARS6). A standard curve was prepared from a 1000 mg/L stock solution of selenium (Merck) at concentrations ranging from 0 to 40 µg/L.

The detection and quantification of total aflatoxins (B1, B2, G1, and G2) were carried out through solid-phase extraction followed by liquid chromatography analysis coupled to sequential mass spectrometry (UPLC–MS/MS).[25]

Diet preparation

Brazil nuts were purchased from local producers in Itaúba, MT, Brazil, at room temperature, outside the hedgehog with bark, and were stored in natura at −20°C until use in diet preparation. The diets were formulated based on standard rodent chow (Nuvilab CR-1, Nuvital®, Curitiba, PR, Brazil), as described in Table 1.

The obesogenic diet was established according to previous methods,[26] in which the ingredients were weighed and added to standard rodent chow as follows: 85% standard rodent chow, 8% pork lard, 2% soybean oil, and 5% sucrose (a high-fat diet). The Brazil nut-supplemented diet was formulated with 95% standard rodent chow and 5% Brazil nut, while the high-fat diet supplemented with Brazil nut was formulated with 80% standard rodent chow, 8% pork lard, 2% soybean oil, 5% sucrose, and 5% Brazil nut. Thus, when 5% was added to the standard chow, there was an increase of 32.2 g/kg of Brazil nuts in the groups fed this oleaginous diet.

Regarding selenium, the recommendation in the standard diet for laboratory rats (AIN-93) is 0.15 mg/kg, increasing to 0.2-0.5 mg/kg during pregnancy and lactation.[27] In our work, 5% Brazil nut was added to standard rodent chow, and the average concentration of Se was 0.195 mg/kg. The variability in selenium levels in the soil reflects the need for the consumption of this micronutrient, which varies according to the geographical region.[28] In Brazil and the United States, the recommended dietary intake is 55 µg/day in adult women, which increases to 60 µg/day during pregnancy and 70 µg/day during lactation.[12,29] In rats, the need for selenium seems to be greater throughout pregnancy and the nursing period, considering the greater proportion of demand for this micronutrient.

Maternal dietary intervention and experimental design

At 60 days of age, female rats (190 g–200 g) were randomly divided into four groups according to dietary intervention: rats were fed standard rodent chow (control group, CT); rats were fed standard rodent chow supplemented with 5% Brazil nut (BN group); rats were fed a high-fat diet (HF group); and rats were fed a high-fat diet supplemented with 5% Brazil nut (HF/BN group).

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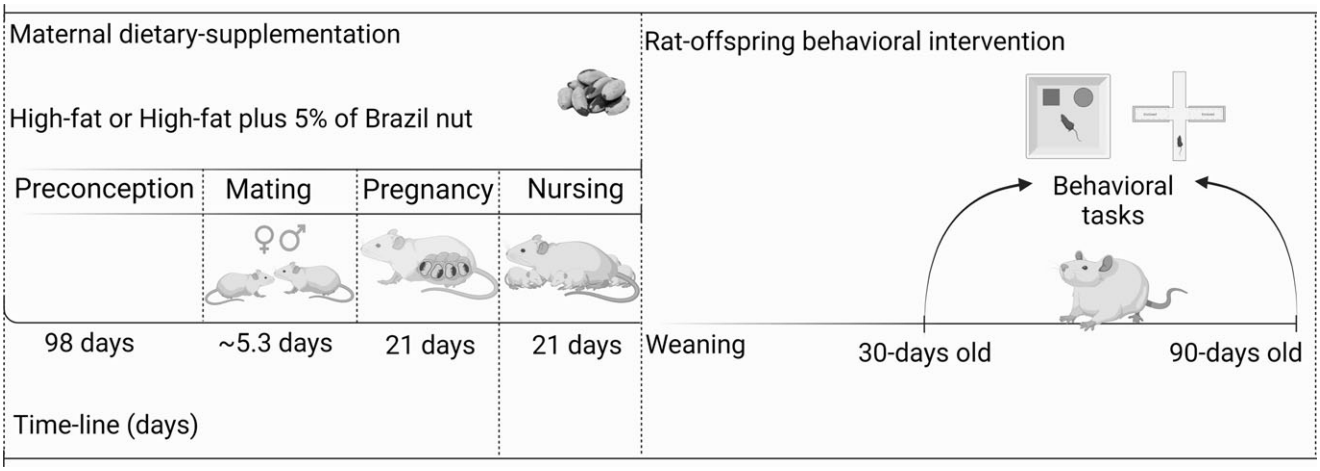

Figure 1. Timeline of *in vivo* procedures.

The maternal dietary intervention was carried out for approximately 21 weeks, as depicted in Figure 1. At the 14th week, after beginning dietary intervention, virgin females were mated with proven male breeder rats (two females per male for 5.3 ± 3.7 days). Maternal body mass was weighed weekly throughout the dietary intervention period (digital balance, SF 400). At the end of the nursing period, mothers were euthanized by decapitation, and the visceral fat pads (mesenteric and retroperitoneal fat masses) were dissected and weighed to assess body fat accumulation. The fat pads were normalized to the body mass of the rats and are shown as g/100 g of body weight.

Litter size and groups of rat offspring

On the 3rd day after birth, the litter size was adjusted to eight pups per mother. Preferably, male pups were used; however, when the number of male pups was insufficient, female pups were used only to complete the litter size and only during lactation.

At 21 days of age, the rat offspring were weaned and then given a standard rodent diet and water *ad libitum*. At least 4 litters were included in each maternal group. The groups of rat offspring were established according to maternal dietary treatment as follows: offspring from control group mothers (oCTL), offspring whose mothers were fed Brazil nut (oBN), offspring from mothers fed a high-fat (oHF), and offspring whose mothers were fed a high-fat/Brazil nut diet (oHF/BN).

Behavioral assessment

In all the tasks, the rat offspring ($n = 11–18$) were previously habituated to the experimental location in the laboratory for at least 30 minutes. The tasks were performed at the onset of adolescence, at 30 days of age, and in adulthood, at 90 days of age.

Elevated plus maze

Initially, the animals were subjected to the elevated plus maze test. The apparatus was 50 cm above the floor and comprised two open arms (50 × 10 cm) perpendicular to two closed arms (50 × 10 × 30 cm) with a central platform. The rats were individually placed on the central platform of the maze, previously cleaned with 10% ethanol, facing one of the open arms, and allowed to move freely about the maze for 5 minutes. The behavior of the animals was recorded, and the following parameters were evaluated: the number of entries into each arm, the time spent in each arm as well as on the central platform, and head-dipping and stretched attend posture as a risk assessment.[30] The anxiety index was calculated according to the following formula: 1 - [([time the animal remained in the open arms, in seconds/time of duration of the task, in seconds (300 s)] + [frequency of entry into the open arms/total number of entries])/2].[31] It should be noted that the total number of entries was defined as the sum of the frequency with which the animal entered the open and closed arms when the four legs exceeded the initial limit of the arm. The stretched attend posture was considered when the rat stretched forward and retracted to the original position and head-dipped when the animal projected the lateral commissure of the eye over the edge of an open arm and down toward the floor.

Object recognition task

At the end of the elevated plus maze test, the animals were subjected to an object recognition task[32] in an open field, which consisted of a circular arena 120 cm in diameter and 45 cm in height, with the floor divided into 12 areas. This task consisted of three sessions: habituation, training, and retention. Twenty-four hours after free exploration of the open field for 5 minutes in the absence of objects, training was conducted by placing individual rats for 10 minutes into the field in which two identical objects were positioned on adjacent sides. In the retention sessions to evaluate short- and long-term memories, which were carried out three and 24 h after training, respectively, the rats were allowed to explore the open field for 5 minutes, during which one of the familiar objects used during training was replaced by a novel object. All the objects were made of odorless plastic, were similar in size, and were previously tested for how much they aroused curiosity, fear, or interest. Between each trial, the objects and the field were cleaned with a 10% ethanol solution. The total time spent sniffing or touching each object with the nose and/or forepaws; the number of peripheral and central crossing, rearing, or fecal buns; and the number of episodes and self-cleaning times were recorded. Recognition memory was expressed as the percentage of exploration time for the new object in the short- and long-term memory sessions. The absence of exploration in any session was considered an exclusion criterion.

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Table 2. Biometrical parameters of mothers throughout experimental period (preconception, pregnancy, and nursing periods)

Parameters	mCTL	mBN	mHF	mHF/BN
Diet consumption	367.1 ± 17.0	418.2 ± 22.2	357.0 ± 36.4	349.0 ± 13.6
Body weight variation (AUC)	1963 ± 135.4	2090 ± 85.1	2182 ± 79.1*	1793 ± 224.4
Adiposity index	0.038 ± 0.012	0.047 ± 0.010	0.052 ± 0.006*	0.051 ± 0.009*

Data are expressed as mean ± *SD*, analyzed by two-way ANOVA and considering.
*$p < 0.05$ compared to mCTL group ($n = 10$–11).

Marble-burying task

After object recognition task completion, the rats were subjected to the marble-burying task.[33] The rats were placed individually in a polypropylene box (42 × 24 × 12 cm) closed with a metal grid, where 20 evenly spaced marbles (diameter 1.5 cm) were placed 5 cm above the depth of the sawdust without food or water. In this task, the number of buried marbles was evaluated, and up to at least two-thirds of the marbles were buried after 30 minutes.

Blood and tissue collection

One day after the marble-burying test, eight-hour-fasting rat offspring were euthanized via decapitation. Whole blood was collected and immediately centrifuged at 3,000 r.p.m. for 10 minutes. The brain, liver, kidneys, retroperitoneal, periepididymal, and mesenteric fats were dissected and weighed. Serum and all dissected tissues were stored in a freezer at −80°C for biochemical and redox balance assessment.

Serum selenium assessment

The serum selenium levels were determined by HG-AAS.[24] The analyses were performed in duplicate.

Evaluation of oxidative stress markers

To evaluate the repercussions of metabolic programming and investigate the role of oxidative stress in the behavioral effects of the offspring, the levels of enzymatic antioxidants and biomarkers of lipid and protein damage were determined in the brain, adipose tissue, liver, kidney, and serum.

Lipid peroxidation levels were evaluated in the brain, adipose tissue, liver, and kidney by determining the levels of substances reactive to thiobarbituric acid (TBARS). TBARS levels in brain and adipose tissues were measured as previously described,[34] except that the reaction pH was 3.4. In the liver and kidneys, TBARS levels were evaluated[35] with modifications.[36] The TBARS concentration was expressed as nmol of malondialdehyde (MDA) g tissue^{-1}, following the calibration curve for MDA.

The other analyses were performed in the serum as follows: protein carbonylation was determined by spectrophotometry after 2,4-dinitrophenylhydrazine (DNPH) derivatization,[37] with some modifications. The total carbonyl content was calculated using a molar extinction coefficient of 22,000 per M per cm and is expressed as nmol carbonyls/mg protein. Superoxide dismutase (SOD) activity was assessed by the inhibition of adrenaline oxidation and is expressed as UI SOD mg protein^{-1}.[38] Catalase (CAT) activity was determined based on the decomposition of H_2O_2, which is expressed in μmol H_2O_2 min^{-1} mg protein^{-1}.[39] Protein determination was performed by the Coomassie blue method using bovine serum albumin as a standard. The absorbance of the samples was measured at 595 nm.[40]

Statistical analysis

The data were statistically analyzed by two-way analysis of variance (ANOVA), followed by Tukey's post hoc test or Student's *t*-test, when appropriate. Differences between groups were considered significant when $p < 0.05$. The results are expressed as the mean ± standard deviation (*SD*).

Results

Chemical characterization and analysis of aflatoxins in Brazil nuts

The centesimal analysis was performed with a sample from the 2018/2019 harvest, obtaining the following percentages: 14.57 ± 0.003% for protein, 64.44 ± 0.446% for fat, 3.61 ± 0.037% for humidity, 3.62 ± 0.005% for ashes, and 13.76% for total carbohydrates. Moreover, the average selenium content of the samples from the 2018/2019 and 2019/2020 harvests was 3.855 ± 0.018.

The presence of aflatoxins B1, B2, G1, and G2 was analyzed in the samples used in the preparation of the diets (2018/2019 and 2019/2020 crops), obtaining results "below the limit detected by the equipment", i.e., almost zero or absence of aflatoxins for both. Among the wide range of known mycotoxins, aflatoxins are among the most important in Brazil due to the incidence and magnitude of aflatoxin contamination in foods and because of their toxic effects.[41] Considering these facts, the Brazilian government established a maximum limit for aflatoxins in Brazil nuts.[42] After that, and considering the good practices in Brazil nut production, several studies did not detect mycotoxins or found them below the limits established by the Brazilian government,[11] as observed in the present study.

Biometrical evaluation

Despite all mothers showing normophagy in relation to the control group, an increase of 11.1% in the initial body weight of mothers fed a high-fat diet was observed when compared to the control diet. This effect was prevented by supplementing the diet with Brazil nuts ($p < 0.05$; Table 2). Considering the adiposity index, compared with the control diet, the high-fat diet and the Brazil nut/high-fat diet induced obesity in mothers, with accumulations of excessive fat of 67.6% and 78.8%, respectively ($p < 0.05$, Table 2). Unlike body weight, body fat was not prevented by using Brazil nuts.

Behavioral assessment of offspring

Anxiety behavior

The anxiety behavior of the offspring in the elevated plus maze test is shown in Table 3. Statistical analysis revealed no significant differences in any of the evaluated parameters ($p > 0.05$).

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Table 3. Parameters analyzed in the elevated plus maze test with the offspring at 30 and 90 days of age

	oCTL	oBN	oHF	oHF/BN
30 days				
Entries in closed arms	6.7 ± 3.1	7.5 ± 3.4	7.5 ± 3.3	6.7 ± 2.7
Number of lurks	4.2 ± 3.0	5.3 ± 2.4	5.5 ± 4.1	4.3 ± 1.7
Number of dives	13.8 ± 7.0	15.5 ± 7.3	15.0 ± 6.6	14.5 ± 8.5
Anxiety index	0.68 ± 0.1	0.75 ± 0.1	0.75 ± 0.1	0.73 ± 0.1
90 days				
Entries in closed arms	5.4 ± 1.8	5.9 ± 2.4	5.0 ± 2.8	4.2 ± 2.4
Number of lurks	5.9 ± 3.2	6.5 ± 3.5	6.0 ± 2.8	4.2 ± 2.8
Number of dives	8.2 ± 3.9	9.4 ± 5.5	8.3 ± 2.7	8.3 ± 6.3
Anxiety index	0.78 ± 0.1	0.85 ± 0.1	0.80 ± 0.1	0.74 ± 0.2

Data are expressed as mean ± *SD*, analyzed by two-way ANOVA (30 days: $n = 1518$, and 90 days: $n = 1315$).

Table 4. Burying marble task with the offspring at 30 and 90 days of age

	oCTL	oBN	oHF	oHF/BN
Number of buried marbles at 30 days	17.5 ± 1.9	18.6 ± 1.9	17.5 ± 2.7	19.2 ± 0.8
Number of buried marbles at 90 days	12.2 ± 5.4	13.4 ± 5.0	13.5 ± 2.8	13.7 ± 4.2

Data are expressed as mean ± *SD*, analyzed by two-way ANOVA (30 days: $n = 1518$, and 90 days: $n = 1315$).

Therefore, maternal diet did not influence anxiety-like behavior in offspring; i.e., metabolic programming induced by high-fat or Brazil nut diets did not modify this behavior. In the present work, the marble-burying test was performed as a complement to the elevated plus maze test, and the results are presented in Table 4. Statistical analyses revealed no significant difference in the number of buried marbles ($p > 0.05$).

Cognition

Figures 2 and 3 show the effects of the different maternal diets on the cognition of the offspring in the object recognition task. Among the young animals (30 days old), the oHF group presented cognitive impairment in short-term memory (31.4%) compared to the oCTL group (Fig. 2a). However, this effect was prevented by adding Brazil nuts to the high-fat maternal diet (oHF/BN group). Two-way ANOVA revealed significant main effects of diet [$F_{(1, 60)} = 6.051$, $p < 0.05$] and treatment [$F_{(1, 60)} = 5.394$, $p < 0.05$]. Post hoc analysis revealed significant differences between all the groups and the oHF group. According to the long-term memory evaluation, compared with the oCTL group, the oHF group also exhibited cognitive impairment (19.6%) (Fig. 2b). Two-way ANOVA revealed a significant interaction effect between diet and treatment factors [$F_{(1, 68)} = 5.876$, $p < 0.05$]. Post hoc analysis revealed a significant difference between the oCTL and oHF groups.

Among the adult animals (90 days old), the oHF group presented cognitive impairment in short-term memory (30.4%) compared to the oCTL group (Fig. 3a). Two-way ANOVA revealed a significant main effect of diet [$F_{(1, 56)} = 5.226$, $p < 0.05$]. Post hoc analysis revealed a significant difference between the oCTL and oHF groups. In addition, maternal diet did not influence the cognitive behavior of the offspring during long-term memory. Two-way ANOVA revealed no significant difference ($p > 0.05$; Fig. 3b).

Locomotor (crossing scores), vertical exploration (rearing scores), and grooming activities were observed in the open field during all object recognition task sessions (data not shown). A statistical analysis revealed a significant effect of diet [$F_{(7, 239)} = 6.782$, $p < 0.05$] on the number of crossings in the central area of adult offspring in the open field. Post hoc tests showed that the oHF and oHF/BN groups were different from the oCTL group and presented a greater number of crossings. Among the other parameters observed and in all the evaluated sessions, maternal diet did not significantly influence the behavior of the respective offspring ($p > 0.05$).

Serum selenium dosage

The dosage of serum selenium administered to the 90-day-old offspring is shown in Figure 4. Two-way ANOVA revealed a significant main effect of diet [$F_{(1, 25)} = 10.46$, $p < 0.05$], and post hoc analysis revealed a difference between the oHF group and the oCTL and oBN groups ($p < 0.05$). These data showed that a maternal high-fat diet significantly decreased the serum selenium concentration in the offspring (21.6%). However, since the oHF/BN group was not different from the oCTL group, BN intake by mothers seems to mitigate the selenium deficit in the offspring.

Evaluation of oxidative stress markers

Table 5 shows the TBARS levels in the brain, adipose tissue, liver, and kidneys of the offspring. Two-way ANOVA revealed no significant difference among the groups ($p > 0.05$). Maternal diet did not influence the TBARS levels of their respective offspring in adulthood. The results of the analysis of the serum protein carbonylation and SOD and CAT activities of the offspring at 90 days are shown in Table 6. Maternal diet did not influence the serum carbonyl protein or SOD levels of the respective offspring. Two-way ANOVA revealed no significant difference ($p > 0.05$). Conversely, a reduction in CAT activity was observed in the oBN (57.4%), oHF (71.8%), and oHF/BN (52.8%) groups compared to the oCTL group. Two-way ANOVA revealed significant effects of diet and treatment interaction [$F_{(1, 28)} = 39.97$, $p < 0.05$], diet factor [$F_{(1, 28)} = 30.84$, $p < 0.05$], and treatment factor [$F_{(1, 28)} = 10.07$, $p < 0.05$]. Post hoc analysis revealed significant differences between the oBN, oHF, and oHF/BN groups and the oCTL group.

Discussion

The present study showed that supplementing the Brazil nut diet offered to mothers during critical stages of life development protects rat offspring against long-term cognitive impairment, which is programmed early by maternal obesity and seems to be associated with blood levels of selenium.

Even though anxiety did not change among young rat offspring (at 30 days old) or adults (at 90 days old) in the present study, it has been shown that a high percentage of calories from lipids in maternal obesity models increased anxiety in adult rat offspring from mothers fed a high-fat diet (60% lipids) by four weeks prior to pregnancy and until lactation.[43] Similar results were found in a high-fat diet (60% lipids)-induced obesity model in which maternal lipid intake for six weeks prior to pregnancy until lactation resulted in changes in anxiety, hyperactivity, and sociability in offspring.[44] In fact, a high-fat diet acts as a maternal

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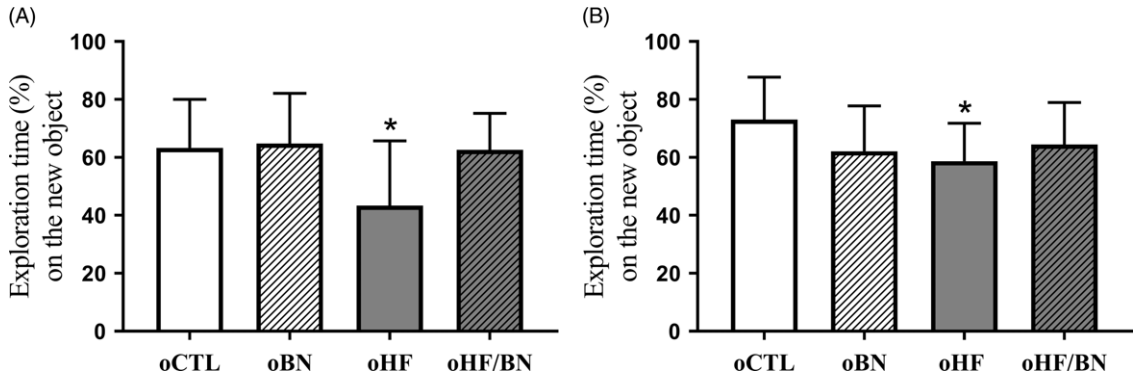

Figure 2. Exploration time of the new object (%) on short (A) and long-term memory (B) in the test sessions of the object recognition task with the offspring at 30 days of age. Data are expressed as mean ± SD, analyzed by two-way ANOVA and considering. *$p < 0.05$ compared to oCTL group ($n = 14$–17).

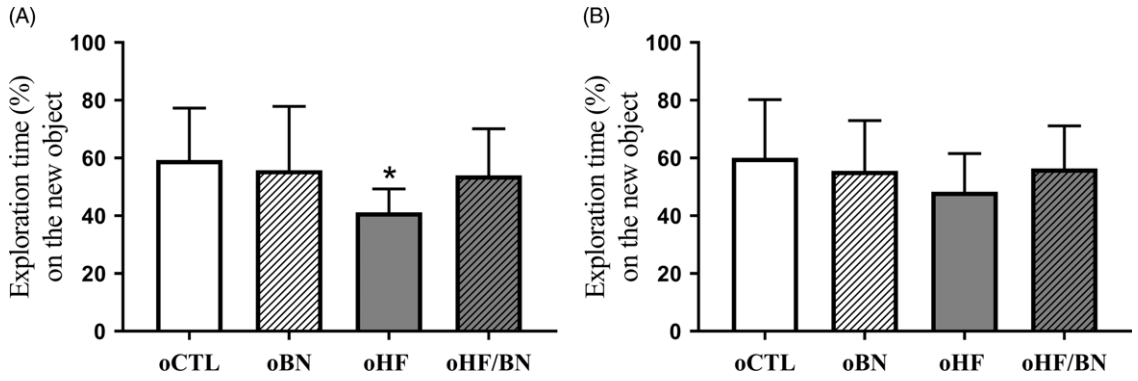

Figure 3. Exploration time of the new object (%) on short (A) and long-term memory (B) in the test sessions of the object recognition task with the offspring at 90 days of age. Data are expressed as mean ± SD, analyzed by two-way ANOVA and considering. *$p < 0.05$ compared to oCTL group ($n = 11$–14).

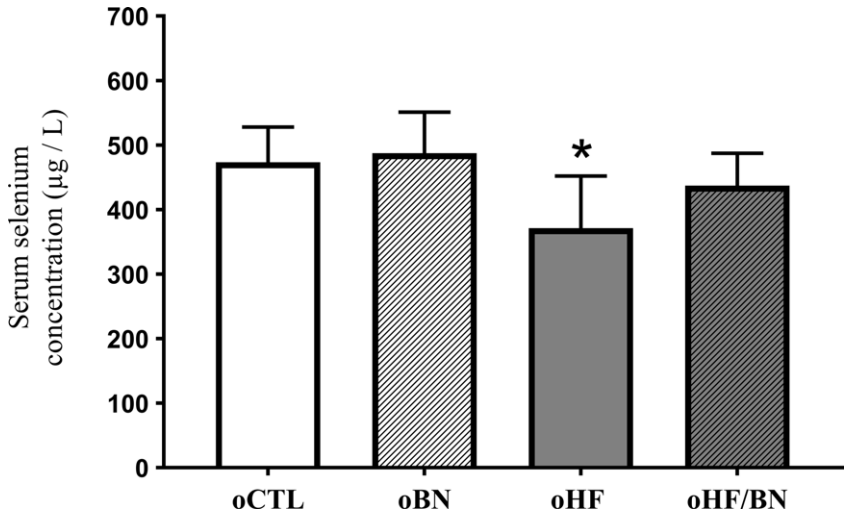

Figure 4. Serum selenium concentration (µg/L) of the offspring at 90 days of age. Data are expressed as mean ± SD, analyzed by two-way ANOVA and considering. *$p < 0.05$ compared to oCTL group ($n = 7$–8).

stress factor, impairing the neuroendocrine system and neural activity of some brain regions and negatively affecting maternal physiology and behavior.[45] These results seem to support the participation of inflammatory mechanisms underlying the changes in anxiety-related behavior. Thus, contrasting our results with the literature, it is supposed that the fat percentage (30% lipids) in the high-fat diet in the present study was insufficient to induce behavioral changes in offspring anxiety, even though it was offered

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Table 5. TBARS levels (nmol MDA g tissue^{-1}) of the offspring at 90 days of age

Tissue	oCTL	oBN	oHF	oHF/BN
Brain	20.0 ± 1.0	20.6 ± 1.6	20.1 ± 2.3	20.8 ± 1.3
Adipose tissue	11.4 ± 3.0	9.9 ± 3.5	11.8 ± 2.8	12.5 ± 3.8
Liver	44.0 ± 8.7	45.3 ± 4.5	46.8 ± 9.8	43.7 ± 6.6
Kidneys	21.7 ± 7.0	17.9 ± 4.5	19.8 ± 5.0	24.3 ± 6.8

Data are expressed as mean ± *SD*, analyzed by two-way ANOVA ($n = 68$).

Table 6. Protein carbonyl, SOD, and CAT activity in serum of the offspring at 90 days of age

	oCTL	oBN	oHF	oHF/BN
Protein carbonyl (mnol/mg of protein)	35.2 ± 4.9	34.0 ± 2.9	38.6 ± 6.2	34.2 ± 3.0
SOD (UI SOD/mg of protein)	5.4 ± 1.1	6.4 ± 1.6	5.7 ± 0.7	6.1 ± 2.7
CAT (µmol/min/mg of protein)	0.24 ± 0.05	0.10 ± 0.04*	0.07 ± 0.02*	0.11 ± 0.04*

Data are expressed as mean ± *SD*, analyzed by two-way ANOVA and considering.
*$p < 0.05$ compared to oCTL group (protein carbonyl: $n = 8$–10, SOD and CAT: $n = 8$).

for a longer time than in previous studies. Additionally, from the comparison of motor activity and risk assessment among the different ages assessed in this study, it was observed that young animals were more active than adults were. These results are consistent with previous studies showing that adolescent rats exhibit greater exploratory motivation and/or a lower degree of anxiety in new environments.[46–48]

Briefly, the results of the present study show that metabolic programming is induced by a maternal high-fat diet (HFD) and that young offspring exhibit cognitive impairment as indicated by short- and long-term memories. However, Brazil nut intake by mothers prevented this impairment of short-term memory in the offspring. In addition, the long-term cognitive impairment in short-term memory induced by the maternal high-fat diet persisted since the adult animals in this group continued to experience the same effect. Other studies have shown that a maternal high-fat diet leads to cognitive impairment in offspring. Young male offspring from obese mothers fed a high-fat diet (60% lipids) showed a decrease in the exploration time for the new object in the object recognition task.[49] Another study showed that adult offspring from rats fed a high-fat diet (45% lipids) during pregnancy and lactation exhibited alterations in the neuronal morphology of the hippocampus and amygdala.[50]

At the cellular level, maternal obesity results in cognitive dysfunction in offspring, which is related to oxidative stress and inflammatory activation.[51–54] In the early stages of life, impaired hippocampal neurogenesis induced by increased TBARS levels was found in offspring from mothers fed a high-fat diet.[51] Persistent synaptic deficiencies in adult offspring from obese rats were observed, which may be due to the accumulation of lipid peroxidation during the lactation period.[52] There were marked changes in anxiety and spatial learning in the experimental groups in adulthood, even after the consumption of a standard diet after weaning, suggesting programming in the early stages of life.[54] Therefore, a maternal high-fat diet leads to oxidative stress in offspring, with increased levels of lipid peroxidation and decreased activity of antioxidant enzymes, including glutathione peroxidase (GPx), during early life.[50,55,56] Nevertheless, it seems that the duration of exposure to diet and the percentage of fat are directly related to such effects. The maternal diet must contain approximately 47% fat to propagate oxidative stress in adult offspring approximately 90 days old.[57] In addition, a comparative study showed that a high-fat maternal diet with 32% fat, offered during the preconception, pregnancy, and lactation periods, triggers oxidative stress in early life, but this effect was not observed later, at 70 days of age.[50] Indeed, in the present work, the adult offspring presented no differences in the oxidative stress biomarkers evaluated but only a reduction in catalase activity. A reduction in the serum catalase concentration suggests increased consumption of antioxidant defenses since the role of this enzyme is to remove H_2O_2, which mainly attacks the polyunsaturated fatty acids present in cell membranes.[58]

However, despite the deleterious effects of maternal obesity on offspring, our data showed that maternal dietetic supplementation with Brazil nuts prevents cognitive damage. Corroborating this result, a randomized controlled trial showed that daily consumption of Brazil nuts for 6 months improved cognitive function in older adults with mild cognitive impairment. In the supplemented group, an increase in the serum selenium concentration and an improvement in the antioxidant status were also observed, suggesting that the intake of Brazil nuts restores selenium deficiency with positive effects on some cognitive functions.[59] Nevertheless, the beneficial effect of nut intake on cognition seems to be more favorable for individuals who are at higher risk of cognitive impairment, as nut intake has inconsistent effects on adults and older people with no cognitive decline.[18] In fact, our results showed a positive effect only in the offspring with cognitive impairment (oHF group).

To our knowledge, this is the first study to show that the adult offspring of obese mothers have low serum selenium levels. In turn, supplying Brazil nuts to obese mothers prevented a reduction in the serum selenium concentration in adult offspring. It is known that selenium requirements increase during pregnancy when there is an increased demand for oxygen in the body of the mother and fetus, which can lead to increased production of ROS.[60] These micronutrients are incorporated into selenoproteins, which are involved in cellular antioxidant mechanisms,[61] especially as cofactors of GPx.[62] During lactation, there is a new increase in the selenium requirements of mothers.[12,29] This event is related to the increased demand for this micronutrient in the offspring because, despite the neonate having a selenium reserve, it still depends on its supply through breast milk.[63,64] Therefore, breastfeeding becomes crucial for maintaining optimal selenium status in offspring, considering that it is provided through maternal milk.[64,65] Several studies have shown the importance of selenium in the understanding of offspring. Organic selenium improves lead-induced deficiencies in spatial learning and memory in a time-dependent manner.[66] Nevertheless, the effects of selenium on cognition are complex. A study from the Spanish Childhood and Environment Project (INMA, 2003–2012) showed a nonlinear association between the concentration of serum selenium in mothers at the end of the 3rd trimester of pregnancy and the neuropsychological development of children at 5 years of age. Generalized additive models indicated inverted U-shaped relationships between the serum selenium concentration and verbal and global memory scale scores. Thus, high and low maternal selenium concentrations seem to be detrimental to

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neuropsychological development in children.[67] Additionally, selenium also plays an essential role during gestation due to its involvement in the metabolism of thyroid hormones, given that a deficiency of this micronutrient is closely associated with a reduction in triiodothyronine levels.[68] A review study indicated that clinical or subclinical hypothyroidism leads to neurodevelopmental impairments in the cerebral cortex, hippocampus, and medial ganglionic eminence in fetuses. Considering that such changes are correlated with behavioral and cognitive impairments in offspring,[3] the absence of thyroid function assessment in both mothers and their offspring constitutes one of the limitations of our study.

In turn, Brazil nuts are recognized as an important source of selenium.[11,12] In the present work, concerning neurobehavioral development, it is assumed that the offspring of the oHF/BN group benefited from maternal intake of this micronutrient. Previous works have shown that the consumption of Brazil nuts improves the deficiency in plasma selenium levels observed in obese women[69] and increases the concentration in obese women[70] and adolescents[71] with adequate nutritional status of this mineral. However, the beneficial effects of Brazil nuts must not be limited to selenium supplementation since the presence of polyunsaturated fatty acids and phenolic compounds also plays a role in oxidative and inflammatory events.[11,72,73] This is another limitation of our study because, although we highlighted selenium, we cannot ensure that the effects observed in this study were exclusively attributed to this micronutrient under these experimental conditions. In addition, analysis of oxidative stress and antioxidants in other tissues, as well as plasma selenium concentrations in mothers, newborns, or young animals, could help clarify a causal relationship with the observed cognitive effects. Furthermore, we consider it important to expand our investigation to compare the sexes, despite the added variability due to the oestrus cycle.

Conclusion

Maternal obesity due to consumption of a high-fat diet during preconception and throughout pregnancy and nursing periods reduced the serum selenium concentration in adult rat offspring and led to cognitive impairment of short- and long-term memory in the rat offspring at young ages and in adulthood, which was prevented by maternal consumption of a diet supplemented with Brazil nuts. Given that the long-term effects of maternal obesity can have profound implications for public health, additional investigations are needed to elucidate the underlying mechanisms involved. Our data highlight the importance of adequate maternal nutrition, including functional foods, especially during the preconception, pregnancy, and rat offspring suckling phases, for the programming of younger rats' health status throughout life.

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Competing interests. None.

Ethical standards. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national guides on the care and use of laboratory animals (Comissão de Ética no Uso de Animais – CEUA) and has been approved by the institutional committee (process no 23,108.017929/2019-93).

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