Effect of the consumption of a hypoproteic diet in pregnancy on brain and behavioral disorders in the postnatal stage

Efecto del consumo de una dieta hipoproteica en el embarazo sobre las alteraciones cerebrales y conductuales en la etapa posnatal

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Abstract

Feeding behavior during pregnancy is essential to fetal development, growth, and survival. The consumption of hypoprotein diets in this stage has a negative impact on the central nervous system (CNS) and sensorimotor skills, learning, and memory. This research summarizes the effects of low-protein diet intake during pregnancy in the brain and the behavioral responses of offspring. This review used information with the inclusion of some terms such as pregnancy, maternal, neonatal, and postnatal events, in combination with consumption, high protein diet, CNS, and behavior. The search was carried out in PubMed (May - Jun 2021) and was limited to the period from 1996 to 2021; consensus, positioning publications, reviews and meta-analyses were excluded. Twenty-three original research articles, in murine and human models, identified to integrate the current importance of low-protein diet intake. On the morphological and functional alterations in the postnatal brain and behavior.

Hippocampus, Low-protein, Malnutrition, Pregnancy, Postnatal age

Resumen

La alimentación durante la gestación es fundamental en el desarrollo, crecimiento y supervivencia fetal. El consumo de dietas hipoproteícas en dicha etapa impacta negativamente en estructuras del sistema nervioso central (SNC) y en la ejecución de habilidades sensoriomotoras, aprendizaje, memoria, entre otras. Esta investigación resume e integra la evidencia científica de los efectos del consumo de una dieta hipoproteica durante el embarazo sobre los mecanismos cerebrales y conductuales de la descendencia en la etapa posnatal. En la revisión se empleó la búsqueda de información con la inclusión de términos: embarazo, eventos maternos, neonatales y postnatales, en combinación con consumo, dieta hipoproteica, SNC y conducta. La búsqueda se realizó en PubMed (mayo - jun de 2021) y se limitó al periodo de 1996 a 2021; se excluyeron consensos, publicaciones de posicionamiento, revisiones y metaanálisis. Se identificaron artículos de investigación original, en modelos murinos y en humanos, para integrar la importancia actual de consumir dietas hipoproteicas durante el embarazo sobre las alteraciones morfológicas y funcionales en el cerebro posnatal y como esto repercute en la conducta.

Hipocampo, Baja proteína, Malnutrición, Embarazo, Edad posnatal

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Introduction

Proteins are macronutrients in the diet that present nutritional characteristics such as digestive kinetics, amino acid sequence, bioactive peptides; these molecules determine structural functions in the body (Mansilla et al., 2020). Protein intake during pregnancy is a topic of current interest in health, because the functions that manifest during this reproductive stage are involved in the development and formation of structures, organs, and systems, as well as in intermediary metabolism (Waksmańska et al., 2020). Previous studies indicate that an adequate intake of protein during pregnancy should be in a proportion of 18 to 20% of the total macronutrient (Sellayah & Cagampang, 2018). In humans, it is suggested that normal protein intake during pregnancy should be 60–100g per day ("Maternal Anthropometry and Pregnancy Outcomes. A WHO Collaborative Study", 1995). The recommended protein intake is 0.9 g of protein/kg of body weight/day in the first-second trimester and 1.0 g of protein/kg of body weight/day in the third trimester (Butte & King, 2005; «Maternal Anthropometry and Pregnancy Outcomes. A WHO Collaborative Study», 1995).

Diets with a low proportion of protein or hypoproteinic are those whose content is lower than required; these diets can be isocaloric, hypocaloric, or hypercaloric, among the diets that have been described, they were found in a range of 6 to 9% protein (Sellayah & Cagampang, 2018). The low protein diet consumed during pregnancy indicates a negative impact on the maternal environment and the health of the offspring, which were investigated in experimental preclinical models (Langley-Evans et al., 1996; Rees et al., 2000; Zambrano et al., 2006). It has been described that the consumption of low-protein diets during pregnancy produces modifications in body weight, blood pressure, sugar and fat metabolism, intake, adiposity, both in the maternal environment and in offspring (Langley-Evans et al. al., 1996; Rees et al., 2000), In addition, excessive consumption of a low-protein diet during pregnancy is associated with adiposity and obesity increased.

In western countries, obesity affects up to 30% of pregnant women, while 40% of women gain excess weight during pregnancy (Mansilla et al 2020). Consumption of this type of diet is an indicator of negative changes in fetal development, proposed as a factor leading to an increased risk of developing chronic conditions in adulthood. Previous studies indicate that cardiovascular diseases are due to an adverse environment in the early stages of development with a high degree of appearing during adulthood (Barker et al., 2002) It is known that low birth weight is an indicator of protein malnutrition during the perinatal stage and serves as a baseline of slow fetal growth (Intapad et al., 2014). A direct association between low protein intake during pregnancy, placental weight, and birth weight has been reported (Jahan-Mihan et al., 2011, 2012). In humans, studies showed that offspring has a lower body weight compared to pregnant women who consume a higher proportion of protein (Mannion et al., 2006). It is not only important to consider the proportion of proteins in the diet inthe health of the mother and the offspring, but also the source of them since it has been suggested that the consumption of proteins of plant origin has a differential influence on weight and the body composition of the offspring (Waksmańska et al., 2020). In the postnatal stage, it was observed that the risk of developing the metabolic syndrome caused by the consumption of a low-protein diet during pregnancy increases (Moore et al., 2004).

Prospective studies in women with singleton pregnancies indicate that the body composition of the fetus can be modified through nutritional intervention in the mother. This suggests that maternal nutrition during pregnancy cannot only lead to variations in the proportion of fetal fat and muscle, but also in fat deposits in different body areas of the maternal environment (Blumfield et al., 2012). There is a positive relationship between the abdominal visceral adiposity of the fetus and the consumption of maternal protein and it is suggested that carbohydrate metabolism could also intervene in the body composition of the fetus; the result could be reflected in the postnatal stage.

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In a study in women with a normal body mass index (BMI), it has been reported that the replacement of carbohydrates by an animal protein source could have a greater risk of developing a BMI> 25, both in male and female offspring, which resulting in a risk of obesity in the postnatal stage (Maslova et al., 2014).

Currently, this field investigates the role of protein metabolism.

The proteins could be involved in:

1) Regulation of digestion.

2) The absorption of specific amino acids in embryonic growth.

3) The modification and functioning of the intestine.

4) Conception and development of pregnancy.

5) Changes in the production of metabolites such as nitric oxide (NO) and glutathione.

6) Cell signaling and reproduction.

7) The performance and quality of breastfeeding.

Therefore, an adequate dietary intake of not only protein (taking into account its source and quality) is highly necessary for optimal intestinal and reproductive health (Dai et al., 2015; Gohir et al., 2019).

Methodology to be developed

Material and methods

The bibliographic review was done using the search for information and included terms related to pregnancy, maternal, neonatal, and postnatal events, in combination with several terms related to the consumption of a hypoproteic diet, effects on the nervous system, and behavior. The search was carried out in PubMed (May and June 2021) and was limited to indexed publications in the English language in a period spanning from the year 1996-2021; Consensus, positioning publications, reviews, and articles that report a meta-analysis were excluded.

The review of titles and abstracts were carried out by one person and was corroborated by a second one; four people carried out the information extraction of the maternal and neonatal events and the discrepancies were solved by consensus between the co-authors.

Data analysis

The original articles in which the effect of consuming a low-protein diet during pregnancy and/or lactation on the neurodevelopment of the offspring was reported, as well as their behavioral response, was compiled, organized, and expressed in this review to clarify, summarize and integrate these various reported effects.

The possible effects that have been observed in the works compiled in this review were grouped taking into account the morphological and functional effects that occur in the central nervous system (CNS), as well as the alterations of different types of behavior such as nest behavior. Room, sensory-motor skills, learning, memory, anxiety, and stress responses. The information obtained as described in the different sections of this review and summarized in two tables.

Results

Regarding the effect of the consumption of a low-protein diet during pregnancy, 23 original research articles were identified, to summarize the current importance of the intake of low-protein diets during pregnancy. As well as the general description of low protein diets. This review included a total of 23 original articles, which were conducted in murine models and humans, and published in international journals. Nine articles were identified that presented morphological and functional alterations in murine models in the brains of neonates whose mothers were exposed to a low protein intake, these studies report neurochemical changes in the brains of neonates, a decrease in BDNF expression was observed, this alteration is related to deficit in cognitive processes. In the same way, the effect of the hypoprotein diet during pregnancy on the morphological and functional alterations in the brain of the offspring was identified, 9 original articles were identified, of which 100% were presented in an experimental model of rodents.

Of this total, 88.88% of the studies were evaluated with a comparison between control and experimental groups.

Regarding the effect of the consumption of the low-protein diet during pregnancy on the behavior of the offspring, 20 original research articles were identified, of which 7 publications were discarded since low-protein diets were used and in turn modified these diets to be high in fat and/or high in glucose and fructose; this type of diet can produce effects at the brain level and on the behavior of the offspring associated with the addition of modified elements in the diet that are not specific to the consumption of a low-protein diet during pregnancy. Therefore, in this section of the review, a total of 13 original articles were included, of which 100% were carried out in rodent models (rat and mouse), as well as published in international journals as research articles, 100% of these publications had control groups with which statistical comparisons were made.

Therefore, the consumption of a low-protein diet during pregnancy causes multiple alterations in the offspring, mainly metabolic alterations that can be reflected in the short, medium and long-term, however, it is extremely important to consider that this availability of macronutrients (proteins) put neurodevelopment at risk, observing some morphological and functional alterations in the CNS and these changes, in turn, produce modifications, deterioration, and alterations in various behaviors throughout the life of these malnourished offspring.

Morphological and functional alterations in the CNS

From studies carried out in experimental animal models, it has been possible to identify changes caused by the restriction of proteins in the maternal diet during pregnancy in the CNS, such as alterations in brain chemistry and behavior, in addition , the diet impacts the neuronal and brain structural conformation, that is, alterations at the morphological level that mainly affect the level of neuronal positioning and the connections between nerve cells (Alamy & Bengelloun, 2012). Currently, few studies focus on these morphological changes caused by exposure to a hypoprotein diet during pregnancy in the offspring, however, several authors have pointed out some aspects that highlight these alterations.

In a current study, Marwarha et al. (2017) focused their objective on determining, at the molecular level, the impact of a maternal low-protein diet (8%) on the expression of brain-derived neuronal factor (BDNF) and found a significant decrease in BDNF levels in both male and female offspring whose mothers received a low-protein diet. BDNF is an important modulator of neurotransmitter release and synaptic plasticity (Rosche et al., 2013). Likewise, a decrease in the transactivation of the promoter in BDNF DNA was evidenced, driven by CREB (cAMP response element-binding protein), an intracellular protein that regulates the expression of genes that are important for synaptic plasticity. A decrease in BDNF expression in the brain results in induction and maintenance of long-term potentiation (LTP) impairment, which influences learning and memory processes; Furthermore, these molecules play an indispensable role in neuronal development and adult neurogenesis.

In another study, Feoli et al. (2008) reported changes in the protein levels used in the glial cells detection in the brain of rats exposed to a low-protein diet (7% casein), specifically the authors relied on glial fibrillar acid protein (GFAP) and the immunocontents S100B, as well as glutamine synthetase (GS) in the cerebral cortex, hippocampus, cerebellum, and cerebrospinal fluid, in PND 2, 15 and 60. In this study, an increase in GFAP, S100B and GS was observed in the cerebral cortex, hippocampus, and cerebellum in DPN 2, 15, and 60, which suggests astrogliosis; the astrogliosis consists of changes in gene expression with a regulation pronounced GFAP, cell hypertrophy, the proliferation of sparse astrocytes, and some loss of individual astrocyte domains with an overlap of neighboring astrocyte processes associated with oxidative stress induced by low levels of protein consumed during gestation (Khakh & Sofroniew, 2015). However, a significant increase in the levels of S100B in the cerebral cortex was only evidenced at P15 in the group with reduced protein. However, after an increase in the levels of S100B in the cerebrospinal fluid, during the early stages of development, its levels remained high until postnatal day 60 compared to the control group (Khakh and Sofroniew, 2015).

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These results infer a neuroinflammatory process induced by oxidative stress that occurs due to the lack of normal protein levels during neurodevelopment and its effects can be maintained in the long-term in the offspring. Research showed results obtained from examining the effect of a protein-limited diet (5% casein) during the first two weeks of gestation on brain development in progeny. In the present study, various alterations were identified in PND 0, 7, and 14, such as 1) delayed astrogliosis demonstrated with reduced GFAP staining, 2) abnormal neuronal differentiation identified by reduced expression of Microtubule-Associated Protein 5 (MAP-5) and increased expression of Microtubule Associated Protein 1 (MAP-1), 3) abnormal synaptogenesis evidenced by increased expression of synaptophysin in the basal ganglia and 4) decreased apoptosis. Furthermore, a reduction in cerebral cortical thickness was observed in progeny with protein malnutrition during embryonic development (Gressens et al., 1997).

In another study, Cintra et al. (1997), carried out an investigation and evaluated the effect of the diet with protein restriction (6%) in the prenatal stage in PND 15, 30, 90, and 220. Specifically, was evaluated the mossy fiber plexus representing the axonal projection of the granule cells of the dentate gyrus to the CA3 and CA4 areas of the hippocampus. The rats exposed to prenatal protein malnutrition showed significant deficits in the total face-caudal extension and volume of the projections at PND 15, 90, and 220, with the most marked effect on PND 220. These results suggest that protein malnutrition during pregnancy is capable of affecting axonal fibers in the trisynaptic circuit of the hippocampus, and alters the information processing of the hippocampal network in the progeny.

Debassio et al., (1994) made a publication where they investigated the effects of protein limitation during gestation in the hippocampus of offspring, on the generation of granular and pyramidal cells. The rodents were administered tritiated thymidine to mark new neurons on embryonic days 12, 16 or 20, since it is incorporated into the DNA at the time of its replication, thus detecting the cells that enter the S phase of the cell cycle (Rahirant et al., 2000), the brains were obtained at DPN 30.

The authors evidenced a significantly decreased neurogenesis in rats injected on embryonic day 20; however, no significant change was demonstrated on days 12 and 16. On the contrary, in the case of pyramidal cells, no significant alteration was found in any group, which indicates a differential effect of prenatal malnutrition in the generation of these two neuronal populations in the formation of the hippocampus in the final stage of gestation.

Later, Debassio et al. (1996), examined hippocampal granule cell neurogenesis in postnatal rodents on the same diet model. Rodents were administered tritiated thymidine at PND 8, 15, and 30, brain tissues were obtained, or, in an independent group, tritiated thymidine was administered at PND 30 and brains were obtained at PND 90. The authors noted that in the dentate gyrus, a significant decrease in granule cells was observed in rats injected with tritiated thymidine at PND 18, but not at PND 15. In contrast, the number of granule cells in rats exposed to the diet was significantly increased on PND 30, as a possible compensatory mechanism for the loss of specific neuronal groups.

Malnutrition in early life, as discussed above, affects serotonergic and dopaminergic neurotransmission, as well as the morphology of the hippocampus structure. One of the key factors on which brain function depends on is the availability of the neurotransmitter molecule for release in the synapse, so this alteration in the neurotransmitter scale may be the basis of the functional deterioration shown by the undernourished groups (5 or 10% protein) (Abey et al., 2019). Guest et al. 2012, analyzed in a murine model, the exposure of a low-protein maternal diet (8%) that induces changes in the concentration of hormones such as insulin and leptin, as well as a decrease in inflammatory and endothelial-related proteins, such as osteopontin, MCSF1 (macrophage colony-stimulating factor 1) and VCAM1 vascular cell adhesion molecule 1). In this study, an analysis was performed to identify altered molecular pathways in the frontal cortex and tissue of the hypothalamus and synaptic remodeling of the hypothalamus (Guest et al., 2012)

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For their part, Reyes-Castro et al. (2018), evaluated the effect of the consumption of a hypoprotein diet (10% casein) during pregnancy and/or lactation on the neural structure of the hippocampus of male offspring, specifically in the area of the mossy fibers of the stratum lucidum, the total density and the type of dendritic spine in the basal dendrites in the stratum oriens in the CA3 area of the dorsal hippocampus. The area of mossy fibers (marked with the Timm technique), the total density of dendritic spines, and the long and fungal dendritic spines were lower in the groups with protein restriction during pregnancy, lactation, or with protein restriction in both periods.

From the evidence presented over the years, it is possible to highlight the changes produced at the morphological level in the brain, which shows the influence of the low protein diet during pregnancy on the brain structure; However, some limitations persist in the literature, since at the moment there are few existing studies, which suggests contributing knowledge about other brain structures different from those mentioned (Table 1).

Behavioral alterations

To determine the effect that the consumption of a low-protein diet during pregnancy and lactation produces on cognitive and non-cognitive behavioral responses, the study of animal models is used, mainly rodents (mice and rats). In these models, diets with a low percentage of protein, mainly casein, have been administered from the preconception, conception, pregnancy, and/or lactation period, depending on the question to be answered.

Belluscio et al. (2014) conducted a study in CF-1 mice that were administered a low-protein diet (9% casein) or a control diet (20% casein) during conception, gestation, and lactation stages. Up to DPN 21, the offspring that developed embryo and that was lactated under the influence of this type of diet and evaluated at different ages in a battery of different behavioral paradigms, between the PND 2-10 neurological reflexes were evaluated, an increase in time was found It took the female and male calf to straighten up once it was placed in a position opposite to normal.

PND 14-18 between the auditory startle was evaluated in male and female offspring and found a decrease in the percentage of individuals with positive responses, however, in the PND 12-20 no change was observed in grip strength. In PND 22-26, a group of tests related to gambling behavior were evaluated, It was found that both female and male offspring decreased the number of events associated with nasal, anogenital, and body sniffing of their congeners, as well as a decrease in the number of frontal focus events with other individuals, a decrease in the number of escape attempts from their box-room, and the number of successful escape episodes, Regarding the behavior of motor activity, an increase was found in the time it takes for the offspring to enter the center area of the box where this test is done, a decrease was also found in the distance traveled and the time of permanence in the center of the arena and a decrease in the number of upright events (Belluscio et al., 2014). To evaluate behaviors associated with anxiety, elevated plus-maze task was performed, in which they found a decrease in the total distance traveled in this maze, as well as a decrease in the distance traveled and the speed in the open arms. Subsequently, a social interaction test was performed in which no effect was found on the preference of exploration and interaction with another congener (Belluscio et al., 2014) (Table 2).

A more recent study was carried out in female mice of the C57BL/6J strain, to which they were administered a low-protein diet (7.5% casein) during the preconception, gestation, and lactation stages (until weaning), to evaluate whether the restriction of the protein during embryonic development and lactation could induce anxiety and stress behaviors, it was found that in the offspring from these mothers there is an impairment in the performance in the open field, the light-dark test and the stress hyperthermia test (anxiety-related behaviors) and forced swimming performance and the response to stress due to movement restriction (stress-related behaviors) (Schoenrock et al., 2018) (Table 2).

Taking into account that the effect produced by these low-protein diets is observed after an administration of these diets in a very wide time window, Gould et al. (2018) used MF-1 mice that were distributed in three groups, in the first a diet low in protein (9% casein) was administered to pregnant females (the entire pregnancy), in the second the same diet was administered to pregnant females from G0.5 to G3.5, this to evaluate a circumscribed time window and in the third, the control diet was administered (18% casein), the offspring were evaluated with the object recognition task in PND 41, 64 and 96 to assess object recognition memory, As a result, a significant decrease in the discrimination index was found only in the group that was administered the low-protein diet in G0.5-G3.5 (Gould et al., 2018) (Table 2).

Most studies in this area of research were performed in rats of different strains. One of the first studies was carried out by Almeida et al. (1996), they administered a low protein diet (6% casein) to Sprague Dawley rats during preconception and pregnancy, since during lactation they were given a control diet (25% casein), the offspring of 70 days old were given a test in the elevated plus-maze to evaluate the anxiolytic response, within the results, a lower number of attempts to enter the open arm was found, a greater number of entries to these same compared to the other arms of the maze, a greater latency of entry to the open arms, these data suggest that malnutrition in pregnancy reduces anxiety behaviors in this test, since in females *per se*, very high anxiety behaviors are observed (Almeida, Tonkiss, & Galler, 1996) (Table 2).

Other research has provided information about the effect of low-protein administration (10% casein) during pregnancy, lactation and both stages in Wistar rats, the offspring of these mothers were evaluated in the DPN 120 in two tests, the first consisted of measuring the free consumption of sucrose, this in order to assess whether these subjects had anhedonia, however, no effect was found, the second test consisted of evaluating a type of associative learning (operant conditioning), in which the rats had to press a lever to obtain a reward (drink), the offspring that received the low-protein diet during pregnancy show a greater trials number to conditioning (Reyes-Castro et al., 2011).

On the other hand, the offspring that received the low-protein diet in pregnancy or lactation or in both stages show a greater requirement of learning trials to achieve conditioning, this occurred given that they presented a lower response to the reinforcer (Reyes-Castro et al., 2011).

A later work by this same research group in which they used the same experimental groups, the offspring in PND 90-92 were evaluated in the highest maze, in which a statistical decrease was found in the time of permanence and the distance in the open arms, suggesting that normal anxiety responses are reduced in animals on a low-protein diet. In PND 91-93, motor activity was measured in the open field, where a decrease in the number of entries and the distance traveled in the center of the field was observed, which shows us that there is an alteration in motor activity. In PND 110-135, operant conditioning was evaluated and it was observed that a greater number of trials are required to acquire this information, which indicates that there is an alteration in associative learning and a lower response to the positive reinforcer in PND 135-146. In DPN 147-150 anhedonia behavior is not observed, nor was a stress response observed in DPN 220 in the movement restriction (Reyes-Castroet al., 2012), However, if this movement restriction is carried out in PND 90-95, there is an exacerbated response to stress in the groups that were under the effect of a protein-restricteddiet (Reyes-Castro, Rodríguez, Rodríguez- González, et al., 2012). In the DPN 110 they were evaluated in the Morris water maze, where an increase in escape latency was observed during learning and an increase in the number of entries, permanency time, and distance traveled in the area opposite the target area where the escape platform is located, these results show that the low-protein diet during embryonic development and/or in lactation or both produces learning and spatial memory deficit (Reyes-Castro et al., 2018) (Table 2).

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Some studies, in which a diet low in protein (6% casein) has been administered to rats of the Wistar strain from G1 to L2 show that at 7 days of lactation they present a change in maternal behavior, reflected as an increase in the recovery of offspring, a higher percentage of time spent caring and grooming the offspring, this was observed after a protocol of maternal separation for 8 hours, in offspring, at PND 5 an increase in ultrasonic vocalizations was reported (Batista, Veronesi, Ribeiro, Giusti-Paiva, & Vilela, 2017), in the PND 84 no effect on the test of elevated plus-maze was observed; however, in DPN 97-100 an increase in the stress response was found as a decrease in the swimming response, as well as an increase in immobilization in the forced swim test and a decrease in food intake after swimming stressful test compared to controls (Ye et al., 2018).

However, if the low-protein diet (6% casein) was administered from G1 to L22, the 5-day-old offspring decreased the ultrasonic vocalizations, in PND 13 an alteration in the "homing" behavior is observed, that is, a decrease in the time spent in the nest and an increase in the time spent searching for the nest when leaving it. In PND 30-32, the object recognition task was carried out and an increase in the exploration time of the familiar object was observed during the learning stage, a decrease in the exploration time of the novel object and a lower recognition index in the retention test, these results show that the offspring present cognitive impairment, a social interaction test is also performed and a decrease in the time and percentage of exploration of a congener is observed, that is, they avoid interacting with other members of their species (Batista, Giusti-Paiva, & Vilela, 2019).

In PND 56, the test of social interaction, stress response after body immobilization, disease behavior was performed and no effect was found. However, if an immune response is induced in these offspring through the administration of an endotoxin known as lipopolysaccharide and an alteration in social behavior is observed, these results demonstrate that the alterations caused by a low-protein diet can synergize with a maternal immune response to modify social behavior (Batista, Ribeiro, Kalil, Giusti-Paiva, & Vilela, 2020) (Table 2).

In recent work, on rats, Sprague Dawley strain administered a low-protein diet (8% protein) during preconception, gestation, and lactation. In the perinatal stage, some tests of sensory-motor skills were carried out on the offspring, in PND 2-8 a decrease in the negative geotaxis reflex was observed, and in PND 3-11 a significant decrease in the cliff-type fall avoidance reflex. The offspring underwent different behavioral tests at 3, 6, and 12 months old. In the motor activity test, an increase in the total distance traveled, a longer movement time, a greater number of entrances to the center of the field, and a decrease in the rest time was observed. In the rotarod test, an increase in wheel fall latency and lower neuromuscular force were observed, demonstrating acceleration in abnormal stereotypic behavior.

In the elevated plus-maze test, an increase in the percentage of time and the number of entries to the open arm was observed, as well as an increase in the percentage of time and the number of entries to the luminous zone in the light / dark test, the results in these tests suggest an anxiety profile, a decrease in the percentage of sucrose preference is also observed, which is typical as an anhedonia response. Concerning associative learning, conditioning was carried out to evaluate conditioned behavior, an increase in the avoidance and escape response and a lower freezing rate was observed, which indicates a deterioration in learning. To evaluate learning and spatial memory, the behavior in the Barnes maze was evaluated, a decrease in the efficiency of the arrival route to the escape box and an increase in the time it takes to get there was observed, to this escape box, all these results were observed in the three ages, at 3, 6 and 12 months old (Sinha, Patro, & Patro, 2020) (Table 2).

With the previously mentioned data, evidence shown that protein restriction during embryonic development and lactation in offspring produces multiple physiological metabolic and brain alterations that are reflected in the behavioral alterations of individuals.

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Conclusion

Adequate protein intake during pregnancy is involved in optimal functions such as development, CNS formation, and intermediate metabolism. A diet with a low proportion of proteins during pregnancy has a negative impact on the health of the offspring and the maternal environment, which has been investigated in experimental models and in humans, damage to morphology and functional mechanisms in the offspring's brains have been reported in these studies, which are reflected in nest behavior, motor behaviors, cognitive functions such as learning, memory, anxiety and stress responses. Therefore, the inadequate consumption of these micronutrients during pregnancy is currently considered a health problem in the reproductive stage with long-term effects.

Abbreviations

DNA Deoxyribonucleic Acid

CAMP Adenosine Cyclic Monophosphate

BDNF Brain Derived Neurotrophic Factor

CREB Response Element Link to cAMP

DPN Postnatal Day

GFAP Glial Fibrillar Acid Protein

GS Glutamine Synthetase

LC - MS Liquid Chromatography with Mass Spectrometry

MAP-1 Microtubule Associated Protein 1

MAP-5 Microtubule Associated Protein 5

MSCF1 Macrophage Colony Stimulating Factor

PKA Protein Kinase A

S100B Calcium-binding protein B S100

CNS Central Nervous System

VCAM-1 Vascular cell adhesion protein 1

Annexes

Timing of exposure to diet	Age of offspring at postnatal analysis	Analyzed characteristic	Effect	Reference
Gestation	PND 30	Pyramidal and granular cells of the hippocampus	Decrease in the cell population	Debassio et al., 1994.
Gestation	PND 90	Granular cells of the dentate gyrus of the hippocampus	Decrease in the cell population	Debassio et al., 1996.
First two weeks of gestation	Embryonic day 15 PND 0, 7 y 14	Brain development, cytoarchitecture, and cell differentiation Brain development, cytoarchitecture, and cell differentiation	Decreased cerebral cortical thickness Decreased cerebral cortical thickness Delayed Astro cytogenesis, abnormal neural differentiation, abnormal synaptogenesis, decreased apoptosis	Gressens et al., 1997.
	PND 21 and PND 63	Brain development, cytoarchitecture, and cell differentiation	Decreased cerebral cortical thickness	
Gestation	PND 15, 30, 90 and 220	Development of the mossy fiber plexus in the formation of the hippocampus	Deficits in total face-caudal extension and plexus volume	Cintra et al., 1997.
Pregnancy, lactation, and up to PND 60 in offspring	PND 2, 15 and 60	Changes in the glial based on GFAP, GS, and S100B	Differential GFAP, S100B, and GS changes in the cerebral cortex, hippocampus, and cerebellum	Feoli et al., 2008.
Three weeks before mating until the end of gestation	PND 0-1	BDNF expression	Decreased expression of BDNF	Marwarha et al., 2017.
Gestation and / or lactation	PND 110	Neural structure of the hippocampus	Decrease in spines of mossy fibers and increase in stubby spines (immature)	Reyes-Castro et al., 2018.
Gestation, lactation and gestation	PND 80	Brain serotonin and dopamine concentrations	Altered neurotransmission in the brain of the F1 generation.	Abey et al., 2019
Gestation and lactation	PND 82-86	Measurement of analyte concentrations in serum of three-month-old rats	Lower levels of adiponectin and increased levels of osteopontin, a tissue inhibitor of metalloproteinases 1 (TIMP1), macrophage colony-stimulating factor 1 (MC SF1) and vascular cell adhesion molecule 1 VCAM1 in low protein offspring	Guest et al., 2012

Table 1 Morphological and functional effects of the low-protein diet during pregnancy on the brain of the offspring

Timing of the low-protein diet	Age of offspring on behavioral assessment (PND)	Behavioral paradigm	Effect on the behavioral test	Reference
Preconception, pregnancy, and lactation	70	Elevated labyrinth	Decreased anxiety response	Almeida et al., 1996
Gestation and lactation	120	Operant conditioning	Decrease in associative learning	Reyes-Castro et al., 2011
		Sucrose preference	No effect	
Gestation and lactation	90-92	Elevated plus-maze	Increased anxiety response	Reyes-Castro et al., 2012
	91-93	Open field		
	110-135	Operant conditioning	Decrease in associative learning	
	135-146	Reinforcement	Decreased responses to stimuli	
	147-150	Sucrose preference	No effect	
	220	Stress response	No effect	
Gestation and lactation	90-95	Elevated plus-maze	Increased anxiety response	Reyes-Castro et al., 2012
		Open field		
		Stress response	Increase	
Mating, gestation, and lactation	2-10	Neurological reflexes	Impaired righting reflex	Belluscio et al., 2014
	14-18	Auditory startle	Decrease	
	12-20	Grip force	No effect	
	22-26	Gambling behavior	Decreased sniffing behavior	
		Escape	Decrease in escape attempts	
		Open field	Decreased motor activity	
		Elevated plus-maze	Increased anxiety response	
		Social interaction	No effect	
		Tail suspension	Increase in immobility time	
Gestation	5	Ultrasonic vocalizations	Increase	Batista et al., 2017
	63	Elevated plus-maze	Decreased avoidance responses	
Gestation	41	Object recognition task	Impaired learning	Gould et al., 2017
	64			
	96			
Preconception, pregnancy, and lactation	68-73	Open field	Alteration in anxiety-like behaviors and stress response	Scoenrock et al., 2017
	72-87	Light-dark test		
		Stress response		
Gestation and lactation	110	Morris water maze	Impaired learning and memory	Reyes-Castro et al., 2018
Gestation	84	Elevated plus-maze	No effect	Ye et al., 2018
	97-100	Forced swim	Greater response to stress	
		Stress response		
Gestation and lactation	5	Ultrasonic vocalizations	Decrease	Batista et al., 2019
	13	Home cage behavior	Nest dwell time decreased	
	30-32	Campo abierto	No effect	
		Gap Board Test	Decreased anxiety response	
		Social behavior	Decreased social interaction	
		Object recognition task	Impaired learning and memory	
Preconception, pregnancy, and lactation	90, 180 y 360	Sensorimotor skills	Decrease	Sinha et al., 2020
		Motor activity	Increase	
		Rotaroad	Abnormal stereotypic behavior	
		Elevated plus-maze	Decreased anxiety response	
		Light-dark test	Increased behavior towards light	
		Conditioned behavior	Impaired aversive learning	
		Sucrose preference	Decrease	
		Barnes Maze	Impaired spatial learning	
Gestation and lactation	56	Open field	No effect	Batista et al., 2020
		Social interaction	No effect	

Table 2 Effect of the consumption of a low-protein diet during pregnancy on the behavior of the offspring

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