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Effect of maternal high-fat diet on serum brain-derived neurotrophic factor and behavioral reactions in male offspring of wistar rats

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Abstract

Maternal diet affects the development and health of future offspring. Recent studies indicate that excess fat in the female's diet can influence the structure and function of the brain of her pups. An important role in the maturation of the central nervous system (CNS) and the maintenance of its functioning is assigned to brain-derived neurotrophic factor (BDNF). Changes in BDNF concentration in the brain are associated with the occurrence of various neurobehavioral and mental disorders. In this work, we studied the behavioral reactions and the level of serum BDNF in male Wistar rats whose mothers before, during pregnancy and during lactation consumed food with excess fat in the form of margarine (20% of the total daily calorie content) and in the offspring of females who consumed a standard vivarium diet. As a result, an impairment of spatial learning and memory was revealed, as well as increased anxiety in the Morris Water maze, Elevated Plus maze and Open Field maze in pups whose mothers consumed margarine. These disorders were combined with a reduced level of BDNF in the peripheral blood. The data obtained indicate that consumption of excess fat in the form of margarine by dams before pregnancy, during prenatal and early postnatal period contributes to the formation of neurobehavioral disorders in offspring, which is probably associated with reduced BDNF levels in peripheral blood and, consequently, in the brain.

Аннотация

Характер питания матери оказывает влияние на развитие и состояние здоровья ее будущего потомства. Результаты недавних исследований указывают на то, что избыточное количество жиров в рационе самки способно оказывать воздействие на структуру и функционирование мозга ее детенышей. Важная роль в созревании центральной нервной системы и поддержании ее функционирования отводится мозговому нейротрофическому фактору (BDNF). Изменение его содержания в мозге связывают с возникновением различных нейроповеденческих нарушений и психических расстройств. В данной работе были исследованы особенности поведенческих реакций и содержание BDNF в сыворотке крови самцов крыс линии Вистар, чьи матери до, во время беременности и в период лактации потребляли пищу с избыточным содержанием жиров в виде маргарина (20% от общей суточной калорийности) и у потомства самок, потреблявших стандартный рацион вивария. В результате было выявлено нарушение пространственного обучения и памяти, а также повышение уровня тревожности в тестах «Водный лабиринт Морриса», «Приподнятый крестообразный лабиринт» и «Открытое поле» у детенышей, чьи матери потребляли маргарин. Эти нарушения сочетались у них со сниженным уровнем BDNF в периферической крови. Полученные данные указывают на то, что потребление самками избыточного количества жиров в виде маргарина до беременности, в пренатальный и ранний постнатальный период способствует формированию у ее потомства нейроповеденческих нарушений, что вероятно связано со снижением BDNF в периферической крови, а, следовательно, и уровня его экспрессии в головном мозге.

Keywords: High-fat diet, offspring, BDNF, behavioral reactions

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1. Introduction

Currently, a large amount of data has been compiled, which show a significant role of a mother's ration in the development and health status of the future generation. Excessive fat intake before pregnancy, during prenatal and/or early postnatal period increases a risk of the offspring's obesity, metabolic syndrome and diabetes mellitus [1]. In addition, recent studies have demonstrated that except metabolic and endocrine effects, mother's ration influences the formation and functioning of a future child's nervous system, and, consequently, can contribute to the development of mental and behavioral disorders [2, 3]. So, experiments on animals revealed that offspring of female rats which received food with surplus contents of fat before and after pregnancy had sensory-motor and neurobehavioural disorders, and was also prone to depressive and aggressive behavior [4]. Studies on mice models demonstrated that adult offspring had neurogenesis disturbance, changes in the morphology of neurons in the hippocampus and amygdala, which play a significant role in memory, emotional and cognitive processes [5]. Experiments on non-human primates have shown that the offspring of high-fat diet females exhibits a disorder in the functioning of neurotransmitter systems (serotonin and GABA-ergic). As a result there was an impairment of signaling through synapses in the fetus during its development and an increase in the risk of neuropsychiatric and neurobehavioral disorders [6]. The mechanisms of such deviations remain to be insufficiently explored up to date. Currently, there are several ways to explain the effect of maternal high-fat diet on offspring neurodevelopment: neuroinflammation; increased oxidative stress, dysregulated insulin, glucose, and leptin signaling; impaired serotonergic and dopaminergic signaling; and perturbations in synaptic plasticity [7].

Neurotrophins play a significant role in synapsis functioning and brain development, BDNF being one of the key and most studied of them. In the brain, BDNF is active in the hippocampus, amygdala and cerebral cortex – areas, which are responsible for learning and memory. BDNF plays an important role in the development of the nervous system, neurogenesis, neurons survival and synaptic plasticity, which determines its role in the mechanisms leading to different mental and neurological diseases [8]. Results of some researches show the influence of a high-fat diet on BDNF level and cognitive functions of adults [9, 10, 11, 12, 13]. So, Molteni et al. [11] in experiments on rats consuming excessive saturated fatty acids and refined carbohydrates revealed a decrease in the efficiency of spatial learning, neurogenesis suppression and reduced BDNF level in hippocampus. There was also revealed a decline in synapsin 1 and CREB proteins that are involved in the formation of neuronal plasticity and synaptic transmission, levels of which are controlled by BDNF. Similar results were obtained in the works of Wu et al. [12] who found that a diet high in saturated fat decreases the level of BDNF and proteins regulated by it. This was shown to due to oxidative stress arising under such conditions. Modeling the "western" diet rich in fats and carbohydrates on rats, Stranahan et al. [13] revealed cognitive deterioration, decreased synaptic plasticity and dendritic spines density as well as reduced level of BDNF in the hippocampus. Therefore, it can be assumed that high-fat diet causes synapsis function disorder through the mechanism which includes decreased BDNF level and dendritic spines atrophy.

However, the data regarding the effects of maternal diet on the offspring's brain, neurotrophins levels and cognitive functions are more limited and mixed. Page et al. [14] have shown that consumption of food with high fat content by female rats deteriorates spatial memory of their pups in Morris Water maze test. At the same time, Bilbo and Tsang [15] obtained opposite results. They found improvement of spatial memory of rats, when their mothers' ration contained excessive saturated or trans-fats. Tozuka et al. [16] studied the effect of maternal high-fat diet induced obesity in female mice on the presence of cognitive impairment and the production of BDNF in the hippocampus of their offspring. As a result, spatial memory deterioration was revealed as well as neurogenesis alteration and BDNF level decrease in the hippocampus of pups during the early postnatal period. However, such changes were not observed in the adult offspring of these mice. On the contrary, Rincel et al. [17] described protective effects of a mother's high-fat diet on the nervous system of the offspring. Thus, it was found that excess fat in the female's diet before, during pregnancy and during lactation helps to reduce the effects of early postnatal stress (prolonged separation from the mother) in pups. Offspring revealed normalization of some proteins involved in the development of the nervous system including BDNF as well as reduced anxiety, improved spatial memory and social behavior. In addition, this research did not find changes of BDNF level in the brain of unstressed offspring of mothers consuming high-fat diet. The authors explain this finding by the fact that mother's obesity, rather than a high-fat diet, has an adverse effect on the offspring's brain structure and function.

So, the question about the influence of a mother's diet on the BDNF level and behavioral reactions of its offspring has not been studied completely and requires further research. The literature describes a number of models of high-fat diets with various sources of fat (lard, vegetable oils, margarine) and varying fat percentages from daily calories (from 20 to 60 %) [18, 19, 20]. In the Republic of Belarus, margarine is widely used both in the food industry (baking, confectionery and culinary production) and home cooking. In this regard, the diet with the addition of fat in the form of margarine (20 % of the total daily calories) was used as a model of a high-fat diet for our experiment [19]. Margarine of the selected composition is most widely represented on the Belarusian market.

The aim of this work was to study the effect of maternal

high-fat diet using margarine on behavioral reactions and serum BDNF in male offspring of Wistar rats.

2. Material and Methods

2.1. Animals and diets

All experiments were conducted and approved by the Institute of Physiology of the National Academy of Sciences of Belarus and were in accordance with the guidelines set forth by the European Convention for the Protection of Vertebrate Animals.

The study was conducted on 20 immature female Wistar rats at the age of 1,5 months. The animals were divided into two groups and kept under 12/12-h light-darkness cycle at a temperature of 22 ± 2 °C with free access to water and food (at libitum). The first group (SD, n=10) received a standard vivarium diet. The second group (HFD, n=10) consumed a high-fat diet rich in fats in the form of margarine (20 % of the total daily calories) for 8 weeks. The margarine used in the experiment had the following composition: refined deodorized vegetable oils in natural and modified form (sunflower oil, rapeseed oil, palm oil), water, edible salt, dry whey, sugar, emulsifier: mono- and diglycerides of fatty acids, preservative, dye (beta-carotene) and flavoring. Mass fraction of fat was 82%.

After 8 weeks, female rats in the estrus phase mated with males. Pregnancy was confirmed by the presence of sperm in vaginal smears. During pregnancy and lactation, females from SD group continued to consume the standard diet of vivarium, and HFD females – food with excess fat content. On the 30th day of life, offsprings were separated from their mothers into another cages and divided into two groups depending on the female's diet. The first group (offspring SD, n=19) were males whose mothers were kept on a standard vivarium diet, and the second (offspring HFD, n=27) were males whose mothers consumed a high-fat diet. In the present study, only male offspring were used. Till the end of the experiment, the pups were kept under normal conditions, being on the standard diet of the vivarium, and were removed from the experiment at the age of 3 months by decapitation with prior anesthesia .

2.2. Determination of Visceral Fat Mass

One day after weaning, the female rats were weighed and removed from the experiment by decapitation with prior anesthesia. Visceral fat mass was assessed by weighing the total perirenal and inguinal adipose tissues after dissection.

2.3. Morris Water maze

The Morris Water maze (MWM) consists of a round tank 60 cm in diameter and 40 cm deep, filled with water. The water temperature was 24 ± 2 °C. The tank was divided into four sectors with four equidistant from each other points, marked as North (N), East (E), South (S) and West (W). A circular platform (10 cm in diameter) was submerged in the center of the target sector (South-West sector). The platform remained in the same sector throughout the experiment. High contrast visual signals were placed on the pool wall in each quadrant.

Using the Morris Water maze spatial learning and memory in male offspring of 51-56 days old were tested. The experiment lasted two days [21]. On the first day, the animals were trained using a visible platform. The platform had a bright color and rose 2-3 cm above the surface of water. Each animal was subjected to four tests with an interval of 30 ± 10 min. The rat was carefully placed in the pool water at points N or E between sectors equidistant from the platform (the start of the rat alternated first from point N, then E, then again N and E). The test time was 180 sec to find the visible platform. Animals that could not cope with the task in this period of time were sent to the platform manually. The rats remained on the platform for 10 sec before being removed. The animals were dried with towels and placed in cages. On the second day, a series consisting of three trials was conducted to find the hidden platform. The platform was placed 2 cm below the water surface in the same sector as on the first day of testing. As before, the interval between tests was 30 ± 10 min. The rat was launched from point N, then E, then again N.

In each test, the time from the animal's immersion in water to finding a hidden platform was measured (escape latency time) to assess the development of spatial memory.

2.4. Open Field maze

Open Field maze is a circular arena with a diameter of 120 cm and a wall height of 55 cm, divided into 12 peripheral and 7 central quadrants. In the Open Field test, we studied explorative and motor activity, as well as the level of anxiety in male offspring of 57-62 days old. The test time was 3 min. Before placing the rat, the maze was each time cleaned with 70 % ethanol. The rat was placed in the center of the arena. The latent period of the animal's exit from the center was recorded, the number of line crossings in the periphery and in the center, the quantity of rearings, acts of grooming and droppings (boluses) were counted.

2.5. Elevated Plus maze

Elevated Plus maze is a cross-shaped labyrinth located at a height of 60 cm from the floor. It consists of four opposite sleeves, two open (50 cm x 10 cm) and two closed (50 cm x 10 cm). Closed sleeves are surrounded by walls 40 cm high, open sleeves have no walls. Using this test, we studied explorative and motor activity, as well as the level of anxiety in male offspring of 63-69 days old. Before each rat was placed in the labyrinth, all sleeves were cleaned with 70 % ethanol. The rat was placed in the center of the maze facing the open sleeves. The test duration was 5 min. The results

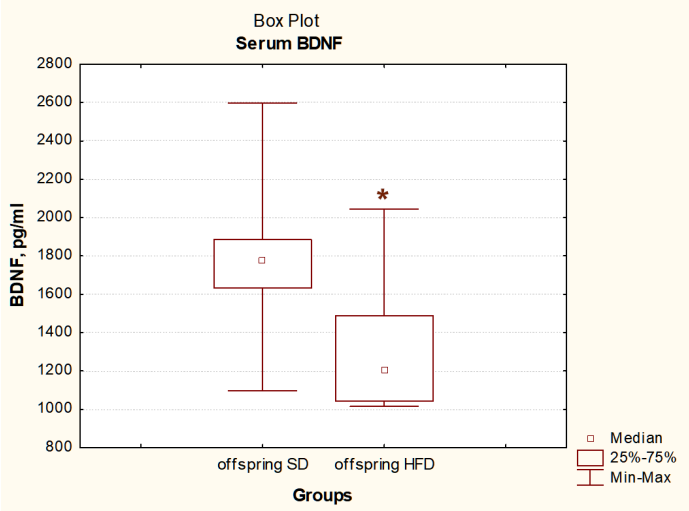

Figure 1 | Serum BDNF in offspring. Values are Median [25%; 75%]. * p ≤ 0,05 offspring HFD versus offspring SD. (Mann–Whitney U test). Уровень сывороточного БДНФ у потомства. Значения представлены в виде медианы [25%; 75%]. * p ≤ 0,05 достоверные отличия группы «Потомство ВЖД» от группы «Потомство СД». (U критерий Манна-Уитни).

were recorded using a Logitech Webcam 905 camera (Logitech, China). Data processing was carried out using the program ANY-Maze (Stoelting Ing., USA).

2.6. BDNF protein level determination

For serum preparation, the blood was collected into sampling tubes and left to coagulate for 1 h. Then it was centrifuged at 2500 g for 10 min to obtain serum. Sera were divided into aliquots and stored at −80°C until BDNF measurements. BDNF were measured by ELISA (FineTest, China) in accordance with the manufacturer's instructions.

2.7. Statistical analysis

Statistical analysis was performed using Statistica 7.0. Normality was defined by the Shapiro-Wilk test. Parametric variables were expressed as means ± standard error, and analyzed by independent t-test. The non-parametric variables were expressed as median, 25 and 75 percentile and analyzed by Mann–Whitney U test. For all statistical tests p≤0,05 was considered significant.

3. Results

The weight of mothers who consumed high-fat diet (margarine) did not differ from the weight of mothers on a standard diet of vivarium (230 ± 10 g and 253 ± 5 g, respectively). The results of weighing visceral adipose tissue (VAT) of female rats from two groups also did not reveal significant differences. So, VAT mass in the HFD group was 3,03 [2,69; 6,67] g, and in the SD group - 2,65 [1,80; 3,20] g. Thus, the diet used didn't lead to the development of obesity in female rats.

According to the results of the experiment in the Morris Water maze, the HFD offspring showed a significant (p ≤ 0,05) increase in the escape latency time in the first two trials (T1, T2) on the second day of the test (24 hours after training using the visible platform). So, the time to reach the platform hidden under water in the T1 test was 10 sec, in the T2 test – 5 sec, and in the control group (offspring SD) – 5 and 4 sec, respectively (Table 1). The data obtained indicate impairment of the spatial learning and memory in offspring HFD.

The Open Field test is designed to assess the behavioral reactions in rodents under stressful conditions that occur in response to placing an animal in open space with intense lighting. The results of our experiment showed an increased anxiety and motor activity in offspring HFD, which revealed a significant (p ≤ 0,05) increase in the number of line crossings in the center and quantity of rearings, as well as a decrease in the number of grooming acts (Table 2).

An increased anxiety in HFD offspring was also confirmed by the Elevated Plus maze test. The method is based on the fact that rodents by their nature stay in secluded places (closed zone) and avoid open spaces. In the conditions of our experiment, HFD offspring showed a passive behavior in the "safe" closed zone: increase in inactivity time, decrease in activity time, decrease in the number of grooming acts and its duration (Table 3).

Concerning the level of serum BDNF, its significant (p ≤ 0,05) decrease compared to control (offspring SD) was found in the offspring of rats whose mothers consumed margarine before and during pregnancy, as well as during lactation (Fig. 1). So, the level of BDNF in the offspring HFD was 1208 [1042; 1489] pg/ml, and in the offspring SD − 1778 [1631; 1887] pg/ml.

4. Discussion

In this study, it was found that the consumption of excess fat in the form of margarine the above-mentioned composition by female Wistar rats before and during pregnancy, and also during the lactation period facilitates the formation of its offsprings's neurobehavioural disorders.

Table 1 | Effect of maternal high-fat diet on offspring's spatial learning and memory in Morris Water maze. Values are Median [25%; 75%]. * p ≤ 0,05 offspring HFD versus offspring SD (Mann–Whitney U test). T1, T2, T3 – series hidden platform trials.
Влияние высокожировой диеты матери на пространственное обучение и память у потомства в тесте «Водный лабиринт Морриса».Значения представлены в виде медианы [25%; 75%]. * p ≤ 0,05 достоверные отличия группы «Потомство ВЖД» от группы «Потомство СД» (U критерий Манна-Уитни). T1, T2, T3 – серии испытаний со скрытой платформой.

Groups	Escape latency time (s)		
	T1	T2	T3
offspring SD	5 [3;12]	4 [2; 6]	4 [2; 9]
offspring HFD	10 [5; 21]*	5 [4; 8]*	4 [3; 8]

So, in the tests Morris Water maze, Elevated Plus maze and Open Field maze an impairment of spatial learning and memory was revealed, as well as an anxiety level increase in rats pups, whose mothers had excessive fat in their diet. These tests are in line with the results obtained by Page et al. [14] and contradict with the data of Bilbo and Tsang [15], which, on the contrary, gave evidence of more successful test performance by pups in the Morris Water maze. It could be due to the fact that Bilbo and Tsang [15] used in their experiment sexually mature female animals which were on a high-fat diet during a shorter period of time (4 weeks before impregnation). Improved spatial memory and a decreased anxiety level in pups of female animals, which were on a high-fat diet, were also observed in the experiments of Rincel et al [17]. But these authors started to use such a ration only from the first day of gestation. Consequently, it can be assumed that not only excessive fat in the ration but also duration of its consumption by a mother before pregnancy influences cognitive functions of its offspring.

As for BDNF level, we revealed its significant decrease in the peripheral blood of the offspring of female rats which consumed a high-fat diet. Today, there are few researches concerning the influence of mother's nutrition on the BDNF level of its young ones and they mainly concern BDNF content in the brain tissues. Concentration of serum BDNF is known to be positively correlated with its content in the brain [8]. Consequently, the results obtained by us can reflect the changes of this neurotrophin in the central nervous system. Our results are in line with the data of Tozuka et al. [16] who revealed a decrease of BDNF in the hippocampus of pups from female rats with obesity provoked by a high-fat diet. At the same time, Rincel et al. [17] did not reveal such changes in their works. They explained it by the fact that a mother's obesity (rather than excessive content of fats in its ration) causes a disturbance of this neurotrophin expression. According to the results of our research, obesity did not develop in female rats. Consequently, a conclusion can be drawn that in our case, a change in the BDNF level is related to the excessive content of fat in a mother's ration. Since this neurotrophin plays an important role in maturation of the central nervous system and maintaining of its functioning, neurobehavioural disorders observed in this experiment can be explained by BDNF level decrease.

Table 2 | Effect Effect of maternal high-fat diet on offspring's behavioural reactions in Open Field maze. Values are Median [25%; 75%]. * p≤0,05 offspring HFD versus offspring SD (Mann–Whitney U test).
Влияние высокожировой диеты матери на поведенческие реакции у потомства в тесте «Открытое поле». Значения представлены в виде медианы [25%; 75%]. * p ≤ 0,05 достоверные отличия группы «Потомство ВЖД» от группы «Потомство СД» (U критерий Манна-Уитни).

Groups	Line crossing: center square	Rearing (act)	Grooming (act)
offspring SD	4 [1;6]	9 [6; 14]	4 [3; 7]
offspring HFD	6 [2; 13]*	16 [9; 23]*	1 [1; 2]*

Table 3 | Effect of maternal high-fat diet on offspring's behavioral reactions in Elevated Plus maze. Values are Median [25%; 75%]. * p ≤ 0,05 offspring HFD versus offspring SD. (Mann–Whitney U test).
Влияние высокожировой диеты матери на поведенческие реакции у потомства в тесте «Приподнятый крестообразный лабиринт». Значения представлены в виде медианы [25%; 75%]. * p ≤ 0,05 достоверные отличия группы «Потомство ВЖД» от группы «Потомство СД». (U критерий Манна-Уитни).

Groups	Time active (s)	Time inactive (s)	Grooming (act)	Time grooming
offspring SD	190 [141; 208]	110 [92;159]	7 [5; 13]	46 [21;80]
offspring HFD	146	154	4	21 [7; 35]*

5. Concluding Remarks

Thus, based on the data obtained, it can be concluded that neurobehavioral disorders (increased anxiety level, impared spatial learning and memory) are observed in male Wistar rats whose mothers consumed excessive fat in the form of margarine before, during pregnancy and during lactation. One of the mechanisms of the formation of such disorders can be related to decreased levels of BDNF protein. In addition, taking into account the results presented in this work, as well as the data of other authors [14, 15, 16, 17], it can be assumed that the duration of a high-fat diet consumed by a mother before pregnancy is also of great importance for the emergence of the nervous system functioning disorders in its offspring.

Заключение

Таким образом, исходя из полученных данных можно сделать вывод, что у крыс мужского пола линии Вистар, матери которых потребляли избыточное количество жиров в виде маргарина до, во время беременности и в период лактации, наблюдаются нейроповеденческие нарушения (повышение уровня тревожности, ухудшение пространственной памяти и способности к обучению). Одним из механизмов формирования таких нарушений может быть снижение уровня белка БДНФ. Кроме того, учитывая результаты, представленные в этой работе, а также данные других авторов [14, 15, 16, 17], можно предположить, что длительность применения высокожировой диеты матерью до беременности также имеет важное значение в возникновении нарушений функционирования нервной системы у ее потомства.

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