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MATERNAL HIGH-FAT DIET DURING PREGNANCY AND LACTATION PROVOKES DEPRESSIVE-LIKE BEHAVIOR AND INFLUENCES THE IRISIN/BRAIN-DERIVED NEUROTROPHIC FACTOR AXIS AND INFLAMMATORY FACTORS IN MALE AND FEMALE OFFSPRING IN RATS

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A balanced maternal diet is necessary for the proper health and development of offspring. Recent clinical and preclinical studies have strongly indicated that maternal exposure to a high-fat diet (HFD) can have an irreversible impact on the structure and function of the offspring's brain and affect the immune system, which may predispose the offspring to brain disorders, including depression. The irisin/brain-derived neurotrophic factor (BDNF) axis is a pathway that influences several neurobehavioral mechanisms involved in the pathogenesis of mental disorders. The aim of the present study was to evaluate the influence of a maternal HFD during pregnancy and lactation on depressive-like behavior, serum irisin concentration and hippocampal levels of irisin, BDNF and inflammatory factors (interleukin-1α, interleukin-6 and tumor necrosis factor-α) in adolescent and adult male and female offspring. The main findings indicate that offspring exposed to a maternal HFD are characterized by an increased immobility time in the forced swimming test at both stages of life. Our results showed that a maternal HFD decreased serum and hippocampal irisin levels in females on postnatal day (PND) 28 and decreased the level of interleukin-1α at postnatal days 28 and 63 in the hippocampus. Interestingly, significant age-dependent changes were observed in irisin, BDNF and interleukin levels. To summarize, our study indicates that a maternal HFD during pregnancy and lactation provokes depressive-like behavior in the offspring. However, despite the observed changes in the levels of irisin and IL-1α in females, further investigations are required to identify the underlying molecular mechanism associated with depressive-like behavior in the offspring of HFD-fed dams.

Key words: *brain-derived neurotrophic factor, cytokines, forced swimming test, high-fat diet, irisin, lactation, maternal diet, pregnancy*

INTRODUCTION

According to the fetal origins hypothesis, intrauterine nutrition has long-term effects on the health of the offspring (1). A maternal high-fat diet (HFD) may contribute to changes in the physiology of the offspring in early life, such as metabolic syndrome and increased body weight, reduced leptin levels, insulin resistance and increased circulating total cholesterol levels (2). However, recent studies have shown that the maternal diet affects development and predisposes individuals to brain diseases (3, 4). Further evidence from animal models has indicated that a maternal HFD affects the behavioral programming of offspring, increasing depressive-like behaviors and impairing cognitive functions, among other effects (5, 6). The burden of mental disorders, such as depression, is growing, while the pathogenesis of this illness remains unknown. Research suggests that peripheral and central inflammation play an important role in the pathophysiology of depression (7). Moreover, numerous studies have suggested that the programming of the brain and behavior by a maternal HFD is associated with inflammatory factors (4, 8-11).

Irisin was discovered as a transmembrane protein in muscle tissue by Bostrom and colleagues in 2012 (12). This novel myokine secreted by myocytes containing 112-amino acid glycosylated protein is formed by the proteolytic cleavage of fibronectin type III domain containing 5 (FNDC5) that is produced in response to the activation of peroxisome proliferator activated receptor-c coactivator-1a (PGC-1a) (13-16). Irisin is a myokine and adipokine that has gained much attention recently due to its mechanisms of action (17). Moreover, irisin can cross the blood brain barrier and act as a neurokine to protect brain function. Irisin is involved in the regulation of neuronal differentiation, the learning process and metabolism. Currently, due to its interesting physiological role, irisin is a very popular subject of research. Clinical data support the idea that FNDC5 is essential for maintaining metabolic homeostasis, and its dysregulation may lead to systemic metabolic imbalance and eventually may result in the onset of metabolic disorders (15, 16). Irisin signaling is expected to be a potential therapeutic target for the treatment of diabetes, obesity, and mental disorders and may act as a potential biomarker of the above diseases. Deeper knowledge

of the functions of irisin may be the key to a better understanding of many diseases and their development.

Preclinical and clinical evidence has shown that irisin could induce brain-derived neurotrophic factor (BDNF) expression in the ventral tegmental area and the hippocampus, which confirmed the existence of a functional irisin/BDNF axis as well as a link between physical activity and reward-related learning and motivation (18). BDNF is a neurotrophin that is expressed mainly in the hippocampus, amygdala and neocortex and plays a significant role in the maturation, preservation and plasticity of the brain. Moreover, BDNF plays an important role in the pathophysiology of depression (19). In addition, a large amount of evidence from human and animal studies linking depression with immune system disturbances and from patients with diagnosed depression has revealed an increased level of proinflammatory cytokines, interleukin IL-1α, IL-6, tumor necrosis factor (TNF)-α (20).

In light of the above information, the major aim of the present study was to determine the effect of maternal HFD consumption during pregnancy and lactation on behavioral patterns related to a depressive-like phenotype. We evaluated the impact of a maternal HFD on the development of depressive-like behavior in the forced swimming test. To explain the potential mechanism of behavioral changes, we investigated the serum concentration of irisin and the hippocampal levels of irisin, BDNF and proinflammatory factors (IL-1α, IL-6 and TNF-α). To provide insight into maternal programming in offspring development, behavioral and biochemical tests were performed in two life stages (adolescence and adulthood) with both male and female offspring.

MATERIALS AND METHODS

Animals and diets

All experiments were performed in accordance with the EU directive 2010/63/EU and with the approval of the Local Ethics Committee in Cracow, Poland.

Wistar rats were delivered by a licensed breeder (Charles River, Germany) and were housed individually in standard cages in an animal colony room maintained at 22 ± 2°C and at 55 ± 10% humidity under a 12-h light-dark cycle (lights on at 6.00 a.m.). The animals had free access to food and water.

Sixteen virgin female rats (Charles River, Germany, 200 – 240 g), after the acclimatization period and during the proestrus phase, were mated with males (Charles River, Germany) overnight, and gestation was confirmed by examination of the vaginal smears for the presence of sperm. Pregnant females were housed individually and randomly assigned to groups fed a standard diet (SD, 13% fat, 3.4 kcal/g; VRF1; Special Diets Services, UK) or a HFD (60% fat, 5.31 kcal/g; C1057 mod.; Altromin, Germany). Dams were fed these diets *ad libitum* during gestation and lactation (21 days). After weaning, offspring were separated by sex and housed with 6 rats per cage and were fed a SD. Male and female offspring were used in the present study. Offspring body weight was monitored at postnatal days (PNDs) 28 and 63. The scheme of the experiment is presented in *Fig. 1*.

Locomotor activity

Before the forced swimming test (FST), spontaneous locomotor activity was recorded individually for each animal from all groups at PNDs 28 and 63 (n = 8 for each group) in Opto-Varimex cages (Columbus Instruments, USA) linked online to an IBM-compatible PC, as described previously (21). Locomotor activity was defined as horizontal activity and is presented as the distance traveled in cm during 5- and 30-min trials.

Forced swimming test

The FST was carried out according to the protocol described by Frankowska *et al*. (21). Briefly, at PNDs 33 and 68, rats (separate subset for each age) were individually placed in a cylinder (height 50 cm, diameter 23 cm) filled with water to a depth of 30 cm (25 ± 1°C) for a 15-min pretest. Next, rats were removed from the water and dried using towels and then returned back to their home cages. The cylinders were emptied and cleaned between tests. Twenty-four hours after the pretest, the rats were retested under the same conditions for 5 min, and the immobility time was measured manually. The data are expressed as the mean (± SEM) time of behavior under study within the 300-s observation period. Eight animals were used per group.

Tissue and blood collection

Groups of male and female offspring (n = 9 in each group) were sacrificed through decapitation at PNDs 28 and 63. The entire hippocampi (both hemispheres) were dissected according to The Rat Brain Atlas (22) and were immediately frozen on dry ice and stored at –80°C until analysis. Trunk blood samples were collected and centrifuged at 3500 rpm at 4°C for 20 min, and serum was stored at –20°C until assessment. All samples were collected between 9.00 and 12.00 a.m.

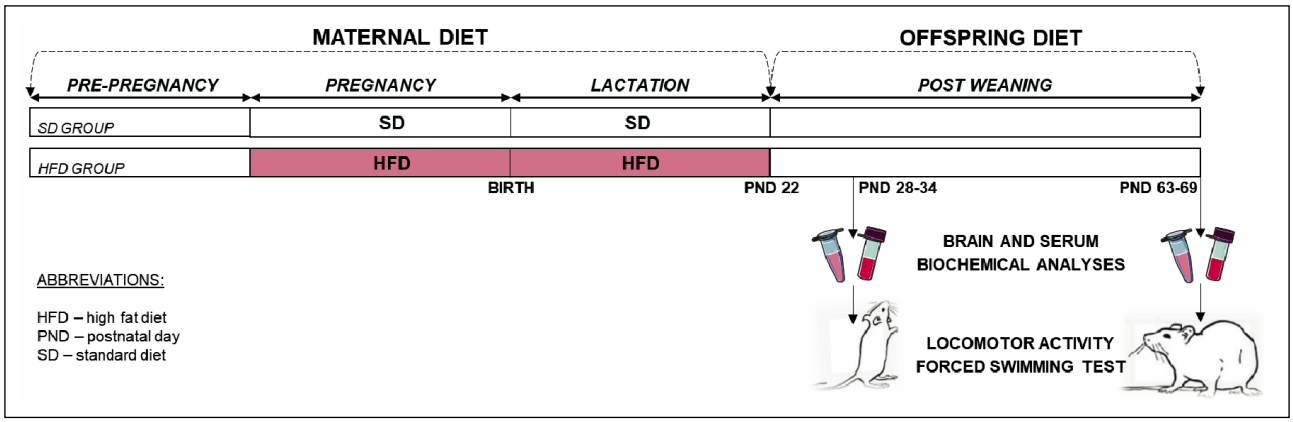

Fig. 1. Study design.

Enzyme-linked immunosorbent assay (ELISA)

The concentration of irisin in the serum was measured using ELISA (Cat.# ER1486, FineTest, China) according to the manufacturer's instructions. A 100 µL portion of each sample (n = 5 for each group) was transferred to 96-well ELISA plates in duplicate with standards (0; 1.56; 3.125; 6.25; 12.5; 25; 50; 100 ng/mL). The absorbance was measured at a wavelength (λ) of 450 nm using a Multiskan Spectrum spectrophotometer (Thermo Labsystems, USA). The concentration of irisin was calculated from a standard curve and is expressed as ng/mL. The detection limit was < 0.938 ng/mL. The interassay precision was < 10%. The intra-assay precision was < 8%.

Luminex microbeads fluorescent assays

The entire hippocampi were homogenized using a sonicator (EpiShear™ Probe Sonicator; Active Motif, USA) in ice-cold lysis buffer (100 mM Tris-HCl, pH 7.5, 200 mM NaCl, 0.2% sodium dodecyl sulfate, 5 mM EDTA) with a protease inhibitor cocktail (Complete, Roche, Germany). The determination of BDNF, irisin and cytokines was performed as follows: the TNF-α, interleukin IL-1α, and IL-6 concentrations in the rat hippocampi (n = 9 for each group) were measured using Luminex micro bead fluorescent assays (EMD Millipore, MILLIPLEX MAP Kit, Rat Myokine Magnetic Bead Panel Cat.# RMYOMAG-88K; Cat.# RECYTMAG-65K, USA) and a Luminex 200 system (Luminex Corp., USA) in accordance with the manufacturers' instructions. A 25 µL aliquot of each sample was transferred to 96-well plates in duplicate with the following standards: BDNF (0, 7, 29, 117, 469, 1875, 7500, 30000 pg/mL), irisin (0, 0.5, 2, 7.8, 31.3, 125, 500, 2000 ng/mL), TNF-α (0, 2.4, 9.8, 39.1, 156.3, 625, 25000, 10000 pg/mL), IL-1α (0, 12.2, 48.8, 195.3, 781.3, 3125, 125000, 50000 pg/mL), and IL-6 (0, 73.2, 293, 1172.9, 4687, 18750, 75000, 300000 pg/mL). The concentrations of the analyzed factors were calculated from calibration curves and are expressed as pg/mg of protein. For protein determination, a bicinchoninic acid assay (BCA) protein assay kit (Thermo Scientific, USA) was used. The detection limits were as follows: BDNF < 3.56 pg/mL, irisin < 0.28 ng/mL, TNF-α < 1.9 pg/mL, IL-1α < 4.2 pg/mL, and IL-6 < 30.7 pg/mL. The interassay precision was as follows: BDNF and irisin < 20%, TNF-α < 10.8%, IL-1α < 4.8, and IL-6 < 12.7%. The intra-assay precision was as follows: BDNF and irisin < 10%, TNF-α < 2.7%, IL-1α < 2.2%, and IL-6 < 2.3%.

Table 1. Body weight of male and female offspring at postnatal day (PND) 28 and 63.

MALE

	SD	HFD
PND 28	65.4 ± 1.83	67.7 ± 2.30
PND 63	262.6 ± 2.72	**276.2 ± 3.72****
	$F_{(1,32)} = 4.390$, $P < 0.05$	

FEMALE

	SD	HFD
PND 28	63.9 ± 3.02	65.0 ± 2.45
PND 63	166.4 ± 2.98	171.0 ± 2.85
	$F_{(1,32)} = 0.370$, $P = 0.547$	

Body weight (g) were measured at PND 28 and 63 in male and female offspring whose mothers were standard (SD) or fed high-fat (HFD) during pregnancy and lactation. The results are expressed as means (± SEM). N = 9 rats/group. Data were analyzed by two-way ANOVA and the post hoc Newman-Keuls test. **P < 0.05 versus SD group.

Statistical analyses

Statistical analyses were performed using GraphPad Prism 7.04 software (GraphPad Software, USA). All data are expressed as the means ± SEMs. Statistical analysis was performed using two-way analysis of variance (ANOVA) for diet and age or using Student's t-test. The *post hoc* Newman-Keuls tests were used to examine differences between group means. P < 0.05 was considered statistically significant.

RESULTS

Body weight

Exposure to a maternal HFD during gestation and lactation resulted in body mass differences in male ($F_{(1,32)} = 4.390$, $P < 0.05$) but not in female ($F_{(1,32)} = 0.370$, $P = 0.547$) offspring. *Post hoc* analyses performed at PND 63 showed increased weight in males exposed to a HFD ($P < 0.05$) compared to the weight of the rats in the SD group (*Table 1*).

Locomotor activity

Spontaneous locomotor activity (*Fig. 2*) did not change at PND 28 or 63 during the 5-min ($F_{(1,28)} = 0.037$, $P = 0.849$) and 30-min ($F_{(1,28)} = 0.246$, $P = 0.624$) trials in the male offspring of SD- and HFD-fed dams. Likewise, at both stages of life, there were no significant changes in distance travelled among female offspring (5-min trial – $F_{(1,28)} = 0.535$, $P = 0.471$; 30-min trial - $F_{(1,28)} = 0.023$, $P = 0.882$).

Forced swimming test

The influence of a maternal HFD on depressive-like behavior at PNDs 34 and 69 in the male and female offspring was evaluated (*Fig. 3*).

Two-way ANOVA did not show a significant main effect of the maternal diet × age interaction in male ($F_{(1,28)} = 0.011$, $P = 0.919$) or female ($F_{(1,28)} = 0.027$, $P = 0.871$) offspring. However, statistical analysis showed an effect of diet in males ($F_{(1,28)} = 14.640$, $P < 0.001$) and females ($F_{(1,28)} = 12.850$, $P < 0.01$). Feeding dams a HFD during pregnancy and lactation led to an increased immobility time in male ($t = 2.447$, $df = 14$, $P < 0.05$) and female ($t = 2.240$, $df = 14$, $P < 0.05$) offspring when measured at the adolescent stage. Similarly, at PND 69, we observed that maternal HFD-exposed male offspring showed significantly increased immobility time ($t = 3.123$, $df = 14$, $P < 0.01$) compared to that of the control group. Statistical analysis indicated that adult maternal HFD-exposed females demonstrated extended immobility time ($t = 3.341$, $df = 14$, $P < 0.01$) in the forced swimming test. These results suggest that the offspring of dams that consumed a HFD showed depressive-like behavior.

Serum irisin concentration

Fig. 4 shows the serum irisin levels in HFD and SD rats at PNDs 28 and 63. Two-way ANOVA demonstrated significant changes in regard to the diet × age interaction ($F_{(1,16)} = 4.834$, $P < 0.05$) in male offspring. *Post hoc* analysis indicated decreased irisin levels at PND 63 versus the level at PND 28 in offspring from both diet groups ($P < 0.001$). Maternal HFD exposure did not

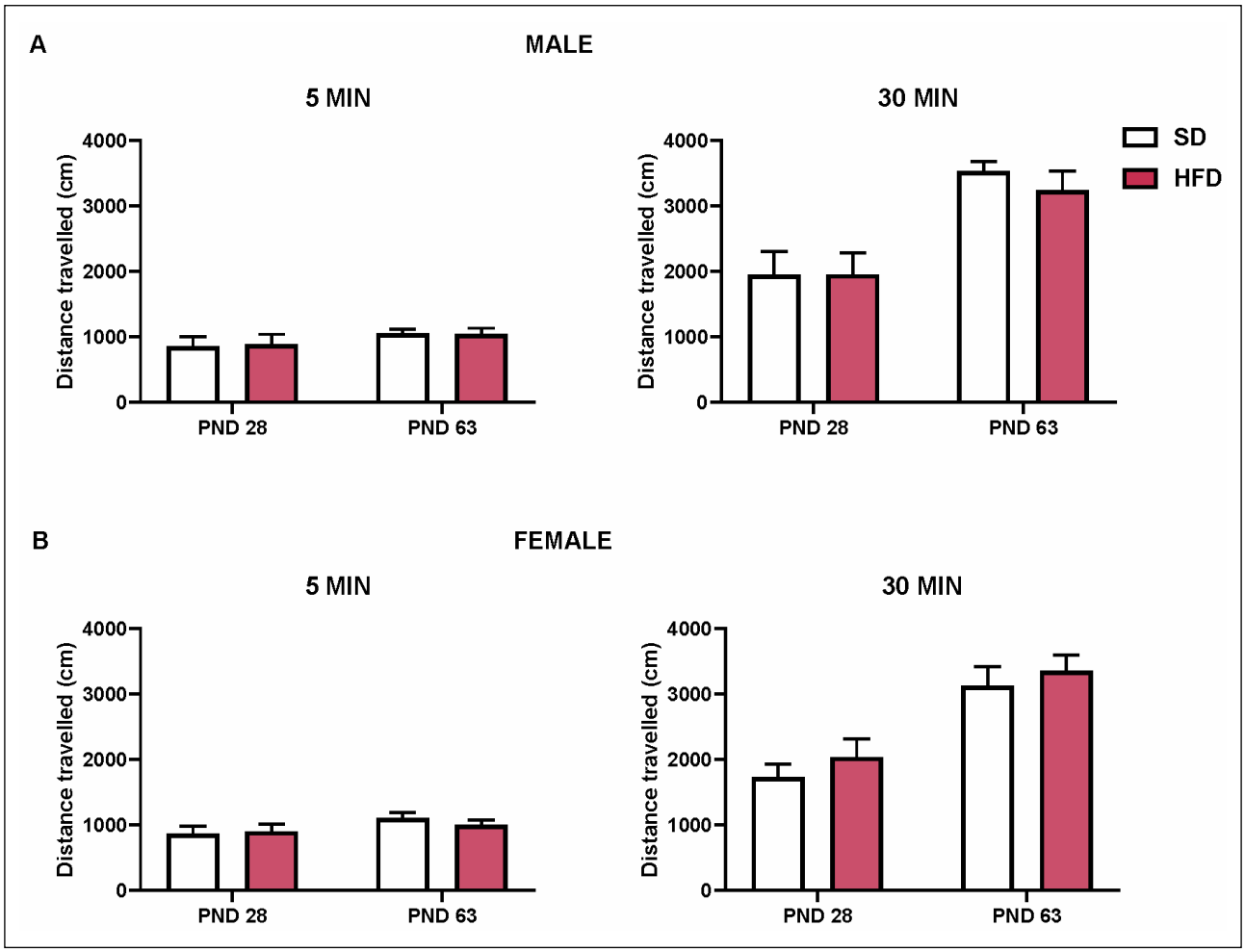

Fig. 2. Effects of maternal high-fat diet (HFD) during pregnancy and lactation on the locomotor activity in male (A) and female (B) offspring at postnatal day (PND) 28 and 63. Distance travelled (cm) was measured after 5- and 30-minute trials. N = 8 rats/group.

change irisin levels in adolescent (P = 0.323) or adult (P = 0.552) offspring. Similarly, two-way ANOVA showed significant changes in regard to the diet × age interaction among females ($F_{(1,16)}$ = 8.156, P < 0.05). *Post hoc* tests showed reduced irisin levels at PND 63 versus the levels at PND 28 in SD (P < 0.001) and HFD (P < 0.01) offspring. Exposure to a maternal HFD did not change the level of irisin in the serum at PND 28 (P = 0.65) or PND 63 (P = 0.567). Statistical analysis using Student's t-test for verification indicated that at PND 28, a maternal HFD decreased the serum irisin concentration in females (t = 2.33, df = 8, P < 0.05).

Irisin concentration in the hippocampus

In male offspring, no changes were observed using two-way ANOVA in regard to the diet × age interaction ($F_{(1,32)}$ = 0.147, P = 0.843) or the type of diet ($F_{(1,32)}$ = 0.449, P = 0.508) on hippocampal irisin levels after exposure to a maternal HFD. Interestingly, statistical analysis showed significant changes in irisin levels within the hippocampus between adolescent and adult rats ($F_{(1,32)}$ = 16.5, P < 0.001) (*Fig. 5A*). Student's t-test showed decreased level in older offspring (SD: t = 2.572, df = 16, P < 0.05; HFD: t = 3.449, df = 16, P < 0.01). Similarly, females did not show significant changes in regard to the diet × age interaction ($F_{(1,32)}$ = 1.764, P = 0.194) or the effect of the modified maternal diet ($F_{(1,32)}$ = 1.999, P = 0.167), but significant changes were observed in terms of the effect of age ($F_{(1,32)}$ = 9.045, P < 0.01). Female offspring from the SD group but not the HFD group manifested a reduction in the level of hippocampal irisin at PND 63 (t = 2.825, df = 16, P < 0.05; t = 1.309, df = 16, P = 0.209; respectively) (*Fig. 5B*).

Brain-derived neurotrophic factor concentration in the hippocampus

Statistical analysis indicated no significant differences in male hippocampal BDNF concentration in regard to the diet × age interaction ($F_{(1,32)}$ = 0.212, P = 0.648) and the diet effect ($F_{(1,32)}$ = 1.928, P = 0.175). The level of BDNF increased with age, independent of maternal diet among males ($F_{(1,32)}$ = 24.790, P < 0.001) (SD: t = 4.241, df = 16, P < 0.001; HFD: t = 3.214, df = 16, P < 0.01) (*Fig. 5C*). Similar effects were observed among females. No significant changes were observed in the statistical analysis in regard to the interaction of diet × age ($F_{(1,32)}$ = 0.024, P = 0.878) and the influence of maternal diet on BDNF level ($F_{(1,32)}$ = 0.398, P = 0.533), but significant changes in terms of the effect of age ($F_{(1,32)}$ = 28.070, P < 0.001) were observed. The hippocampal concentration of BDNF was significantly higher in adult female rats than in adolescent female rats (SD: t = 4.785, df = 16, P < 0.001; HFD: t = 3.234; df = 16, P < 0.01) (*Fig. 5D*). Please note that this is the opposite result as that observed for irisin.

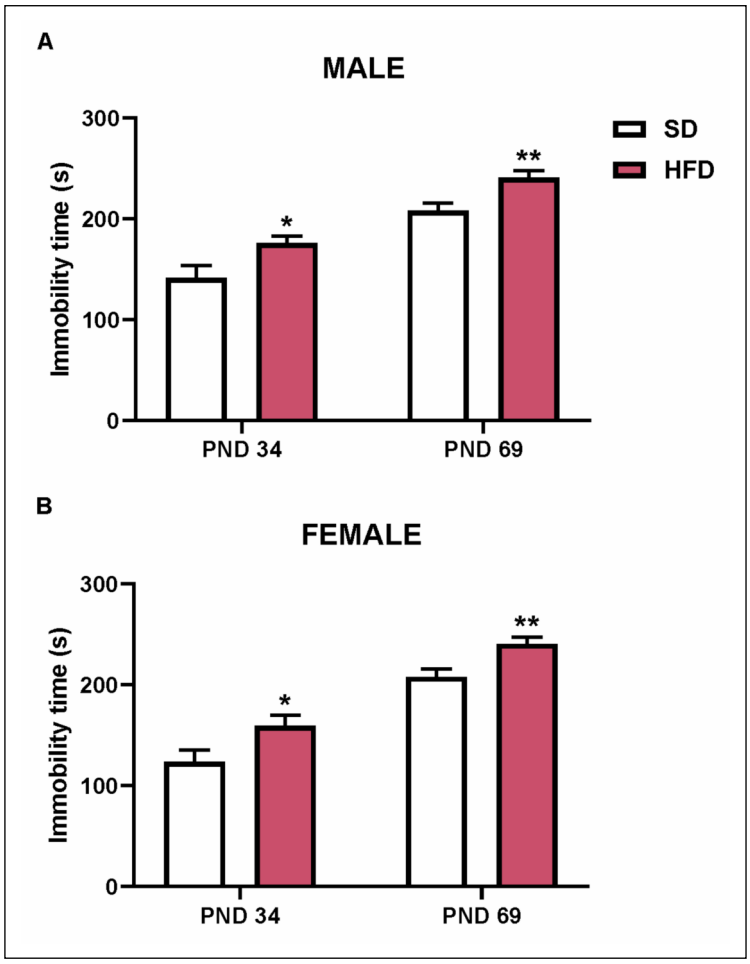

Fig. 3. Effects of maternal high fat-diet (HFD) on the immobility time in the forced swimming test in male (A) and female (B) offspring at postnatal day (PND) 34 and 69. N = 8 rats/group. *P < 0.05; **P < 0.01 versus standard diet (SD) group (Student's t-test).

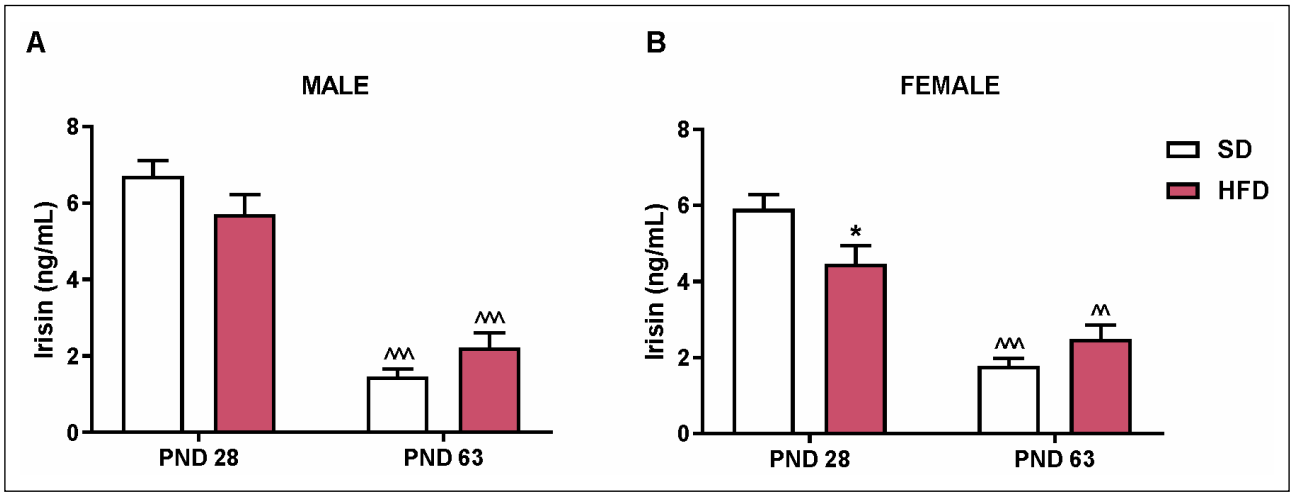

Fig. 4. Irisin serum concentration in male (A) and female (B) offspring at postnatal day (PND) 28 and 63 exposured on maternal high-fat (HFD) and standard diet (SD). N = 5 rats/group. *P < 0.05 versus SD group (Student's t-test); ^^P < 0.01, ^^^P < 0.001 versus corresponding group at PND 28 (two-way ANOVA; *post hoc* Newman-Keuls test).

Cytokine concentrations in the hippocampus

Interleukin-1α

The levels of the proinflammatory cytokine IL-1α within the hippocampus in the evaluated groups are shown in *Fig. 6A* (males) and *Fig. 6B* (females). In male offspring, no changes were observed based on the two-way ANOVA for the diet × age interaction ($F_{(1,32)}$ = 1.601, P = 0.215) or the type of maternal diet ($F_{(1,32)}$ =1.826, P = 0.186). An increased level of IL-1α was observed in adulthood ($F_{(1,32)}$ = 53.760, P < 0.001) in the SD group (t = 6.118, df = 16, P < 0.001) and the HFD group (t = 4.950, df = 16, P < 0.001). Statistical analysis of females did not show any changes in regard to the diet × age interaction ($F_{(1,32)}$ = 3.581, P = 0.068). Effects of maternal diet ($F_{(1,32)}$ = 10.200, P

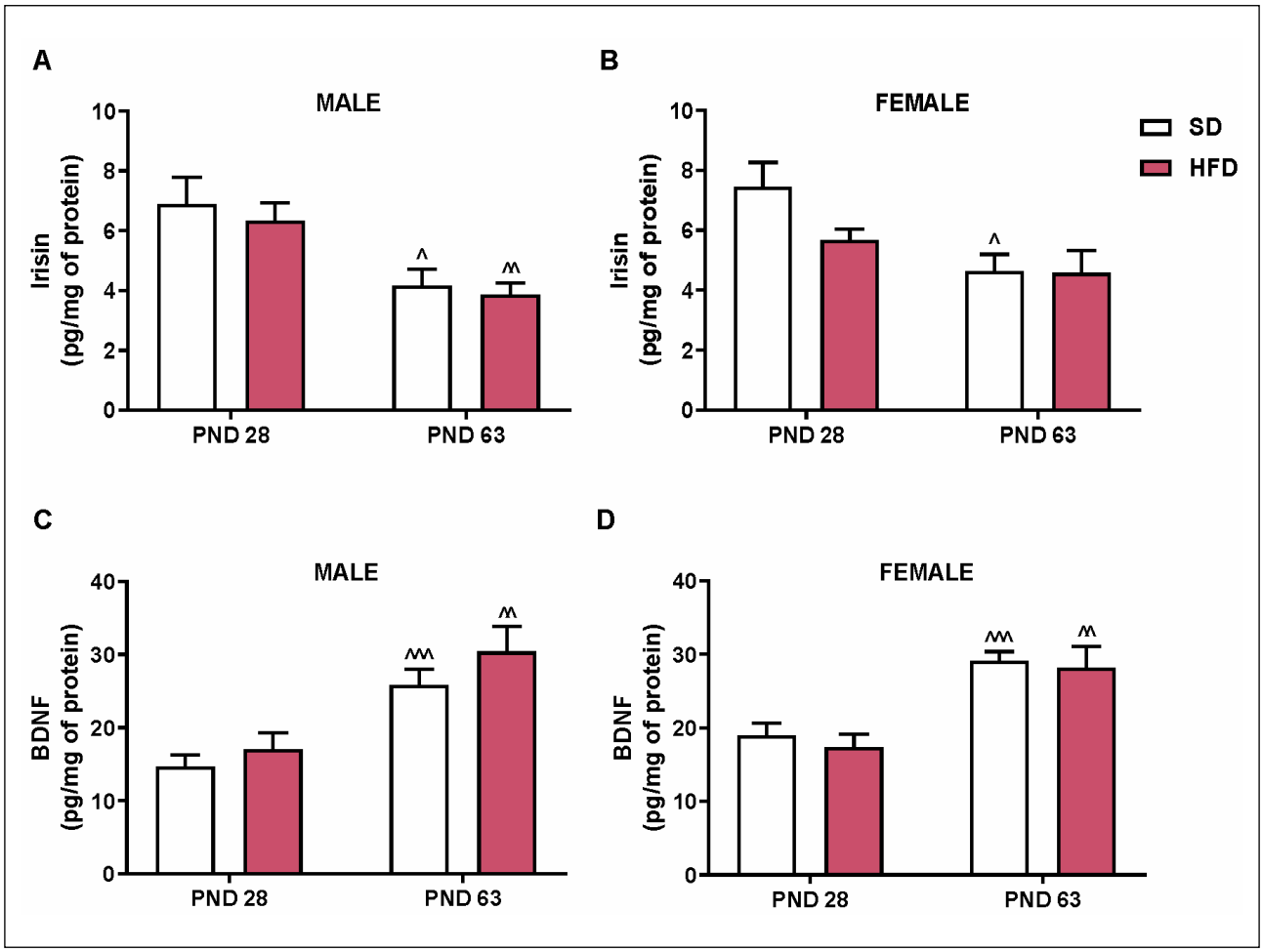

Fig. 5. Irisin (upper panel) and BDNF (lower panel) hippocampal levels in male (A, C) and female (B, D) offspring whose mothers were fed high fat diet (HFD) or standard diet (SD) at postnatal day (PND) 28 and 63. N = 9 rats/group. ^P < 0.05, ^^P < 0.01, ^^^P < 0.001 versus corresponding group PND 28 (Student's t-test).

< 0.01) and the age of the offspring ($F_{(1,32)}$ = 25.070, P < 0.001) on the level of hippocampal IL-1α were observed. Student's t-test analysis showed that maternal HFD exposure decreased the IL-1α level at PND 28 (t = 2.178, df = 16, P < 0.05) and PND 63 (t = 5.284, df = 16, P < 0.001). On the other hand, an increased level was observed at PND 63 in the SD offspring (t = 5.546, df = 16, P < 0.001) and in the HFD rats (t = 3.404, df = 16, P < 0.01).

Interleukin-6

There was no statistically significant difference in the hippocampal IL-6 concentration in regard to the diet × age interaction in males ($F_{(1,32)}$ = 3.580, P = 0.068) or in the maternal diet effect ($F_{(1,32)}$ = 0.029, P = 0.857). However, a significant effect of age was observed ($F_{(1,32)}$ = 27.010, P < 0.001). Increased levels of IL-6 were observed in older animals from the SD (t = 5.349, df = 16, P < 0.001) and HFD (t = 2.206, df = 16, P < 0.05) groups (*Fig. 6C*). In female offspring, no changes were observed in the interaction analysis of diet × age ($F_{(1,32)}$ = 0.177, P = 0.677) or the effect of maternal diet on the level of IL-6 ($F_{(1,32)}$ = 3.950, P = 0.056), but in adult rats, there was a significant increase compared to the effects during the adolescent period ($F_{(1,32)}$ = 20.430, P < 0.001) (Student's t-test for SD: t = 3.530, df = 16, P < 0.01; HFD: t = 3.055, df = 16, P < 0.01) (*Fig. 6D*).

Tumor necrosis factor-α

Fig. 6E (males) and *Fig. 6F* (females) illustrate the effect of maternal HFD on hippocampal TNF-α levels at PNDs 28 and 63. In male and female offspring, no changes were observed using two-way ANOVA for the diet × age interaction ($F_{(1,32)}$ = 2.901, P = 0.098; $F_{(1,32)}$ = 0.040, P = 0.844; respectively). Moreover, the TNF-α concentration did not differ between the HFD males ($F_{(1,32)}$ = 0.018, P = 0.893) and females ($F_{(1,32)}$ = 1.367, P = 0.251) or during the lifetime (male: $F_{(1,32)}$ = 2.221, P = 0.146; female: $F_{(1,32)}$ = 2.455, P = 0.127).

DISCUSSION

In light of the above data, the main goal of this study was to evaluate the impact of maternal consumption of a HFD during pregnancy and lactation on depressive-like behavior. Moreover, we tested the hypothesis that behavioral changes induced by maternal HFD exposure can be associated with an altered irisin/BDNF axis or inflammatory factors. In this paper, we showed that a modified maternal diet consumed during pregnancy and lactation is an important factor that can change the behavioral phenotype of rats, which further confirms the developmental origins of health and disease (DOHaD) theory. The results of the present study

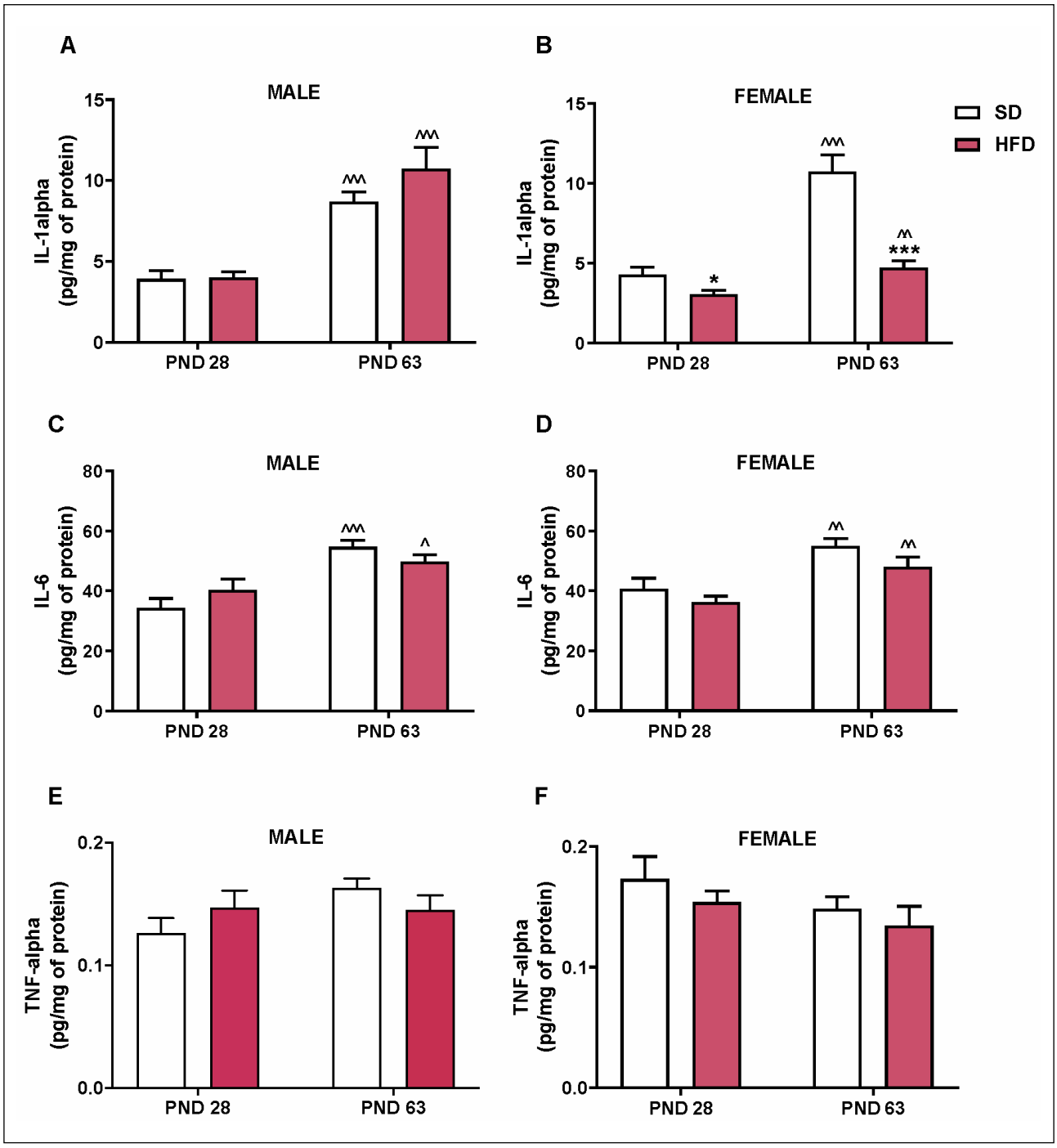

Fig. 6. Interleukin IL-α, IL-6 and TNF-α levels in the hippocampus of male (A, C, E) and female (B, D, F) offspring whose mother fed high-fat diet (HFD) or standard diet (SD) at postnatal day (PND) 28 and 63. N = 9 rats/group. *P < 0.05, ***P < 0.001 versus SD group; ^P < 0.05, ^^P < 0.01, ^^^P < 0.001 versus corresponding group PND 28 (Student's t-test).

demonstrated for the first time that female and male rats exposed to a maternal HFD showed a significantly increased immobility time in the FST. Moreover, no changes were observed in the spontaneous locomotor activity of the animals, which demonstrates the specificity of the FST results and indicates depressive-like behavior in the offspring of HFD-fed dams. Interestingly, the depressive phenotype appeared despite the rats being switched to SD rodent chow after weaning. This finding clearly demonstrates that prenatal exposure to a maternal HFD is a strong factor that can lead to the development of depressive symptoms in adulthood. Our observations correlated with previous reports that demonstrated that offspring showed a depressive phenotype in a maternal model of obesity induced by HFD (23, 24) as well as after maternal HFD consumption limited only to the lactation period (5).

It is well established that proinflammatory cytokines influence several signaling cascades and gene transcription processes, ultimately changing the production and uptake of neurotransmitters, including serotonin, which is involved in the pathogenesis of depression and resistance to treatment (20). On the other hand, it was shown that a diet rich in fat, by promoting

low-grade chronic inflammation, was associated with increased proinflammatory cytokine (*e.g.*, IL-1, IL-6, TNF-α) levels (25), inducing inflammation in the hippocampus (26-28). Clinical studies have reported that increased proinflammatory cytokine levels are also observed in obese pregnant women, lead to dysfunctions in the placenta and are connected with neurodevelopmental and neuropsychiatric disorders, such as depression. A recent study demonstrated that circulating cytokines influence the development of neural circuits critical for the regulation of behavior, which indicates that inflammation is a potential mechanism that impairs proper offspring brain development and leads to risk for mental health disorders (29, 30).

Our paper does not explicitly confirm the relationship between diet and elevated proinflammatory cytokine levels. In fact, our observations indicate that a maternal HFD decreased IL-1α levels in the hippocampus of female offspring at PNDs 28 and 63. Hence, the decreased level of this factor in offspring may suggest adaptive changes, which can be caused by an increased exposure of offspring during the fetal period to proinflammatory factors induced by the maternal diet. Moreover, a maternal HFD did not change the IL-1α level in male offspring or IL-6 in either sex group. Another study evaluating the IL-1β and IL-6 mRNA expression levels in the brains (lamina terminalis and paraventricular nucleus) of 10-week-old male rats after maternal exposure to a HFD during pregnancy and/or lactation also demonstrated no significant changes in their expression (31), which supports our present findings. The lack of changes in proinflammatory cytokine levels may result from the restoration of the balance in the immune system after switching to a standard chow after weaning.

The cytokine TNF-α was the first to be identified as a molecular link between obesity and inflammation (32). HFD consumption for 12 or 24 weeks in rats resulted in a 1.5-fold or twofold increase in TNF-α levels in the rat hippocampus (33). Moreover, recent data have indicated that the proinflammatory cytokine TNF-α may be one of the factors that can modulate BDNF synthesis (34). Previous reports have shown increased TNF-α in depressed individuals (35). However, in our study, the TNF-α levels did not differ significantly between the offspring rats in the maternal HFD and SD groups. This could be caused by differences in depression severity and some other species and specific factors in cohorts. Similarly, Zhang and colleagues (31) noted no changes in the lamina terminalis of male offspring of HFD-fed dams, with a simultaneous increase in TNF-α mRNA expression in the paraventricular nucleus. A maternal HFD did not change the TNF-α protein level in the cortex of 20-week-old rats (36).

Our study indicates that a diet rich in fats consumed by dams may induce changes in the cytokine levels in the hippocampus; however, these effects were limited only to females and do not explicitly confirm a direct relationship between a maternal HFD and factors such as elevated proinflammatory cytokine levels and depressive-like behavior in the offspring. The fact that the changes were limited only to the female group may result from important sex differences in the activation, regulation and distinct kinetics of cytokine networks in the hippocampus (37).

There is strong neurochemical support for the pathophysiological role of neuronal plasticity and BDNF in depression (38). Moreover, data suggest that the consumption of a HFD may increase the risk of the occurrence of depressive symptoms and that HFD consumption leads to atrophy of the hippocampus (39). Animal studies have indicated that HFD consumption decreases neural progenitor cell proliferation, hippocampal neurogenesis and BDNF levels (39, 40). On the other hand, the current literature has reported that irisin, like BDNF, might act in the central nervous system to modulate mood-related behaviors. It was shown that intracerebroventricular irisin administration elicited antidepressant-like behavior in forced swimming and tail suspension tests of antidepressant potential in mice (41).

The latest report demonstrates decreased serum irisin levels in adult patients with depression (42). However, clinical study focused on adult obese women no differences were found in plasma irisin level between groups with low and high depressiveness score (using PHQ-9, Patient Health Questionnaire) (43). Our findings demonstrated decreased serum irisin levels in female offspring exposed to a maternal HFD when tested at PND 28, and the same trend was observed in the hippocampus. However, a maternal HFD did not change the serum or hippocampal irisin levels in adolescent male or adult offspring. Sex differences were observed in offspring exposed on maternal HFD intrauterine and during suckling period in plasma adiponectin level (44). This confirms the different effects of maternal exposure to external factors on the development of male and female offspring. We also report that a maternal HFD during pregnancy and lactation did not affect the hippocampal BDNF levels in the examined groups. As other literature data reported maternal HFD consumption during gestation lowered BDNF in the offspring brain after birth. Then, it was shown that maternal HFD did not affect the BDNF in the adult offspring (45). The latter observation may indicate that the decreased level of irisin in the brains of young females may contribute to symptoms of depression. However, the irisin/BDNF axis was not changed by a maternal HFD in males and adult females, so it could not be the main factor constituting the pathophysiological basis of the observed depressive-like behavior. Furthermore, on the basis of the obtained results, one can draw an interesting conclusion that for both sexes, in the hippocampus, there is a relationship between the increased irisin level and the decreased level of BDNF at PND 28 and a converse relation at PND 63, providing further support for the existence of an interaction between the two factors. The possible existence of the irisin-BDNF axis may be confirmed by the fact that the intracerebroventricular administration of irisin changed BDNF mRNA expression, while BDNF injection modified FNDC5 (irisin precursor) mRNA expression in the hippocampus and prefrontal cortex (41). Szilasi *et al*. (46) showed that irisin is involved in the ability of BDNF to influence mood and anxiety *via* changed contextual learning in structures under BDNF control. Moreover, it was found that the molecular pathway that may link affective constructs such as anxiety to reinforcement learning is the irisin-BDNF axis, based on its potential role in reinforcement learning, given its modulatory effect in structures related to the generation of context frames inherent to reinforcement learning (46, 47).

Interestingly, our study indicated that the serum irisin level was closely associated with the hippocampal irisin level, which may lead to the conclusion that serum irisin could be used as a potential biomarker of the central levels of this myokine. Another very important finding is that the basal levels of the examined factors in the hippocampus, such as irisin, BDNF, IL-1α, and IL-6, changed with age; namely, in older animals (irrespective of sex and maternal diet), basal levels of these factors were significantly increased/decreased in all studied groups of offspring, which suggests physiological changes. Supporting the present data, age-related changes in the hippocampal BDNF level have also been reported, namely, the concentration of BDNF increased with age in rats (48-50). Moreover, BDNF may act as a factor reduces expression of FNDC5 as part of an apparent homeostatic loop (51). It is known that irisin may regulate immune system activity and that by downregulating the TLR4/MyD88 pathway, irisin reduces the release of IL-1α and IL-6, which may be one of the explanations for the increased levels of these cytokines in adult rats (reduced irisin level) and decreased concentrations of proinflammatory factors in younger animals (elevated irisin level) (52, 53).

The increased serum irisin level in adolescent animals could be associated with muscle development, which can support the hypothesis that irisin plays a role as a promyogenic factor. In the study evaluating serum irisin levels in different age groups of humans, the highest level was observed in young people compared to the levels in the middle-age and elderly groups, which corresponds to our observations (54).

Despite the lack of confirmation of the main hypothesis about the involvement of the irisin/BDNF axis and proinflammatory factors in depressive-like behavior provoked by a maternal HFD during pregnancy and lactation, one of the potential mechanisms that can be responsible for the observed changes in factors described above may be related to epigenetic machinery. In fact, the role of nutrients in epigenetics is supported by studies demonstrating that nutritional factors may change the methylation of gene-specific promoters associated with the expression of genes (55). Current experimental studies conducted with animal models have focused on hypotheses concerning the potential role of a maternal HFD on changes in the methylation of gene-specific promoters, which are closely associated with gene expression (3, 55). The consumption of a HFD during the gestation and lactation periods has an impact on epigenetic and plasticity regulators in the brain. Animal studies indicate that the epigenetic approach may be an important mechanism underlying programming (56), but more studies on this subject are still needed.

In summary, our results indicate that a maternal HFD during pregnancy and lactation may trigger long-term depressive-like behavior in male and female offspring. Although the levels of irisin, BDNF and proinflammatory factors could not explain the observed behavioral changes, in females, they may correlate with depressive-like behavior; however, further investigations are required to elucidate the mechanism of the effect of a maternal HFD on depressive-like behavior. Our findings highlight the importance of looking for similarities and differences between the sexes, confirming the current tendency to design studies including experimental groups of females. The analysis of biological factors at various stages of individual growth contributes to a deeper understanding of changes occurring in the lifetime, which may be implicated in the development and pathophysiological bases of diseases as well as adaptive changes occurring with age.

In recent years, human and animal studies have suggested that the risk of lifestyle-related diseases is associated with the *in utero* environment, but little is known about the effects of HFD consumption during fetal development, which may lead to many kind of disorders in adult life. In light of the above information, our research may provide novel data in this field.

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