The Mechanism of Fos and Leptin Receptor in Neural Tube Development of Offspring Under The Regulation of Energy Balance

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Research

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Abstract

Background

The occurrence of neural tube defects is a complex process in which genes, internal and external environment and other factors jointly influence and occur interactively. In this experiment, animal models of different energy balance states are constructed. To explore the mechanism of fos and leptin-leptin receptor during neural tube development of offspring under different energy states and its effect on neural tube development of offspring

Methods

Using gene identification technology to obtain Mex3c^{+/-} negative energy balance mice and high-fat diet to obtain positive energy balance mice, and obtain E10.5d, E12.5d, E14.5d embryos. We will verify the expression of fos, leptin, LEPR, nestin, PAX3, and H3K27me3 proteins in the neural tube of the offspring through relevant experimental methods.

Results

We have successfully constructed animal models, Control group (18.82g±1.54g), Mex3c group (18.84g±1.08g), HFD group (22.61g±1.10g). Neural tube HE staining shown that compared with the Control group, the neuronal maturity of the Mex3c group and the HFD group was reduced. Immunohistochemical staining showed that both fos and leptin were expressed on the nucleus, and LEPR was expressed on the cell membrane. Western blot experiments showed that compared with the Control group, the Mex3c group and the HFD group had low expression of fos protein ($P=0.01$), the Mex3c group had high expression of LEPR protein ($P=0.01$) and the HFD group had high expression of LEPR protein ($P=0.01$). Immunostaining experiments showed that nestin was expressed in nerve fibers, and PAX3 and H3K27me3 were both expressed in the nucleus. Western blotting experiment showed that compared with the Control group, the Mex3c group had high expression of nestin protein ($P=0.01$), PAX3 protein ($P=0.01$), H3K27me3 ($P=0.01$), and the HFD group had high expression of nestin protein ($P=0.01$).) And PAX3 protein ($P<0.01$), H3K27me3 ($P<0.01$).

Conclusions

Mex3c regulates leptin and LEPR by enhancing the expression of fos mRNA to participate in the neural tube development process of offspring. The neural tube nestin, PAX3, and H3K27me3 of the offspring of Mex3c^{+/-} mice and high-fat diet mice continue to be highly expressed. Mex3c^{+/-} mice express low leptin, and high-fat diet mice highly express leptin; preliminary reveals the regulation of different energy states Leptin-LEPR is involved in the process of neurodevelopment. Mex3c mutant mice and mice on a high-fat diet lead to decreased neurodevelopmental maturity.

Introduction

In neonatal birth defects including neurodevelopmental defects[1], At the 4th weekend of embryonic development, the nerve folds on both sides gradually moved closer to the middle and gradually healed into a hollow structure; that is the process of neural tube development; this process is not closed or incompletely closed leading to neural tube defects (NTDs) including anencephaly, Spina bifida, etc[2]. According to statistics, the global incidence of neural tube defects is 1.23‰[3]and bring significant economic pressure to the family and society. The occurrence of neural tube defects is a complex process that is affected by genes, internal and external environment and other factors, and more and more researchers are exploring the mechanism of preventing neural tube defects by constructing animal models of neural tube defects. Studies have shown that folic acid is involved in the one-carbon unit metabolism of homocysteine and affects neural tube development[4]. In the human, supplementation of folic acid in the first trimester can help reduce the occurrence of malformations. Failure to supplement enough folic acid will increase the incidence of NTDs. Neural tube development is also affected by the internal and external environment. At present, studies have shown the correlation between maternal obesity and increased risk of neural tube defects[5,6]. Studies have shown that the effect of folic acid supplementation in pregnant women with $BMI \geq 25\text{kg/m}^2$ is lower than that of $BMI < 25\text{kg/m}^2$; at the same time, the study found that serum folic acid content in $BMI > 30\text{kg/m}^2$ must be supplemented with 350ug per day to achieve $BMI < 20\text{kg/m}^2$ serum content[7]. The current study because of the intervention of folic acid, although reducing the occurrence of NTDs, but did not make NTDs no longer occur, which also suggests that neural tube development is not a factor, but the interaction of genes and environment.

Energy balance means that energy intake and consumption reach a balanced state. Without changes in internal and external environments, lipid production and consumption in the body are in dynamic balance, providing the body with sufficient energy; the incidence of energy metabolism-related diseases is increasing year by year, leptin (leptin) And leptin receptor (LEPR) are involved in the process of energy metabolism; leptin (leptin) is secreted from white fat[8]; Regulate liver glycogen and fat metabolism, affect energy metabolism process[9]. Studies have confirmed that the central system of energy metabolism is the hypothalamus, which mediates leptin-LEPR to regulate food intake and consumption. Recent studies have shown that obesity is related to hypothalamic damage in humans and rodents[10]; high-fat diet can damage hypothalamus inflammation and increase endoplasmic reticulum stress, which in turn leads to decreased leptin and insulin sensitivity[11,12]. Long-term high-fat diet can cause lipid deposition, increase fat, break the balance of human lipid metabolism, and induce non-alcoholic fatty liver. Studies have shown that long-term high-fat diet during pregnancy can affect offspring neurites by damaging mitochondrial function[13]; that a high-fat diet can lead to changes in mitochondrial function, which in turn leads to lipid metabolism disorders and neurodevelopment. Professor Lu of the research group has proved in previous experiments that Mex3c gene-deficient mice induce the body to form a negative energy state[14] and other studies have shown that without inhibiting food intake, Mex3c mutations can contribute to glucose and lipid metabolism, resulting in decreased blood glucose and blood lipid concentrations in mice, and increased insulin effect, making Mex3c resistant to obesity caused by food[15,16]. Mex3c gene mRNA is expressed most in the brain and testis. Mutations in this gene can protect mice from food-induced obesity, reduce blood sugar and cholesterol in obese mice, and

participate in neurodevelopment[14]. In vivo experiments through the administration of leptin and hunger hormone to form a positive and negative energy state, verified that Mex3c can induce the expression of fos in the hypothalamus to participate in the process of energy metabolism. According to Professor Lu Baisong's research[15], there is no direct regulatory relationship between Mex3c and leptin, but Mex3c can regulate leptin by inducing other energy metabolism factors. From the in vitro experiments of the research group, it was found that Mex3c can enhance the expression of fos mRNA and participate in energy metabolism, and influence the expression of fos through the administration of leptin and hunger hormone.

Studies have shown that the key proteins for neural tube development mainly include (1) Nestin belongs to the sixth type of medium fibrin, and is one of the neural stem cell markers[17]. Nestin regulates mitochondrial metabolism to maintain the state of neural stem cells[18]. (2) Paired box gene family (PAX) is a highly conserved transcription gene family, which plays a key role in embryo formation and organ development[19]. (3) H3K27me3 is a key "switch protein" in the development of neural tube, and its expression is significantly related to the occurrence of neural tube defects. Folic acid can regulate the expression of H3K27me3 and prevent the occurrence of neural tube defects[20]. Based on domestic and foreign research and the research of this research group, in vitro experiments have proved that Mex3c can enhance the expression of fos and regulate leptin to participate in energy metabolism; transmission electron microscopy observed changes in the structure of the offspring's nerve pipeline. We will construct Mex3c^{+/-}, high-fat diet (HFD) and control (Control) mice to explore the mechanism of fos and leptin-leptin receptors during the neural tube development of the offspring under different energy states and the effects on neural tube development.

Methods

Experimental animals the research group used CRISPR and Cas technology to obtain Mex3c gene-deficient mice, and selected 60 wild-type 4-week-old female mice and 30 Mex3c-deficient mice 4-week-old female mice (raised in the Animal Center of Ningxia Medical University, ethics number: 2015-102). The Mex3c group and the Control group were given normal feed, while the HFD group was given high-fat feed. Record how much the three groups of mice eat and drink every day; measure their body weight every week (fasting for 8 hours). When the animals were raised to 16 weeks of age, the weight was measured and statistically analyzed. When the weight of the HFD group was 20% higher than that of the Control group and the Mex3c group, it indicated that the model was successful. All males used for breeding are wild-type. At 20:00, the male and female will mate at a ratio of 3:1. The vagina of the female will be observed at 8:00 the next day. If there is a vaginal plug, it will be recorded as 0.5 days, and the female will be reared alone In the squirrel cage, observe the changes in the amount of water and mammary glands, and the bead-like cysts can be palpable in the lower abdomen at 10.5 days, which is determined to be a successful gestation.

Obtaining experimental specimens When the female mouse is 10.5/12.5/14.5 days pregnant, on the day of taking the experimental tissue, fasting for 4h. Collect three groups of mother mouse liver, abdominal

fat, and serum tissues and collect three groups of embryonic tissues at different time points, and record the total number of embryos, live fetuses, and absorbed fetuses.

Eosin hematoxylin staining and immunohistochemical staining After dewaxing with dimethyl benzene, paraffin sections were exposed to ethanol gradient. We performed conventional dewaxing and hydration and used citrate antigen repair solution for high-pressure repair for 8 min. After treatment with 3% H₂O₂ for 20 min (endogenous peroxidase inhibition) and then goat serum blocking and addition of mouse anti-fos (1:200), mouse anti-nestin (1:200), rabbit anti-leptin receptor (1:200), rabbit anti-leptin (1:300), rabbit anti-Pax3 (1:300), primary antibody were incubation at 4°C for overnight. Next, the tissue was washed with PBS solution according to the secondary antibody kit instructions (ZSGB-BIO, China).

Reverse transcription-quantitative polymerase chain reaction (RT-PCR) The obtained embryos and placentas were ground and the total RNA was extracted with a kit (lot.no.18918913, AXYGEN, Made in China). The total RNA reversely transcribed to cDNA by the TransScript All-in-One First-Strand cDNA Synthesis SuperMix for qPCR (lot.no.30904, AXYGEN, Made in China). According to the manufacturer's protocol, 2 µl cDNA was added to 20 µl of a quantitative fluorescence reaction system (ChamQ Universal SYBR qPCR Master Mix; lot. no. 7E311K9; Vazyme; China) containing, forward primer (10 µM; 0.4 µl), reverse primer (10 µM; 0.4 µl), SYBR Green Real-time PCR Master Mix (10 µl; cat. no. QPK-201; Toyobo Life Science) and nuclease-free water (7.2 µl). BIO-RAD (Model No. CFX Connect Optics Module; Made in Singapore) was used to analyze curves of PCR amplification and solubility. The primer sequences were as follows Table 1. The thermocycling conditions were as follows: 94°C for 30 sec, 94°C for 5 sec and 60°C for 30 sec 40 cycles of amplification. Finally, results were obtained using the $2^{-\Delta\Delta C_q}$ method [21], with β -actin as an internal reference. Experiments were repeated three times for each sample (Table 1).

In situ hybridization At the time of taking embryo samples, all the related equipment was treated with 0.1% DEPC water for two hours, and then sterilized by high pressure steam. The tissues were immersed in 4% paraformaldehyde containing 0.1% DEPC water for 24 hours. The rest of the embedding operation was as same as the immunohistochemistry experiment.

Western blot experiment of leptin, LEPR, fos, nestin, PAX3, H3K27me3 of neural tube

The total lysates were boiled in 2% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) buffer. Prepared protein samples (240 µg/l) were isolated via 8% SDS-PAGE. Electrophoresis (60-120 V) was immediately terminated once bromophenol blue reached the bottom of the gel. Protein was then transferred onto PVDF (0.5mm) membrane for 1 hour at 240mA. After washing with PBS containing 1% Tween 20 (PBST), the membranes were blocked in 5% skimmed milk for 2 hours at room temperature. The following primary antibodies were then added and incubated at 4°C overnight: β -actin, leptin (cat. no. ab16227; 1:3,000), leptin receptor (cat. no. ab5593; 1:2,000), fos (cat.no.66590-1-Ig; 1:3,000), nestin (cat. no. Ab11306; 1:1,000), Pax3 (cat.no.ab18045; 1:3000) and Anti-Histone H3 (tri methyl K27) (cat. no. ab6002; 1:1,000). Membranes were washed three times with PBST (each, 10 min) and incubated with secondary antibodies (horseradish peroxidase-conjugated goat anti-rabbit IgG (cat. no. ab97051; 1:5,000) and goat anti-mouse IgG (1:10000)) at room temperature for 1.5 hours on a shaking table. Leptin, leptin

receptor, Pax3 and anti-Histone H3 were purchased from Abcam. Nestin and C-FOS was purchased from Protintech. The β -actin was purchased from ZSGB-BIO. Membranes were then washed five times with PBST (each, 10 min) and developed using a WesternBright™ ECL detection kit (cat. no. 190725-44; Advansta, USA). ImageJ software (National Institutes of Health, Bethesda, MD, USA) was utilized to quantitatively analyze the molecular weight and net optical density value of target bands, with β -actin used as a reference. Anti- β -actin was used as a reference to normalize the differences in the amounts of protein among samples.

ELISA After obtaining the whole blood, then placed for 30 minutes at 4°C, centrifuged 12,000×g for 5 min, and the supernatant was collected. leptin receptor and leptin levels were detected using ELISA kits. The optical density (OD) value was determined at 450 nm. leptin and leptin receptor levels in samples were calculated using a standard curve. At the same time, microplate reader method to detect TG, HDL and LDL.

Statistical Analysis SPSS16.0 was used for analysis, Image-Pro-Plus6.0 software was used to analyze immunohistochemistry results, GraPhPad Prism 8.0 was used for drawing, and Image J was used to analyze Western blotting results. After the measurement data conforms to the normal distribution, the $\pm$ S standard deviation is used to express, the t test and one-way analysis of variance, LSD pairwise comparison; HDL/LDL/TG use Kruskal-Wallis H test, count data use rank sum test The total number of embryos, the number of live fetuses, and the number of absorbed fetuses were tested by card method, and $P < 0.05$ indicated statistical difference.

Results

Physiological indicators of three groups of animal models

Regularly measure fasting weight (fasting for 8 hours), and draw a trend chart of weight change. The high-fat diet group weight is 20% higher than the control group as a successful model. From Figure 1, that there is no difference in body weight at the beginning. After 10 weeks of different diets, the HFD body weight ($22.61\text{g} \pm 1.10\text{g}$) was significantly higher than the Control group ($18.82\text{g} \pm 1.54\text{g}$) ($P < 0.001$) and the Mex3c group ($18.84\text{g} \pm 1.08\text{g}$) ($P < 0.001$), but there is no statistical difference between the Control group and the Mex3c group (Figure 1-A). Measure the water intake and food intake of each mouse every day per week, but there is no difference after statistical analysis (Figure 1-D, Figure 1-E). Experimental results: fat to body weight ratio. Compared with the Control group, the fat ratio of the HFD group was highly expressed ($P < 0.001$); compared with the Mex3c group, the fat ratio of the HFD group was highly expressed ($P < 0.01$) (Figure 1-B). The ratio of liver weight to body weight. Compared with Control, HFD was highly expressed ($P < 0.05$); compared with Mex3c group, HFD group was highly expressed ($P < 0.01$) (Figure 1-C). The results of TG, LDL and HDL in the serum of the three groups of mother mice showed that compared with the TG and LDL of the Control group, the Mex3c group had low expression ($P < 0.05$) and the HFD group had high expression ($P < 0.05$); compared with the Control group Compared with, the Mex3c group had low HDL expression ($P < 0.05$) and the HFD group had low HDL expression ($P < 0.01$) (Table 2).

Three groups of female mice related experimental results

The general appearance of the three groups of animal models

When the three groups of female mice were raised to 14 weeks of age, one animal was randomly selected from each group, anesthetized, placed on the operating table, and photographed; liver and kidney tissues were obtained and photographed. It can be seen from the Figure2-2 below that compared with the Control group, the HFD group has a larger appearance; compared with the Control group, the Mex3c group has no significant difference in appearance. Compared with the control group, the liver surface of the HFD group showed a typical yellow fatty liver; the appearance of the liver of the control group and the Mex3c group was not significantly different(Figuer 2-2). After opening the abdomen, the kidney tissue and surrounding fat were completely taken out. Compared with the Control group, the HFD group had more fat tissue around the kidney; compared with the Control group, the Mex3c group had more fat tissue around the kidney(Figuer 2-2).

Pathological morphology of liver tissue and abdominal adipose tissue in three groups of female mice

In HE staining(Figuer 2-3), observed under a light microscope, the structure of the liver lobules in the HFD group was damaged but the basic structure was visible. The liver cells were enlarged, the nucleus atrophy, and even the cells were completely vacuolated without nucleus, and were flooded by a large number of lipid droplets; in the Control group, the hepatocyte envelope and nucleus were intact, and the liver lobule structure was clearly visible; in the Mex3c group, the liver lobule structure was seen with small lipid droplets. HE staining of adipose tissue showed that the lipid droplets in the HFD group were the largest among the three groups, and the individual lipid droplets were fused. The lipid droplets in the Mex3c group were smaller than those in the HFD group, but the lipid droplets in the Mex3c group were smaller than those in the Mex3c group. The control group is large, the boundary of the lipid droplets in the Mex3c group is clearly visible; the lipid droplets in the control group are small and dense.

Concentrations of leptin and leptin receptor in the serum of three groups of female mice

Collect the blood of each group at different gestational periods, and determine the leptin receptor content in the serum of mother mice by ELISA method. The change trend of leptin concentration in the Control group, E10.5d~E12.5d~E14.5d ($P \geq 0.05$); the expression trend of the HFD group was the same as that of the Control group, and the change trend of leptin concentration at different developmental time points was different ($P \geq 0.05$). The expression trend at E14.5d was different from that of Control group and HFD group; compared with Control group E14.5d, Mex3c group E14.5d serum leptin content was lower ($P \leq 0.001$); Compared with Control group E14.5d, HFD group E14.5d serum leptin content is low ($P \geq 0.05$) (Table 3).

At the same time point, compared with the Control group, the HFD group had a higher serum leptin receptor concentration ($P \leq 0.001$); compared with the Mex3c group, the HFD group had a higher

expression of LEPR ($P=0.05$), but the same group had no LEPR at different time points. Differential expression (Table 4).

Experimental results of three groups of embryonic neural tubes

The general appearance of the three groups of embryonic tissues

The appearance of the three groups of embryos was photographed under a stereo microscope, and no mice with neural tube defects were seen(Figuer 2-1); during the embryo retrieval process, the fetus was absorbed and only a sac was left(Figuer 2-1). Collect the total number of embryos, live births, and absorbed fetuses of the three groups. After statistical analysis, there is no difference.

Histopathological staining results of three groups of embryonic neural tube

In order to understand the morphological differences of the three groups of mouse embryos, HE staining was designed to observe the neural tube of mouse embryos. The results showed that development at E10.5d, compared with the control group neural tube, HFD has a developmental delay. Compared with the Control group, the Mex3c group had no significant difference in neural tube development(Figuer 2-4). We found that various organs also appeared during this period, such as the heart, liver, and intestines. During E12.5d, compared with the Control group, the expression of cells in the neural tube of the Mex3c group became less and less. The same is true for the HFD group(Figuer 2-4(G/H/I)). When the embryo continued to conceive to E14.5d, we found that the cell expression of the Mex3c group and the HFD group was not as high as that of the Control group. In addition, it was found that the neural tube in the HFD group E14.5d was not completely closed(Figuer-2-4(J/K/L)). By HE staining, the nucleus (blue), cytoplasm (light red), the nucleus becomes smaller and smaller, and the cytoplasmic expression increases.

mRNA expression results of fos and LEPR in placenta tissue

The expression of fos in the three groups all reached a peak at E12.5d ($P=0.05$). In the placenta tissue of E10.5d, compared with the Control group, the Mex3c group expressed higher fos mRNA ($P=0.05$); compared with the Control group, the HFD group expressed lower fos mRNA ($P=0.05$); compared with the Mex3c group, Low expression of fos mRNA in HFD group ($P=0.05$)(Figuer 3-1A). In the placenta tissue of E12.5d, compared with the Control group, the Mex3c group had a higher expression of fos mRNA ($P=0.05$); compared with the Control group, the HFD group had a lower expression of fos mRNA ($P=0.05$); compared with the Mex3c group, The HFD group had low expression of fos mRNA ($P=0.05$)(Figuer 3-1B). In the placenta tissue of E14.5d, compared with the Control group, the Mex3c group had a high expression of fos mRNA ($P<0.0001$); compared with the Control group, the HFD group had a low expression of fos mRNA ($P=0.05$); compared with the Mex3c group, The expression of fos mRNA in the HFD group was low ($P=0.01$)(Figuer 3-1C). In the placenta tissue of E10.5d, compared with the Control group, the Mex3c group expressed high LEPR mRNA ($P=0.01$); compared with the Mex3c group, the HFD group expressed low LEPR mRNA ($P=0.05$); compared with the Control group In the HFD group, LEPR mRNA was highly expressed ($P<0.05$)(Figuer 3-1A). In placental tissues of E12.5d, compared with the

Control group, the HFD group had a low expression of LEPR mRNA ($P=0.01$); compared with the Mex3c group, the HFD group had a low expression of LEPR mRNA ($P=0.01$)(Figuer 3-1B). In placental tissues of E14.5d, compared with the Control group, the Mex3c group expressed high LEPR mRNA ($P<0.0001$), and compared with the Control group, the HFD group expressed low LEPR mRNA ($P<0.05$)(Figuer 3-1C).

mRNA expression results of fos, LEPR, leptin, nestin in neural tube

In order to explore whether the positive and negative energy states during embryonic development can affect the expression of nestin protein and postsynaptic factor fos in neural development by inducing the expression of leptin-LEPR, an RT-PCR experiment was designed for this purpose, at the mRNA level. Verify the expression of nestin and fos in different energy states. The results showed that, compared with the Control group, the fos mRNA expression in the Mex3c group was higher in the E12.5d neural tube ($P=0.001$); compared with the Control group, the fos mRNA expression in the HFD group was lower ($P=0.01$). Combined with the results of the previous experiments, Mex3c can increase the nuclear output of fos mRNA. As shown in Figure X, in the same period, compared with the Control group, the Mex3c group expressed high LEPR mRNA ($P<0.01$); compared with the Control group, The HFD group had low expression of LEPR mRNA ($P=0.01$). By detecting the mRNA expression of leptin, in the embryonic neural tube of 12.5 days, compared with the Control group, the Mex3c group expressed low leptin mRNA ($P<0.05$); compared with the Control group, the HFD group expressed low leptin mRNA ($P<0.01$)(Figuer 3-1D). When the embryo develops to E14.5d, the expression of leptin mRNA in the neural tube of each group is not statistically different. The experimental results show that at this stage, there are certain factors that affect the mRNA expression of leptin-LPER, which in turn affects the expression of leptin-LPER mRNA and mRNA expression of fos. In E12.5d neural tube, compared with Control group, Mex3c group expressed higher nestin mRNA ($P<0.05$); compared with Control group, HFD group expressed higher nestin mRNA ($P<0.05$); when it developed to E14.5d Compared with the Control group, the HFD group expressed higher nestin mRNA ($P=0.001$), but there was no difference in expression between the Control group and the Mex3c group(Figuer 3-1E).

Detection of leptin/LEPR/fos/nestin/PAX3/H3K27me3 protein expression in embryonic neural tube tissue

Detection of leptin/LEPR/FOS/nestin/PAX3/H3K27me3 protein localization in embryonic neural tube

In order to observe the expression position of leptin protein in the neural tube, Immunofluorescence staining showed that leptin expresses in the nucleus. As shown in the figure below, blue represents the nucleus and green represents leptin. After analysis, at E12.5d, compared with the Control group, the Mex3c group had a low expression ($P<0.0001$), compared with the Mex3c group, the HFD group had a low expression of leptin ($P<0.0001$); compared with the Control group, the HFD group Low expression of leptin ($P<0.0001$). When the embryo developed to 14.5 days, compared with the Control group, the Mex3c group still had a low expression of leptin ($P=0.05$), and there was no difference in the pairwise comparison of other groups.(Figuer 3-2)

In order to observe the expression position of leptin receptor protein in the neural tube, immunostaining was performed and it was found that LEPR was mainly expressed in the cell membrane. As shown in the figure below, blue represents the nucleus, and yellow or brown represents leptin receptor protein. The experimental results showed that at E10.5d, compared with the Control group, the Mex3c group had a high expression ($P<0.0001$), and compared with the Control group, the HFD group had a high expression of LEPR protein ($P<0.001$). At E12.5d, compared with the Control group, the Mex3c group had a high expression ($P<0.0001$); compared with the Mex3c group, the HFD group had a low expression ($P<0.0001$); compared with the Control group, the HFD group had a high expression of LEPR protein ($P<0.0001$). At E14.5d, compared with the Control group, the Mex3c group expressed higher expression ($P<0.0001$); compared with the Mex3c group, the HFD group expressed lower expression of the protein ($P<0.0001$); compared with the Control group, the HFD group had LEPR protein No difference in expression.(Figuer 3-4)

Fos is an instant-reactive protein, which is affected by many factors and will be expressed in large quantities in a short time. Through immunohistochemical experiments, we will explore its expression position in the neural tube. Through experiments, we know that fos is mainly expressed in the nucleus. The results obtained through single-factor analysis of variance and pairwise comparison are as follows: At E10.5d, compared with the Control group, fos is highly expressed in the Mex3c group ($P<0.0001$); Compared with the Mex3c group, the HFD group had low expression ($P<0.0001$). At E12.5d, compared with the Control group, the fos expression in the Mex3c group was low ($P=0.0001$); compared with the Mex3c group, the HFD group had a low expression of fos protein ($P=0.0001$); compared with the Control group, the HFD group Low expression of fos protein ($P<0.0001$). At E14.5d, compared with the Control group, the expression of fos in the Mex3c group was low ($P<0.0001$); compared with the Mex3c group, the expression of the HFD group was low ($P<0.0001$); compared with the control group, the expression of the HFD group was low ($P<0.0001$). It can be seen from the figure that the fos protein expression is different in the same group at different time points ($P<0.05$). (Figuer 3-2)

PAX3 protein is one of the embryonic precursor factors. In order to understand the expression of PAX3 in different pregnancy stages of each group, immunohistochemistry technology was used to observe the expression position in cells. Experimental results show that PAX3 is mainly expressed in the nucleus, and the closer to the developmental center of the head and tail, the more its expression, the more concentrated it is. At E10.5d, compared with the Mex3c group, the HFD group was highly expressed ($P<0.0001$); compared with the Control group, the HFD group was highly expressed ($P<0.0001$). At E12.5d, there was no difference in the expression of PAX3 protein in each group. At E14.5d, compared with the Control group, the Mex3c group had a high expression ($P<0.0001$); compared with the Mex3c group, the HFD group had a high expression of PAX3 protein ($P<0.01$); compared with the Control group, the HFD group had a high expression PAX3 protein ($P<0.0001$). (Figuer 3-5)

Nestin is one of the signs of neural stem cells. When the nerves gradually develop, they will be replaced by GFAP and other neural factors, which can indirectly judge the state of nerve development. It can be observed that nestin is mainly expressed on the neural cell stem cell membrane. When E12.5d is known,

compared with the Control group, the Mex3c group has a low expression of the nestin protein ($P<0.0001$), and compared with the Control group, the HFD group has a low expression of the nestin protein ($P<0.05$). At E14.5d, compared with the Control group, the Mex3c group had a higher expression of nestin protein ($P=0.0001$); compared with the Mex3c group, the HFD group had a lower expression of this protein ($P=0.01$). (Figuer 3-6)

It can be seen from Figure X that H3K27me3 is mainly expressed in the nucleus. At E12.5d, compared with the Control group, the H3K27me3 protein was highly expressed in the Mex3c group ($P<0.0001$); compared with the Mex3c group, the H3K27me3 protein was highly expressed in the HFD group ($P=0.05$); Compared with the Control group, the HFD group had a high expression of H3K27me3 protein ($P=0.0001$). At E14.5d, compared with the Control group, the HFD group had a high expression of H3K27me3 protein ($P<0.0001$); compared with the Mex3c group, the HFD group had a high expression of H3K27me3 protein ($P<0.01$). (Figuer 3-7)

The expression of LEPR in neural tube in situ hybridization

In Figure X: the blue in A represents the nucleus, the red in B represents the expression of LEPR in the neural tube, and C represents the merged image of A and B in the software. In the E10.5d neural tube, compared with the Control group, the LEPR protein was highly expressed in the HFD group ($P<0.0001$); compared with the Mex3c group, the LEPR protein factor was highly expressed in the HFD group ($P<0.0001$). In the E12.5d neural tube, LEPR was highly expressed in the Mex3c group compared with the Control group ($P<0.0001$); compared with the Mex3c group, LEPR was lower in the HFD group ($P<0.0001$). In the E14.5d neural tube, compared with the Control group, the HFD group had a higher expression of LEPR protein ($P=0.0001$); compared with the Mex3c group, the HFD group had a higher expression of LEPR protein ($P=0.01$). (Figuer 3-8)

WB test to detect leptin, LEPR, fos, nestin, PAX3, H3K27me3 protein content in embryonic neural tube tissue

In order to further verify whether the passage of leptin-LEPR under different energy states affects the relevant factors in neural development, a western blot experiment was designed. In the E12.5d neural tube, compared with the Control group, the leptin protein expression in the Mex3c group and the HFD group was lower ($P=0.01$) (Figuer 4B). Compared with the Control group, the Mex3c group had high expression of LEPR protein ($P=0.01$) (Figuer 4A). Compared with the Control group, the HFD group had low expression of nestin protein ($P=0.001$); compared with the Control group, the Mex3c group had low expression of nestin protein ($P=0.001$) (Figuer 4C). However, the PAX3 protein as an embryonic precursor factor participates in the process of neural tube development. Compared with the Control group, the Mex3c group highly expressed PAX3 protein ($P=0.001$); compared with the Control group, the HFD group highly expressed PAX3 protein ($P=0.001$) (Figuer 4C). At this stage, compared with the Control group, the Mex3c group had a low expression of fos protein ($P=0.01$); compared with the Control group, the HFD group had a low expression of fos protein ($P=0.001$) (Figuer 4A). Fos protein, as one of the fast-reacting protein factors, is affected by many factors. According to experimental results, in the development stage

of E12.5d, the fos protein of the Control group is affected by the expression level of leptin-LEPR, and there are other factors. Based on the experimental results, it is guessed whether there are other key factors that affect the development of the neural tube. Earlier experiments learned that the H3K27me3 protein is involved in the development of the neural tube. Compared with the Control group, the Mex3c group highly expressed the H3K27me3 protein ($P=0.001$); compared with the Control group, the HFD group also highly expressed this protein ($P=0.001$); compared with the Mex3c group, the HFD group highly expressed H3K27me3 protein ($P=0.01$)(Figuer 4B). This result can infer that positive and negative energy states can activate the expression of H3K27me3, which in turn affects the development and maturation of nerve cells.

Based on the E12.5d experimental results of the three groups of embryonic neural tubes, the positive and negative energy states affect the trend of neural development. Combined with the total number of embryos, live fetuses, and absorbed fetuses, there is no difference between the three groups. There are other factors, so the experiment continued to E14.5d, and the western blotting experiment was designed to detect each protein factor such as E12.5d again. In the experimental results, compared with the Control group, the Mex3c group had a high expression of LEPR protein ($P=0.01$); compared with the Control group, the HFD group had a high expression of LEPR protein ($P=0.05$)(Figuer 4D). Compared with the Control group, the Mex3c group had a low expression of fos protein ($P<0.0001$). Compared with the Control group, the HFD group had a low expression of fos protein ($P<0.0001$)(Figuer 4D). This expression trend was consistent with E12.5d. At this stage, compared with the Control group, the HFD group had a high expression of leptin protein ($P=0.05$); compared with the Mex3c group, the HFD group had a low expression of leptin protein ($P=0.01$)(Figuer 4E); the expression of leptin protein was expressed at different developmental stages inconsistent, suggesting that there are some factors affecting the development of leptin at this stage. Compared with Control group, Mex3c group highly expressed nestin protein ($P<0.0001$) and PAX3 protein ($P<0.001$); compared with Control group, HFD group highly expressed nestin protein ($P<0.001$) and PAX3 protein ($P<0.001$); Compared with the Mex3c group, the HFD group has low expression of nestin protein ($P<0.0001$) and PAX3 protein ($P<0.001$); the results show that when the embryo develops to E14.5d, the positive and negative energy state inhibits neurodevelopment and maturation(Figuer 4F). Also at this stage of development, the positive and negative energy states activate the H3K27me3 protein, as shown in Figure4E. Combining the results of 12.5 days and 14.5 days, in addition to the different expression trends of leptin protein, the expression trends of other protein factors are consistent, suggesting that energy status is not the only factor that affects the expression of leptin protein during embryonic development, and there is some mechanism to correct the expression during development. Errors in the developmental process lead to the completion of development.

Discussion

Energy balance means that intake and consumption reach a stable state. When the two are unbalanced, energy is out of balance. Positive energy balance means intake is greater than consumption, and negative energy balance means intake is less than consumption. In order to construct a positive-negative energy

balance animal model, wild-type female mice are given high-fat diet, and the model is successfully constructed when their body weight is 20% higher than the control group. Knock out the *Mex3c* gene by gene editing technology to construct *Mex3c*^{+/-} mice with negative energy balance. Figure 1A shows the weight change trend of three groups of female rats at different time points, which indicates that the animal model was successfully constructed. The positive energy balance group (HFD) had more abdominal fat and the liver appeared fatty liver. The appearance of liver in the negative energy balance group (*Mex3c*^{+/-}) was not significantly different from that in the control group. From the results of fat around the kidney, compared with the control group, the negative energy balance group had less and the positive energy balance group had more.

The nervous system is the most complex and the most rigorous in the entire embryonic development. There should be no mistakes, especially the development of the central nervous system. The development process is mainly in E4.5-7d (the first stage) and E8-E10.5d (the second stage). The first stage neuroectoderm is developed from the epiderm. At this time, the stem cells are totipotent and have the potential to develop into neural stem cells. After that, in the second stage, the neural plate cells in different positions have undergone different degrees of development and folding, and then closed[22]. Based on the HE staining results, the general appearance of embryos at 10.5 days and the poor development of the HFD group. This result indicates that a high-fat diet can affect embryonic development. Studies have shown that long-term high-fat diets can cause mitochondrial dysfunction and affect the offspring's synapses development[23]. When the embryos of the three groups continued to develop to 12.5d and 14.5d, it was observed that the cells in the neural tube of the *Mex3c* group and the HFD group were small and scarce. In the HFD group, there was no cell migration gap in the neural tube. However, there is no difference in the appearance of embryos at 14.5 days from the three groups. There is no difference in the number of absorbed fetuses and live fetuses through the analysis of the three groups. Nutritional imbalance will affect the development of nerve cells, but ultimately did not induce neural tube defects; which is same to Tauheed J researched[24].

Based on previous studies, it is known that *Mex3c* can induce the nuclear export of mRNA from *fos*. The immediate early genes (Immediately Early Genes, IEGs), also known as rapid response genes, are members of the proto-oncogene family and can be induced to express rapidly by the second messenger in the cell. C-FOS is the first to be called the immediate early gene (IEG), whose function is to combine short-term cell surface stimulation signals with long-term cell responses by regulating specific target genes, and participate in the regulation of cell proliferation, differentiation and apoptosis[25]. C-FOS mRNA may play an important role in early embryonic development[26]. C-FOS is a family of proto-oncogenes that is evolutionarily conserved in eukaryotic cells. C-FOS proto-oncogenes regulate transcription factors during cell transformation to lead to cell morphology. Changes in nematode cells are related to nerves, thoracic cavity development, and wound closure through extracellular matrix and cytoskeleton, which may also cause epithelial cell-to-interstitial transfer[27]. C-FOS is one of the early genes induced by neurotransmitters. It is generally considered to be the result of post-synaptic C-FOS inducing progress and mediating the transient stimulation of neurons, so C-FOS is used as an indicator of

neuron activation[28]. The experimental results show that C-FOS is expressed in the nucleus. C-FOS is a transient stress protein that participates in the process of normal cell growth, differentiation, information recognition and division. In our study, the expression of C-FOS was less in the Mex3c group and the HFD group. When stimulated by a variety of factors, C-FOS can be expressed transiently. It may be that the experimental group received less stimulation than the control group, resulting in less C-FOS expression.

In in vitro experiments, the Mex3c variant protein can enhance the nuclear export, stability and translation of C-FOS mRNA. Experiments have shown that the expression of C-FOS increases after leptin injection[29-31]. Professor Lu Bosong's research shows that Mex3c does not directly mediate leptin's involvement in food regulation and energy expenditure, but affects insulin and other factors on energy regulation. Through this experiment, the Control group was constructed, the negative energy state induced by Mex3c^{+/-}, and the positive energy state induced by high-fat diet were used to obtain mouse embryos. The concentration of leptin and LEPR in the serum of mother mice and the leptin and leptin in the neural tube of mouse embryos were detected. The expression of LEPR and fos protein can be concluded from experiments: the expression level of leptin is affected by different energy states, and high-fat diet can inhibit the expression of leptin, which is consistent with other scholars' research that high-fat diet induces leptin resistance in the body. Leptin works by inhibiting energy intake and increasing energy output. When obesity occurs, it induces leptin resistance, thereby inhibiting energy balance[32]. Leptin needs to bind to its receptor (LEPR) to act in the arcuate nucleus of the hypothalamus, and then stimulate a series of neural activities. The specific mechanism is currently unclear. Based on this understanding, if you want to explore the relationship between leptin and FOS, you must Check the expression of LEPR and understand the relationship between the two. From the results, it can be concluded that the mouse embryonic neural tube LEPR expression was at different time points. Compared with the Control group, the Mex3c group expressed LEPR higher; compared with the Mex3c group, the HFD group expressed lower LEPR. This expression trend was consistent, but in Changes in expression levels were observed at different developmental time points, and the corresponding expression levels of FOS in the neural tube also changed. Just as the FOS mentioned above is an immediate response factor, it will react immediately when the environment changes. From the results, it can be observed that compared with the control group, the peak of FOS expression in the Mex3c group was at E10.5d, while the expression trend in the HFD group was the same as that in the control group, suggesting that the negative energy state will affect the development of FOS earlier, and there is a certain mechanism. Affect the development of nerve postsynaptic FOS. Therefore, the in situ hybridization experiment was used to detect the expression of LEPR mRNA level, and it was found that only the expression trend at E12.5d was consistent with the LEPR protein level at E12.5d, which suggests that other factors are involved in the LEPR protein translation process. Furthermore, RT-PCR experiments were conducted to verify LEPR transcription at the DNA level. The experiments found that the DNA transcription trends of the three groups of LEPR were consistent with the protein level. The experimental results indicated that there were other factors involved in the process from cDNA to mRNA, which led to changes in the transcription results, but mRNA There is a mechanism in the process of translation into protein that once again corrected the previous transcription errors. Corresponding to in vitro experiments that Mex3c variant protein can enhance the nuclear output

of FOS mRNA, Mex3c enhances fos expression and regulates leptin-LEPR to participate in energy metabolism during the development of the offspring's neural tube.

In 1996, during the study of the embryonic development of *Caenorhabditis elegans*, it was discovered that Mex3c is a conserved RNA binding protein from nematodes to humans. It contains hnRNA homology (KH) RNA binding domains and regulates pal-1, rme-2 and target RNA such as nos-2[33]. The human Mex3c gene has four homologues, namely MEX3A, MEX3B, MEX3C and MEX3D, all of which contain a KH RNA binding domain at the N-terminus[34]. Some studies have shown that Mex3c can induce the body to show a strong negative energy state, and regulate food intake and energy expenditure by regulating insulin and leptin[28,29,35]. Mex3c induced negative energy state and high-fat diet induced positive energy state on the development of offspring's neural tube; designed corresponding experiments to verify one of the neural stem cell markers nestin (nesin), embryonic precursor factor (PAX3), histone 3 alpha Basicization factor (H3K27me3). PAX is a highly conserved transcriptional gene family that plays a key role in embryo formation and organ development[19]. The PAX family contains a paired domain (PD) and a transcription activation domain (TD) that bind to DNA sequences. In addition, some members of the family contain homology domains and octapeptide structures. In the PAX family, only PAX3 and PAX7 contain all the structures, and their functions are closely related to nerve and muscle development. Studies have shown that in mouse animal experiments, PAX3 participates in the process of neural tube development and closure by maintaining neuroepithelial cells in a proliferating and undifferentiated state. If PAX3 expression is insufficient or delayed during the critical period of neural tube development, it will cause neural tube Incomplete closure[36]. During embryonic development, PAX3 and PAX7 share a high degree of homology. Two genes are expressed on sensory neurons and melanocyte progenitor cells. These two genes are involved in the regulation of neural tube closure and subsequent differentiation[37]. PAX3 continues to maintain the stemness of muscle cells, and still allows certain muscle-derived cells to maintain the characteristics of stem cells after adulthood; PAX3 protein can be detected during the development of the dorsal neural tube; it is proposed that PAX3 maintains the characteristics of myogenic stem cells mediates the P53 gene, but the specific mechanism is not clear[38]. Therefore, by detecting the expression of PAX3 protein in the embryonic neural tube at different stages, the development and maturity of the neural tube can be judged. The experimental results showed that PAX3 was mainly expressed near the closed center of the front and back ends. Combined with the results of immunohistochemistry and western blotting, it was found that the HFD group expressed the most PAX3 at E10.5d, the Mex3c group expressed the most at E14.5d, and the control group was at E14.5d. There was no expression peak at these three time points. Combined with the closure of mouse embryonic neural tube before E10.5, it can be seen that the high-fat diet induced positive energy state and Mex3c^{+/-} induced negative energy state inhibited PAX3 in neural tube. Close the key period expression. This experimental study showed that nestin was expressed in the three groups of embryonic neural tubes. The embryonic neural tube of the control group was highly expressed at 12.5 days, and was gradually replaced by factors such as GFAP at 14.5 days. The HFD group and Mex3c group expressed low expression at 12.5 days. Compared with group E14.5d, the positive and negative energy group had higher expression of nestin protein. Nestin is the sixth type of medium fibrin, as a marker of neural stem cells. Studies have

shown that this protein is specifically expressed in the development of precursors in the central nervous system of mice[39]. Intermediate filament protein can provide structure and stability for cells. In mature neurons, intermediate filament protein maintains the normal structure of axons[40]. Vimentin and nestin proteins are strongly expressed in neural progenitor cells, so nestin protein can be used as one of neural progenitor stem cell markers. In different stages of neurodevelopment, nestin protein responds differently. Nestin's regulation of Sema3a responsiveness may be one of the mechanisms that regulates the cortical differential axon-guided decision in time and space; nestin protein is continuously expressed on immature nerve cells; Decrease expression as it matures[41]. GFAP is a marker of astrocytes that is not expressed in the early embryonic stage until the embryo develops to 17 or 18 days[42]. Therefore, in this experiment, nestin protein was used to judge the maturity of nerve cells.

It was found that nestin was not expressed in adult mice, except for the expression in the mouse brain, and other tissues did not express under normal conditions. It was verified that the mRNA expression of nestin in the mouse embryonic brain reached a peak at 14 days, and then fell into a downward trend. GFAP replaces. Nestin is expressed during neural tube development. When spinal cord injury occurs in adulthood due to various reasons, nestin, GFAP and BrdU can be used as signs of nerve cell regeneration, or signs of stem cells such as neural stem cells (progenitor cells); nestin can regulate Mitochondrial metabolism maintains the state of neural stem cells[18]. Based on the experimental results, compared with the Control group, the HFD group and the Mex3c group had a higher expression of nestin protein at 14.5 days. It can be seen that the positive and negative energy states inhibit the maturation of nerve cells. In the previous study, transmission electron microscopy observed that the embryonic neural mitochondria in the HFD group and Mex3c group at 14.5 days were underdeveloped and autophagy, indicating that the positive and negative energy status affects the development of offspring mitochondria, energy production and thus neural stem cells nestin expression.

Based on research at home and abroad, it is known how much PAX3 and nestin expression in the critical period of neural tube development will affect neural tube development. In addition, the research team found that H3K27me3 (histone 3 lysine 27 trimethylation status)) Is a key factor for neural tube development. The higher the expression during the closure period, the closure of the neural tube will be inhibited, and the intervention of folic acid will reduce its expression[20]. The expression of H3K27me3 protein is low after adding folic acid in animal models, which effectively prevents the occurrence of neural tube defects, indicating that the lack of folic acid is one of the inducements of neural tube defects[24]. Mammalian sperm and oocytes are different in the process of formation and have different epigenetic backgrounds[43]. During the fertilization process of the paternal genetic material, the cytoplasm is left outside the fertilized egg, so the maternal histone-related modifications will continue to exist[44]. Analyzed the H3K27me3 and DNA methylation status during embryonic development, and found that the methylation status of H3K27me3 will mostly decrease gradually. What is interesting is that H3K27me3 is mostly reduced in 6.5-day embryos but is found in the E9.5d placenta. DNA methylation status will persist[45]. During diploid development, most chromosome alleles are transcribed from two alleles. For a small part of genes, their parental alleles are transcriptionally silenced by genomic imprinting, depending on whether this allele is From oocytes or sperm cells; because genetic imprinting can continue to the next

generation, genomic imprinting and XCI imprinting are related to epigenetics; these two processes are essential to control the amount of gene expression involved in the embryonic development process, and they are dysregulated. Can cause malformations in embryonic development[46,47]. Based on research at home and abroad, histone methylation or DNA methylation status will affect epigenetics and continue to the next generation through a certain mechanism. According to experimental results, compared with the Control group, the HFD group and the Mex3c group are higher. Expression of H3K27me3 protein, this sustained methylation state will affect neural tube development, combined with the high expression of H3K27me3 protein in the neural tube malformation model group induced by the administration of all-trans retinoic acid in rats, and the folic acid supplement model. The group will reduce the expression of this protein; it can be concluded that Mex3c can activate the H3K27me3 protein to affect neural tube maturation.

In summary, Mex3c can regulate the leptin-leptin receptor molecular mechanism in the process of energy metabolism by enhancing fos mRNA to participate in the neural tube development of the offspring. The HFD group induces a positive energy state and the Mex3c group induces a negative energy state. Despite the continuous high expression of nestin, PAX3, and H3K27me3, it initially revealed that different energy states regulate leptin-LEPR to participate in the process of neurodevelopment, and the imbalance of positive and negative energy leads to the decrease of neurodevelopmental maturity. This experiment mainly explores the effects of neural tube fos and leptin receptors in the offspring of different energy states at the animal level and its influence on the neural tube development of offspring. It has not been verified at the cellular level. For the experimental results, the positive and negative energy states cause offspring. The decrease in the developmental maturity of nerve cells in the neural tube is the main innovation of this experiment. Insufficiency of the experiment, according to the results of this experiment, the lentiviral vector was transduced to construct Mex3c nerve cells. Western blotting experiments, RT-PCR, transmission electron microscopy and other experiments were used to further verify that the induction of energy imbalance in the offspring under different energy states affects neural tube development.

Conclusion

1. Mex3c regulates leptin and LEPR by enhancing the expression of fos mRNA to participate in the neural tube development process of offspring.
2. The neural tube nestin, PAX3, and H3K27me3 of the offspring of the Mex3c group and the HFD group are consistently highly expressed, which preliminarily reveals that different energy states regulate leptin-LEPR to participate in the process of neurodevelopment, and the imbalance of positive and negative energy reduces the maturity of neurodevelopment.

Abbreviations

abbreviation	Full name
Mex3c	Caenorhabditis Elegans Muscle Excess
HFD	High-Fat Diet
E10.5d	Embryonic Day 10.5
E12.5d	Embryonic Day 12.5
E12.5d	Embryonic Day 12.5
LEPR	Leptin Receptor
H3K27me3	Trimethylation of Lysine 27on Histone 3
PAX	PairedBox
LDL	Low Density Lipoprotein
HDL	High Density Lipoprotein
TG	Triglycerides

Declarations

1. **Ethics approval and consent to participate** Ethical certification has been submitted to the system supplementary materials.
2. **Consent for publication** If such an article is accepted, agree to publish.
3. **Availability of data and materials** Regarding the feasibility and authenticity of the data in the article, the author can guarantee that it is accurate and usable. Ningxia Medical University can prove it.
4. **Competing interests** All data and results in this article do not have any interest disputes with any institution.
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7. **Acknowledgements** The corresponding author indicates that the above information is accurate, and the author's order has been agreed by all authors.
8. **Authors' information (optional)**

Author Contributions

Yong DU designed and conceived experiments; Ting Li, Quan Huo ,Dongjun Sun and Zhiguo Lu performed the experiments; Meigang Li,Xinran Xing and Lu Ding analyzed the data and wrote the paper.

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References

1. Dute J: ECHR 2012/9 Case of Biziuk v. Poland (No. 2), 17 January 2012, no. 24580/06 (Fourth Section). *Eur J Health Law* 2012;19:311-314.
2. Detrait ER, George TM, Etchevers HC, Gilbert JR, Vekemans M, Speer MC: Human neural tube defects: developmental biology, epidemiology, and genetics. *Neurotoxicol Teratol* 2005;27:515-524.
3. Salih MA, Murshid WR, Seidahmed MZ: Epidemiology, prenatal management, and prevention of neural tube defects. *Saudi Med J* 2014;35 Suppl 1:S15-S28.
4. Petersen JM, Parker SE, Benedum CM, Mitchell AA, Tinker SC, Werler MM: Periconceptional folic acid and risk for neural tube defects among higher risk pregnancies. *Birth Defects Res* 2019.
5. KJ S, PW T, R B, J R: Maternal overweight and obesity and the risk of congenital anomalies: a systematic review and meta-analysis. *JAMA* 2009;301:636-650.
6. A E, S H, K F, S D, M A, Z H, F N, M EK: Risk factors associated with lipomyelomeningocele: a case-control study. *Pediatric neurosurgery* 2013;49:202-207.
7. M W, ZP W, LJ G, R G, XH S, ZT Z, AIM To Study The Influence Of Maternal Body Mass Index BMI On The Association Between Folic Acid Supplementation And Neural Tube Defects NTDs Risk In Offspring. MAHC: Maternal body mass index and the association between folic acid supplements and neural tube defects. *Acta paediatrica (Oslo, Norway : 1992)* 2013;102:908-913.
8. Mullen M, Gonzalez-Perez RR: Leptin-Induced JAK/STAT Signaling and Cancer Growth. *Vaccines (Basel)* 2016;4.
9. X C, Z F, W M, R Y, H C, OBJECTIVE To Observe The Short-term And Long-term Effects Of Leptin On Hepatic Glucose Oxidation And Glucokinase Gene Expression. MRHC: Effects of acute or prolonged exposure to leptin on hepatic glucose oxidation. *Chinese medical journal* 2001;114:592-595.
10. Thaler JP, Yi CX, Schur EA, Guyenet SJ, Hwang BH, Dietrich MO, Zhao X, Sarruf DA, Izgur V, Maravilla KR, Nguyen HT, Fischer JD, Matsen ME, Wisse BE, Morton GJ, Horvath TL, Baskin DG, Tschop MH, Schwartz MW: Obesity is associated with hypothalamic injury in rodents and humans. *J Clin Invest* 2012;122:153-162.
11. Ozcan L, Ergin AS, Lu A, Chung J, Sarkar S, Nie D, Myers MJ, Ozcan U: Endoplasmic reticulum stress plays a central role in development of leptin resistance. *Cell Metab* 2009;9:35-51.
12. Eletto D, Chevet E, Argon Y, Appenzeller-Herzog C: Redox controls UPR to control redox. *J Cell Sci* 2014;127:3649-3658.
13. Penna E, Pizzella A, Cimmino F, Trinchese G, Cavaliere G, Catapano A, Allocca I, Chun JT, Campanozzi A, Messina G, Precenzano F, Lanzara V, Messina A, Monda V, Monda M, Perrone-Capano C, Mollica

- MP, Crispino M: Neurodevelopmental Disorders: Effect of High-Fat Diet on Synaptic Plasticity and Mitochondrial Functions. *Brain Sci* 2020;10.
14. Han C, Jiao Y, Zhao Q, Lu B: Mex3c mutation reduces adiposity partially through increasing physical activity. *J Endocrinol* 2014;221:457-468.
 15. Y J, SK G, Q Z, MW H, SM H, CE B, B L, The Function Of MEX C TMHO: Mex3c mutation reduces adiposity and increases energy expenditure. *Molecular and cellular biology* 2012;32:4350-4362.
 16. Jiao Y, Bishop CE, Lu B: Mex3c regulates insulin-like growth factor 1 (IGF1) expression and promotes postnatal growth. *Mol Biol Cell* 2012;23:1404-1413.
 17. Cattaneo E, McKay R: Proliferation and differentiation of neuronal stem cells regulated by nerve growth factor. *Nature* 1990;347:762-765.
 18. Wang J, Huang Y, Cai J, Ke Q, Xiao J, Huang W, Li H, Qiu Y, Wang Y, Zhang B, Wu H, Zhang Y, Sui X, Bardeesi ASA, Xiang AP: A Nestin-Cyclin-Dependent Kinase 5-Dynamin-Related Protein 1 Axis Regulates Neural Stem/Progenitor Cell Stemness via a Metabolic Shift. *STEM CELLS* 2018;36:589-601.
 19. Paixao-Cortes VR, Salzano FM, Bortolini MC: Origins and evolvability of the PAX family. *Semin Cell Dev Biol* 2015;44:64-74.
 20. Zhai S, Zhao M, Zhou C, Lu F, Zhang H, Na L, Feng S, Qiang X, Du Y: The association and significance of H3K27me3 and a folate metabolic gene Acat2 in neural tube defects. *Nutrition Journal* 2016;15.
 21. Livak KJ, Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 2001;25:402-408.
 22. Tang K, Yang J, Gao X, Wang C, Liu L, Kitani H, Atsumi T, Jing N: Wnt-1 promotes neuronal differentiation and inhibits gliogenesis in P19 cells. *Biochem Biophys Res Commun* 2002;293:167-173.
 23. Penna E, Pizzella A, Cimmino F, Trinchese G, Cavaliere G, Catapano A, Allocca I, Chun JT, Campanozzi A, Messina G, Precenzano F, Lanzara V, Messina A, Monda V, Monda M, Perrone-Capano C, Mollica MP, Crispino M: Neurodevelopmental Disorders: Effect of High-Fat Diet on Synaptic Plasticity and Mitochondrial Functions. *Brain Sci* 2020;10.
 24. Tauheed J, Sanchez-Guerra M, Lee JJ, Paul L, Ibne Hasan MOS, Quamruzzaman Q, Selhub J, Wright RO, Christiani DC, Christiani DC, Coull BA, Baccarelli AA, Mazumdar M: Associations between post translational histone modifications, myelomeningocele risk, environmental arsenic exposure, and folate deficiency among participants in a case control study in Bangladesh. *Epigenetics* 2017;12:484-491.
 25. Ji M, T C: Immediate-early genes: ten years on. *Trends in neurosciences* 1995;18:66-67.
 26. Li X, Li Y, Liu C, Jin M, Lu B: Oocyte-Specific Expression of Mouse MEX3C652AA in the Ovary and Its Potential Role in Regulating Maternal Fos mRNA1. *Biology of Reproduction* 2016;94.
 27. Alfonso-Gonzalez C, Riesgo-Escovar JR: Fos metamorphoses: Lessons from mutants in model organisms. *Mech Dev* 2018;154:73-81.

28. Dhillon H, Zigman JM, Ye C, Lee CE, McGovern RA, Tang V, Kenny CD, Christiansen LM, White RD, Edelman EA, Coppari R, Balthasar N, Cowley MA, Chua SJ, Elmquist JK, Lowell BB: Leptin directly activates SF1 neurons in the VMH, and this action by leptin is required for normal body-weight homeostasis. *Neuron* 2006;49:191-203.
29. MA C, JL S, M R, MG C, S D, TL H, RD C, MJ L: Leptin activates anorexigenic POMC neurons through a neural network in the arcuate nucleus. *Nature* 2001;411:480-484.
30. Qiu J, Zhang C, Borgquist A, Nestor CC, Smith AW, Bosch MA, Ku S, Wagner EJ, Ronnekleiv OK, Kelly MJ: Insulin excites anorexigenic proopiomelanocortin neurons via activation of canonical transient receptor potential channels. *Cell Metab* 2014;19:682-693.
31. FC C, F JR, BT H: Using c-fos to study neuronal ensembles in corticostriatal circuitry of addiction. *Brain research* 2015;1628:157-173.
32. Deng G, Morselli LL, Wagner VA, Balapattabi K, Sapouckey SA, Knudtson KL, Rahmouni K, Cui H, Sigmund CD, Kwitek AE, Grobe JL: Single-Nucleus RNA Sequencing of the Hypothalamic Arcuate Nucleus of C57BL/6J Mice After Prolonged Diet-Induced Obesity. *Hypertension* 2020;76:589-597.
33. BW D, CC M, B B, J H, JR P, After The First Division Of The C. Elegans Embryo TPBC: MEX-3 is a KH domain protein that regulates blastomere identity in early C. elegans embryos. *Cell* 1996;87:205-216.
34. Buchet-Poyau K, Courchet J, Le Hir H, Seraphin B, Scoazec JY, Duret L, Domon-Dell C, Freund JN, Billaud M: Identification and characterization of human Mex-3 proteins, a novel family of evolutionarily conserved RNA-binding proteins differentially localized to processing bodies. *Nucleic Acids Res* 2007;35:1289-1300.
35. Donnini M, Lapucci A, Papucci L, Witort E, Jacquier A, Brewer G, Nicolin A, Capaccioli S, Schiavone N: Identification of TINO: a new evolutionarily conserved BCL-2 AU-rich element RNA-binding protein. *J Biol Chem* 2004;279:20154-20166.
36. Orriss IR, Lanham S, Savery D, Greene N, Stanier P, Oreffo R, Copp AJ, Galea GL: Spina bifida-predisposing heterozygous mutations in Planar Cell Polarity genes and Zic2 reduce bone mass in young mice. *Sci Rep* 2018;8:3325.
37. Huo B, Yang Y, Li M, Wan J, Zhang W, Yu B, Chen X: Pax3 inhibits Neuro-2a cells proliferation and neurite outgrowth. *Journal of Cellular and Molecular Medicine* 2020.
38. Buckingham M, Relaix F: PAX3 and PAX7 as upstream regulators of myogenesis. *Semin Cell Dev Biol* 2015;44:115-125.
39. Lendahl U, Zimmerman LB, McKay RD: CNS stem cells express a new class of intermediate filament protein. *Cell* 1990;60:585-595.
40. RC L, JP J: Functions of intermediate filaments in neuronal development and disease. *Journal of neurobiology* 2004;58:131-148.
41. CJ B, CG J, CC Y, ND D, KA L, B W: Nestin in immature embryonic neurons affects axon growth cone morphology and Semaphorin3a sensitivity. *Molecular biology of the cell* 2019;30:1214-1229.
42. Miller FD, Gauthier AS: Timing is everything: making neurons versus glia in the developing cortex. *Neuron* 2007;54:357-369.

43. A B, ME T: Chromatin dynamics in the regulation of cell fate allocation during early embryogenesis. Nature reviews. Molecular cell biology 2014;15:723-734.
44. A I, Y Z: Nucleosome assembly is required for nuclear pore complex assembly in mouse zygotes. Nature structural & molecular biology 2014;21:609-616.
45. A I, L J, F L, T S, Y Z: Maternal H3K27me3 controls DNA methylation-independent imprinting. Nature 2017;547:419-424.
46. JT L, MS B: X-inactivation, imprinting, and long noncoding RNAs in health and disease. Cell 2013;152:1308-1323.
47. D M, DJG M, T E, ER M, A R: Genomic imprinting disorders: lessons on how genome, epigenome and environment interact. Nature reviews. Genetics 2019;20:235-248.

Tables

Tabel 1 Primers used for real-time PCR

Gene	Forward primer	Reverse primer
<i>Leptin</i>	TGTTCAAGCAGTGCCTATCC	GGACCTGTTGATAGACTGCCA
<i>Fos</i>	ATCTGTCCGTCTCTAGTGCCA	CATTCCCGCTCTGGCGTAA
<i>Nestin</i>	GAACAGAGATTGGAAGGCCG	AGCCACTTCCAGACTAAGGG
<i>LepR</i>	CGTGGTCAGAAGATGTGG	CAGGAAAGGATGACACAGC
<i>β-actin</i>	TTGCCGACAGGATGCAGAAGGA	AGGTGGACAGCGAGGCCAGGAT

Tabel 2 Contents of TG, HDL and LDL in serum of pregnant mice in each group

Groups	TG	HDL	LDL
Control group	3.021±0.114	3.246±0.432	0.435±0.071
Mex3c group	2.275±0.432*	2.556±0.139*	0.147±0.018***
HFD group	6.669±0.691**##	1.039±0.139**##	1.022±0.041**###

*for Control mice versus Mex3c^{+/-}, # for Mex3c^{+/-} mice versus HFD mice; */# $P \leq 0.05$, **/## $P \leq 0.01$, ***/### $P \leq 0.0001$.

Table 3 The concentration of leptin in the serum of the three groups of female mice (pg/ml)

Group	E10.5d	E12.5d	E14.5d	<i>P</i>
Control group	181.51±61.53	1289.40±476.43	2120.29±504.19	0.05
Mex3c group	421.35±250.95	1442.01±468.62	494.36±170.37***	0.01
HFD group	487.32±243.50	1070.79±99.22	1267.52±728.73*###	0.05

*for Control mice versus Mex3c^{+/-}, # for Mex3c^{+/-} mice versus HFD mice, **P*0.05, **/###*P*0.001. These data are tested by rank sum test .

Table 4 The concentration of LEPR in the serum of the three groups of female mice (pg/ml)

Group	E10.5d	E12.5d	E14.5d
Control group	2105.38±673.20	1927.19±523.92	1955.30±585.23
Mex3c group	1901.22±977.98	2388.09±1180.43*	1859.82±452.30
HFD group	7705.05±1929.55***#	6619.01±1005.26***##	4122.30±598.70***###

*for Control mice versus Mex3c^{+/-}, # for Mex3c^{+/-} mice versus HFD mice, **P*0.05, **/##*P*0.01 ***/###*P*0.001. These data are tested by rank sum test.

Figures

Figure 1 Physiological indexes of pregnant mice. A Body weight of three group mice. B Abdominal fat to body weight ratio . C Liver weight to body weight ratio . D Three groups of mice food intake . E Three groups of mice water intake .
 * Represents compared with Control group ; # Represents compared with Mex3c group; */# $P < 0.05$, **/## $P < 0.01$,
 ***/### $P < 0.001$.

Figure 1

Physiological indicators of three groups of animal models Regularly measure fasting weight (fasting for 8 hours), and draw a trend chart of weight change. The high-fat diet group weight is 20% higher than the control group as a successful model. From Figure 1, that there is no difference in body weight at the beginning. After 10 weeks of different diets, the HFD body weight ($22.61\text{g} \pm 1.10\text{g}$) was significantly higher than the Control group ($18.82\text{g} \pm 1.54\text{g}$) ($P \leq 0.001$) and the Mex3c group ($18.84\text{g} \pm 1.08\text{g}$) ($P \leq 0.001$), but

there is no statistical difference between the Control group and the Mex3c group(Figuer 1-A). Measure the water intake and food intake of each mouse every day per week, but there is no difference after statistical analysis(Figuer 1-D, Figuer 1-E). Experimental results: fat to body weight ratio. Compared with the Control group, the fat ratio of the HFD group was highly expressed ($P \leq 0.001$); compared with the Mex3c group, the fat ratio of the HFD group was highly expressed ($P \leq 0.01$)(Figuer 1-B). The ratio of liver weight to body weight. Compared with Control, HFD was highly expressed ($P \leq 0.05$); compared with Mex3c group, HFD group was highly expressed ($P \leq 0.01$)(Figuer 1-C). The results of TG, LDL and HDL in the serum of the three groups of mother mice showed that compared with the TG and LDL of the Control group, the Mex3c group had low expression ($P \leq 0.05$) and the HFD group had high expression ($P \leq 0.05$); compared with the Control group Compared with, the Mex3c group had low HDL expression ($P \leq 0.05$) and the HFD group had low HDL expression ($P \leq 0.01$)(Tabel 2).

Figure 2

The general appearance of the three groups of animal models When the three groups of female mice were raised to 14 weeks of age, one animal was randomly selected from each group, anesthetized, placed on the operating table, and photographed; liver and kidney tissues were obtained and photographed. It can be seen from the Figure2-2 below that compared with the Control group, the HFD group has a larger appearance; compared with the Control group, the Mex3c group has no significant difference in

appearance. Compared with the control group, the liver surface of the HFD group showed a typical yellow fatty liver; the appearance of the liver of the control group and the Mex3c group was not significantly different(Figuer 2-2). After opening the abdomen, the kidney tissue and surrounding fat were completely taken out. Compared with the Control group, the HFD group had more fat tissue around the kidney; compared with the Control group, the Mex3c group had more fat tissue around the kidney(Figuer 2-2).

Figure 3

mRNA expression results of fos and LEPR in placenta tissue The expression of fos in the three groups all reached a peak at E12.5d ($P \leq 0.05$). In the placenta tissue of E10.5d, compared with the Control group, the Mex3c group expressed higher fos mRNA ($P \leq 0.05$); compared with the Control group, the HFD group expressed lower fos mRNA ($P \leq 0.05$); compared with the Mex3c group, Low expression of fos mRNA in HFD group ($P \leq 0.05$)(Figuer 3-1A). In the placenta tissue of E12.5d, compared with the Control group, the Mex3c group had a higher expression of fos mRNA ($P \leq 0.05$); compared with the Control group, the HFD group had a lower expression of fos mRNA ($P \leq 0.05$); compared with the Mex3c group, The HFD group had low expression of fos mRNA ($P \leq 0.05$)(Figuer 3-1B). In the placenta tissue of E14.5d, compared with the Control group, the Mex3c group had a high expression of fos mRNA ($P < 0.0001$); compared with the Control group, the HFD group had a low expression of fos mRNA ($P \leq 0.05$); compared with the Mex3c group, The expression of fos mRNA in the HFD group was low ($P \leq 0.01$)(Figuer 3-1C). In the placenta tissue of E10.5d, compared with the Control group, the Mex3c group expressed high LEPR mRNA ($P \leq 0.01$); compared with the Mex3c group, the HFD group expressed low LEPR mRNA ($P \leq 0.05$); compared with the Control group In the HFD group, LEPR mRNA was highly expressed ($P < 0.05$)(Figuer 3-1A). In placental tissues of E12.5d, compared with the Control group, the HFD group had a low expression of LEPR mRNA ($P \leq 0.01$); compared with the Mex3c group, the HFD group had a low expression of LEPR mRNA ($P \leq 0.01$)(Figuer 3-1B). In placental tissues of E14.5d, compared with the Control group, the Mex3c group expressed high LEPR mRNA ($P < 0.0001$), and compared with the Control group, the HFD group expressed low LEPR mRNA ($P < 0.05$)(Figuer 3-1C).

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Figure 4 The total protein levels of LEPR/ C-FOS/ H3K27me3/ Leptin/ Nestin/ PAX3 examined by western blot in the Neural tube of E12.5d(A/ B/ C) and E14.5d (D/ E/ F) region. β -actin was used as a loading control. The data are represented as mean $\pm$ SEM of three experiments. For each group, n = 5. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

Figure 4

WB test to detect leptin, LEPR, fos, nestin, PAX3, H3K27me3 protein content in embryonic neural tube tissue In order to further verify whether the passage of leptin-LEPR under different energy states affects the relevant factors in neural development, a western blot experiment was designed. In the E12.5d neural tube, compared with the Control group, the leptin protein expression in the Mex3c group and the HFD group was lower ($P \leq 0.01$)(Figuer 4B). Compared with the Control group, the Mex3c group had high

expression of LEPR protein ($P=0.01$)(Figuer 4A). Compared with the Control group, the HFD group had low expression of nestin protein ($P=0.001$); compared with the Control group, the Mex3c group had low expression of nestin protein ($P=0.001$)(Figuer 4C). However, the PAX3 protein as an embryonic precursor factor participates in the process of neural tube development. Compared with the Control group, the Mex3c group highly expressed PAX3 protein ($P=0.001$); compared with the Control group, the HFD group highly expressed PAX3 protein ($P=0.001$)(Figuer 4C). At this stage, compared with the Control group, the Mex3c group had a low expression of fos protein ($P=0.01$); compared with the Control group, the HFD group had a low expression of fos protein ($P=0.001$)(Figuer 4A).