Prenatal choline supplementation attenuates spatial learning deficits of offspring rats exposed to low-protein diet during fetal period

Cui-hong Zhu, Ting Wu, Yu Jin, Bi-xia Huang, Rui-fen Zhou, Yi-qin Wang, Xiao-lin Luo, Hui-lian Zhu*

Running title: choline supplementation and spatial learning deficits

¹ School of Public Health, Sun Yat-sen University, Guangzhou (510080), People's

Republic of China

* The corresponding author

AUTHORS:

Cui-hong Zhu: Email: zhuch3@mail2.sysu.edu.cn

Ting Wu: Email: 13760638645@163.com

Yu Jin: Email: jinyu@mail.sysu.edu.cn

Bi-xia Huang: Email: huangbix@mail2.sysu.edu.cn

Rui-fen Zhou: Email: 1123487506@qq.com

Yi-qin Wang: Email: gallicawang@163.com

Xiao-lin Luo: Email 16137515@qq.com

Hui-lian Zhu: Email: zhuhl@mail.sysu.edu.cn

Key words: choline, spatial learning, low-protein

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Abstract

Prenatal intake of choline has been reported to lead to enhance cognitive function in offspring, but little is known about the effects on spatial learning deficits. The present study examined the effects of prenatal choline supplementation on developmental low-protein exposure and its potential mechanisms. Pregnant female rats were fed either a normal or low-protein diet containing sufficient choline (1.1 g/kg choline chloride) or supplemented choline (5.0 g/kg choline chloride) until delivery. The Barnes maze test was performed at postnatal days 31-37. Choline and its metabolites, the synaptic structural parameters of the CA1 region in the brain of the newborn rat, were measured. The Barnes maze test demonstrated that prenatal low-protein pups had significantly greater error scale values, hole deviation scores, strategy scores and spatial search strategy, and lesser random search strategy values than normal protein pups (all $p < 0.05$). These alterations were significantly reversed by choline supplementation. Choline supplementation increased the brain levels of choline, betaine, phosphatidylethanolamine and phosphatidylcholine of newborns by 51.35% ($p < 0.05$), 33.33% ($p < 0.001$), 28.68% ($p < 0.01$) and 23.58% ($p < 0.05$), respectively, compared with the LPD group. Prenatal choline supplementation reversed the increased width of the synaptic cleft ($p < 0.05$) and decreased the curvature of the synaptic interface ($p < 0.05$) induced by a low protein diet. Prenatal choline supplementation could attenuate the spatial learning deficits caused by

prenatal protein malnutrition by increasing brain choline, betaine and phospholipids and by influencing the hippocampus structure.

1. Introduction

Malnutrition during the sensitive period of central nervous system (CNS) development is one of the principal factors adversely influencing brain development and function [1-3]. Early protein deficiency appears to be the most damaging nutritional insult, leading to permanent and unfavorable long-term effects on the formation and maturation of the central nervous system, which further affects cognitive performance[4-6]. Thus, active intervention on maternal protein malnutrition is important to ameliorate potential adverse effects on brain development function in offspring.

Choline is an essential nutrient needed for the structural integrity of the cell membrane, a precursor of cholinergic neurotransmitter, a facilitator of lipid transportation from the liver, and a methyl donor via conversion to betaine[7-9]. Choline is derived not only from the diet but also from de novo synthesis, in which phosphatidylcholine (PC) is formed mostly in the liver by the sequential methylation of phosphatidylethanolamine (PE) via phosphatidylethanolamine N-methyltransferase (PEMT), using S-adenosylmethionine (SAM) as the methyl donor[10]. Choline is well understood to be critical for brain development and function by influencing stem cell proliferation and apoptosis and by altering brain and spinal cord structure and function[11-13]. Increasing evidence indicates that prenatal choline supplementation leads to long-lasting changes in brain structure and enhancements in cognitive functioning[14, 15] . We know, for instance, that prenatal choline supplementation can attenuate learning deficits associated with prenatal alcohol exposure in rats[16] ,

protect rats from seizure-induced spatial memory retention deficits[17], and improve spatial learning and adult hippocampal neurogenesis in the Ts65Dn mouse model of Down syndrome [18]. However, it is unclear if choline supplementation can ameliorate the adverse effects of maternal protein malnutrition on the development of the brain in offspring.

The present study examines the effects of choline supplementation in pregnant rats, fed a low protein diet, on spatial learning and its potential mechanism in choline metabolites and the brain structure of the offspring. The results may provide a basis for the prevention and treatment of the adverse effects of maternal protein malnutrition.

2. Methods

2.1 Animals and diets

Pregnant Sprague-Dawley (SD) rats on gestational day (GD) 1 (appearance of vaginal plugs was designated as GD 0) were obtained from the Guangdong Medical Laboratory Animal Center (GDMLAC) with certificate number SCXK (GuangZhou) 2008-0002. Animals were individually housed in cages in a temperature- and humidity-controlled room with a 12-h light/dark cycle and were allowed ad libitum access to food and water.

All pregnant rats were randomly divided into three groups (3 in each group). One group was fed the AIN-93G diet containing 20% casein and 1.1 g/kg choline chloride (NPD group); the second group was fed an isoenergetic low-protein (10% casein) diet [19] containing 1.1 g/kg choline chloride (LPD group); and the third

group was fed the low-protein with high choline containing 10% of casein and 5.0 g/kg choline chloride (LPD-Cho group). All pregnant rats were fed a diet mentioned above until the day of delivery (postnatal day 0, PND 0) and then fed the AIN-93G diet until weaning day (PND 21). All dams were housed individually with their litter in plastic cages with wood chip bedding from PND 0 to weaning day (PND 21). After weaning, all rat offspring were also fed the AIN-93G diet until the end of the experiment. Compositions of the experimental diets are displayed in Table 1.

On the day of birth, six newborn rats per group from 3 dams (2 rats per dam with medium body weight) were selected and sacrificed. Brain tissues of cerebral cortex were rapidly collected and frozen in liquid nitrogen and stored at -80°C prior to analysis for choline, betaine and phospholipids. The remaining rat offspring were maintained until PND 37. The study was carried out under the guidelines for the use and care of laboratory animals and was approved by the Institutional Animal Care and Use Committee of Sun Yat-Sen University.

Table 1. Composition of the experimental diets (g/kg).

Diet ingredients	NPD*	LPD	LPD-Cho
Casein	200	100	100
Corn starch	398.886	473.886	469.986
L-Cystine	3	3	3
Cellulose	50	50	50
Sucrose	100	100	100
Maltose dextrin	132	157	157
AIN mineral mix 93G	35	35	35
AIN vitamin mix 93	10	10	10

Choline chloride	1.1	1.1	5.0
Soybean oil	70	70	70
<i>tert</i> -Butylhydroquinone	0.014	0.014	0.014

* NPD: Normal protein diet of AIN-93G

2.2 Assessment of spatial learning using the Barnes maze

2.2.1 Apparatus of Barnes maze

The Barnes maze consisted of a white circular Acrylonitrile-butadiene-styrene (ABS) disk (110 cm diameter) with 20 equally spaced holes (10 cm diameter) located 5 cm from the edge of the maze. A black ABS escape box (15 × 15 × 50 cm) was positioned beneath the escape hole, into which rats could escape from the maze surface. The position of the escape box differed randomly between rats but was constant for each animal in relation to distal cues. Rats were reinforced to escape by two flood lights (150 W) and a buzzer (0–37.2 kHz, 89 dB). A camera (Sony AK-025K) was placed 1.4 m above the center of the maze to record (5 frames/sec) the movement of the rats for the Limelight tracking system (Xinruan, Shanghai, China) on a custom-built computer.

2.2.2 Barnes maze procedure

On PND 31–37, all rats were subjected to a Barnes maze modified from the original apparatus [20] to test spatial learning. Rats completed two phases of testing: habituation and acquisition training, as described below. All testing was performed by a single, experienced expert.

Habituation

On PND 31, rats completed one habituation trial to acclimate to the maze environment. During the habituation trial, rats were placed beside their respective escape hole underneath an inverted 2-liter-sized transparent glass beaker for 5 min

and were allowed to practice descending into the escape box. If rats did not enter the escape box freely, they were placed directly into the escape box for 30 s before being returned to their home-cage.

2.2.3 Acquisition training

The day following habituation testing, rats received 6-day acquisition training with two trials per day. For each trial, rats were placed into a start box ($20 \times 20 \times 20$ cm) in the maze. Ten seconds later, the box was lifted away. The rats were stimulated by flood lights and a buzzer, and forced to find and enter the escape box in 4 min. If the rats did not enter the escape box, they were gently guided into it and were allowed to remain in the escape box for 30 s. This trial was repeated twice after a 15 min inter-trial interval and concluded Acquisition Day 1. Using the same procedures and the same location of the goal box, rats received testing in the following five consecutive days (Acquisition Days 2-6). The maze surface and escape box were cleaned with 75% ethanol between each trial to remove intra-maze odor cues.

During acquisition training testing, five measures were recorded: (1) Latency: the time required to first locate the escape hole. (2) Errors: the number of times the rat dipped its head into an incorrect hole before entering the escape hole. We use a semi-quantitative error scale to evaluate this measurement as follows: scale 1 = 0-1 errors; scale 2 = 2-4 errors; scale 3 = 5-9 errors; scale 4 = ≥ 10 errors; scale 5 = no entry to the escape hole. (3) Hole deviation score: the number of holes between the hole the rat first visited and the escape hole, 0-10 range. (4) Mean speed. (5) Search paths [21].

The search paths were classified as spatial, serial and random as described by Barnes CA. [20]. Briefly, spatial: moving directly toward the escape hole from the center of the maze without visiting holes more than two holes away from the escape hole; serial: moving around the edge of the maze past at least three adjacent holes; and random: searching in a nonsystematic search with multiple crossings through the center of the maze. The overall strategy score for one rat was generated as follows: spatial = 1, serial = 2, random = 3, and this score was evaluated every 2 acquisition days. The search strategy was analyzed using the percentage of trials in which each strategy was used and was evaluated every 2 acquisition days (2 days/evaluation).

2.3 Measurement of choline, betaine and phospholipids levels in brain

2.3.1 Choline and betaine analyses

Fifty milligram brains were homogenized in 150 μ l of chloroform/methanol (1:2, v/v) in an ice bath and left at -20°C overnight. Then, the samples were centrifuged at 3,000 rpm for 5 min. Thirty microliters of either the supernatants or standards (choline and betaine) were treated with 90 μ l acetonitrile containing 10 μ M Cho-d₉ as the internal standard, and the samples were centrifuged at 13,000 rpm for 10 min. The resultant supernatants were transferred into spin columns and centrifuged at 3,000 rpm for 2 min to filter impurities. A portion (5 μ l) of this final supernatant was analyzed by high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) [22].

2.3.2 Phospholipids analyses

The brain phospholipids were extracted according to Bligh and Dyer [23].

Briefly, 10 mg brains were homogenized in 500 μ l of chloroform/methanol/water (1:2:0.8, v/v/v) in an ice bath and left at -20 °C overnight. Then, the samples were centrifuged at 3,000 rpm for 5 min. The supernatants were transferred into new tubes and the extraction procedure was repeated two more times. The combined extract solution was centrifuged at 3,000 rpm for 10 min. Sixty microliters of either the final supernatants or standards were spiked with an internal standard of PC-d₉. Then, a 5 μ l aliquot was analyzed by injection onto a normal-phase silica column (100 $\times$ 2.1 mm, 5 μ m particle size, Welch Materials Inc, Maryland, USA) with a guard column containing the same packing (10 $\times$ 2.1 mm, 5 μ m particle size, Welch Materials Inc, Maryland, USA) at a flow rate of 400 μ l/min. A discontinuous gradient was generated to resolve the analytes by mixing solvent A (acetonitrile) with solvent B (10 mM ammonium formate in water at pH 3.0) in the following manner: 0–0.1 min, 15% B; 0.1–10 min, from 15% to 26% B; back to 15% B at 10.1 min; and 10.1–15 min, 15% B. Two scan modes, the precursor ion scan and neutral loss scan, were used to quantify PE, PC, SM and PC-d₉. The coefficient of intra-assay variation for the measurements were 3.6% for PE, 0.6% for PC and 2.5% for SM. The coefficient of inter-assay variation for the measurements were 5.0% for PE, 5.7% for PC and 10.7% for SM.

2.4 Measurement of the synaptic structural parameters of the CA1 region

Upon completion of the Barnes Maze test, the rats were anesthetized with sodium pentobarbital (30 mg/kg) via intraperitoneal injection and perfused transcardially with 0.9% saline (100 ml), followed by a fixative in the mixture of 2%

paraformaldehyde and 2.5% glutaraldehyde in 0.1 mol/L phosphate buffer (150 ml; pH = 7.4). Brain tissue was collected, and the CA1 region was separated from the left side of the hippocampus and fixed with 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 mol/L phosphate buffer (pH = 7.4) over 4 h and followed by post-fixation with 1% osmium tetroxide in 0.1 M phosphate buffer (pH = 7.4) for 1.5 h. Subsequently, the samples were routinely dehydrated using a graded ethanol series and embedded in Epon. Seventy-nm coronal sections were cut and mounted on copper grids. The sections were stained with 4% uranyl acetate and 0.4% lead citrate. Electromicrographs of synapses were taken at a 37,000 $\times$ magnification using a transmission electron microscope (FEI, America) to measure the synaptic structural parameters by selecting typical Gray I synapses [24]. The width of the synaptic cleft, the curvature of the synaptic interface, the length of the synaptic active zone and the thickness of the postsynaptic density (PSD) were determined using Image-Pro Plus 6.1 computer software (Media Cybernetics, Bethesda, MD, USA). Sixty synapses of each group were examined.

2.5 Data analysis

All statistical analyses were performed with SPSS 13.0 (IBM Inc., Armonk, NY, USA). Body weights and measures of acquisition training were presented as the mean $\pm$ SEM. These data were analyzed using the analysis of variance for repeated measurements followed by a Bonferroni t-test. Choline and its metabolites, synaptic structural parameters data were presented as the mean $\pm$ standard deviation (SD) or the median/interquartile range, according to the normal distribution status. These data

were analyzed by one-way analysis of variance or the Kruskal-Wallis H-test followed by Bonferroni t-test. Statistical significance was accepted at $p < 0.05$.

3. Results

None of the pregnant rats is pseudopregnant. There are 4 dead fetus among the total 74 neonatal rats with no significant difference in number of birth and death rate among groups ($P > 0.05$). We selected 54 offspring rats, with 18 rats per group, with medium body weight within the group to performed the experiments.

3.1 Body weights of offspring

Body weights were measured every three days from PND 3 to 36, and the body weight curves are displayed in Fig. 1. During the lactation period (PND 3 to 21), the body weights of all groups increased significantly across days ($F_{(1.56, 51.42)} = 1626.36$, $p < 0.001$; Fig. 1A). There was a main effect across the groups ($F_{(2, 33)} = 4.47$, $p < 0.05$). The Bonferroni t-test showed that the mean body weight of the LPD group was significantly lower than in the NPD group ($p < 0.05$). During the post-weaning period (PND 22 to 36), body weight growth trends of all animals ($F_{(1.61, 53.09)} = 1312.61$, $p < 0.001$; Fig. 1B) and the difference between LPD and NPD groups ($p < 0.05$) (Fig. 1B) were the same as observed in the lactation period. Choline supplementation (LPD-Cho group) seems to attenuate body weight loss induced by low protein diet (LPD group), but no significant difference was observed at any time.

Pregnant rats were weighted every week. The weigh gain were 23.3 ± 8.6 g, 23.2 ± 6.4 g and 21.2 ± 7.0 g in NPD, LPD and LPD-Cho group, respectively ($P > 0.05$)

3.2 Acquisition training

For four learning measures of latency (Fig. 2A), error scale (Fig. 2B), hole deviation scores (Fig. 2C) and mean speed (Fig. 2D), data from six days of acquisition training were analyzed.

Latency ($F_{(1.85, 61.13)} = 13.81, p < 0.001$), error scale ($F_{(5, 165)} = 23.79, p < 0.001$), and hole deviation scores ($F_{(5, 165)} = 3.41, p < 0.05$) decreased, and mean speed ($F_{(5, 165)} = 12.72, p < 0.001$) increased over the days of acquisition training in all rats.

There were pronounced differences in latency ($F_{(2, 33)} = 3.75, p < 0.05$), error scale ($F_{(2, 33)} = 8.93, p < 0.01$) and hole deviation scores ($F_{(2, 33)} = 5.44, p < 0.01$), and the mean speed was relatively consistent among groups. However, the Bonferroni t-test showed that only the error scale ($p < 0.01$; Fig. 3B) and hole deviation scores ($p < 0.05$; Fig. 3C) were significantly higher in the LPD group than in the NPD group. These greater values were significantly reversed by choline supplementation, as the LPD-Cho group had a smaller error scale ($p < 0.05$) and lower hole deviation scores ($p < 0.05$). Although the latency in the LPD group was higher than in the NPD group (but not significantly so), choline supplementation (LPD-Cho group) could obviously reverse the increase in latency produced by the low protein diet ($p < 0.05$).

The results of search strategies are shown in Fig. 4. All animals showed a significant decrease in strategy scores ($F_{(2, 66)} = 8.97, p < 0.001$; Fig. 4A) and percentage of random strategy ($F_{(2, 66)} = 9.27, p < 0.001$; Fig. 4D); there was an increase in percentage of spatial strategy ($F_{(2, 66)} = 3.55, p < 0.05$; Fig. 4B) across three evaluations with obvious group differences (all $p < 0.05$). Compared with the

NPD group, the Bonferroni t-test showed that the LPD group had a significantly higher strategy scores and percentage of random strategy and lower percentage of spatial strategy (all $p < 0.01$). These types of changes were obviously reversed by choline supplementation (LPD-Cho vs LPD: all $p < 0.05$). No effects of evaluation ($F_{(2, 66)} = 2.22$, $p = 0.12$; Fig. 4C) and group ($F_{(2, 33)} = 0.98$, $p = 0.39$) were found using a serial search strategy.

3.3 Levels of choline, phospholipids and betaine in brain

There were no clear differences in brain choline and phospholipids levels between the NPD and LPD groups (Table 2.). However, supplementation of choline to pregnant rats largely increased the brain levels of choline, betaine, PE and PC of newborn rats. Compared with the LPD group, the levels of brain choline, betaine, PE, and PC in the LPD-Cho group increased 51.35% ($p < 0.05$), 33.33% ($p < 0.001$), 28.68% ($p < 0.01$) and 23.58% ($p < 0.05$), respectively. There was no significant difference in SM among all groups.

Table 2. Levels of choline, phospholipids and betaine in brains of newborn rats by groups. *umol/g brain tissue*

	NPD	LPD	LPD-Cho	<i>p</i> value
Choline	0.40 ± 0.10	0.37 ± 0.15	0.56 ± 0.07*	0.023
PE	3.93 ± 0.59	3.94 ± 0.36	5.07 ± 0.55**	0.002
PC	33.22 ± 5.53	32.61 ± 3.26	40.30 ± 4.05*	0.014
SM	2.02 ± 0.31	2.06 ± 0.13	1.96 ± 0.20	0.725
Betaine	0.28 ± 0.03	0.27 ± 0.02	0.36 ± 0.04**	<0.001

Data are present as mean ± SD, (n = 6/group).

* $p < 0.05$, ** $p < 0.01$ compared with the LPD group using Bonferroni t-test.

3.4 Synaptic ultrastructure in the CA1 region of the hippocampus

The synaptic ultrastructure in the CA1 region of the hippocampus was observed, and structural parameters were quantified using the transmission electron microscope. The width in the LPD group was significantly increased compared with the NPD group ($p < 0.05$); this increase was reversed by choline supplementation in the pregnant rats ($p < 0.01$). There were no significant differences in the curvature of synaptic interface, length of active zone or thickness of PSD among the groups, with the exception of a larger curvature of synaptic interface in the LPD-Cho group than the LPD group ($p < 0.01$). The results are shown in Fig. 5 and Table 3.

Table 3. Effects of experimental diets on synaptic structural parameters in the CA1 region of the hippocampus in rats.

Synaptic structural parameters	NPD	LPD	LPD-Cho	<i>p</i> value
Width of synaptic cleft (nm)	16.72 ± 2.23	19.57 ± 2.46#	17.11 ± 2.09*	<0.001
Curvature of synaptic interface	1.04 (1.01-1.05)	1.03 (1.01-1.05)	1.05 (1.02-1.07)*	0.004
Length of active zone (nm)	240.38 (203.09-280.33)	217.09 (190.71-248.74)	243.35 (200.05-284.53)	0.067
Thickness of PSD (nm)	83.96 (74.55-93.00)	83.58 (72.59-89.80)	81.69 (72.09-95.87)	0.764

Data are presented as the mean ± SD or the median/interquartile range, (n = 60/group).

# $p < 0.05$ compared with NPD group by Bonferroni t-test.

* ($p < 0.01$) compared with LPD group by Bonferroni t-test.

4. Discussion

The aim of the present study was to explore the effects of prenatal choline supplementation on the adverse effects of cognitive function induced by prenatal protein malnutrition. The data indicated that the supplementation of choline in

pregnant rats that were fed a low protein diet significantly attenuated spatial learning deficits in offspring, likely by increasing the width of the synaptic cleft and curvature of the synaptic interface in the brain. The related mechanism may occur due to alterations in brain choline and its metabolites betaine, PE and PC.

In the present study, the AIN-93G formula for rat was used for the normal diet, in which there was 1.1g choline chloride (equal to 0.95g choline) per kg diet, and was considered to provide adequate choline intake during normal pregnancy[25]. The high choline diet (5.0g choline chloride) provided approximately 4.5 times the concentration of choline in the normal diet, within the range of dietary variation observed in the human population[26] and demonstrated by previous studies to having cognitive benefits in rodents [18, 27].

The finding that a prenatal low-protein diet (10% protein) led to lower body weights is consistent with previous studies[28, 29]. Prenatal protein-malnourished rats exhibited impaired acquisition learning by measures of error scale and hole deviation scores in the Barnes maze. The use of different search strategies to locate the escape hole may underlie the poor performance of prenatal protein-malnourished rats during acquisition training, as they were worse at finding the escape hole than controls. Overall, these results replicated learning deficits in the Morris water maze [30] and suggest that prenatal protein malnutrition can result in spatial learning deficits on the Barnes maze.

Prenatal choline supplementation attenuated the spatial learning deficits induced by a prenatal low-protein diet in rat offspring. Compared with the prenatal low protein

group, prenatal choline-supplemented rats had a smaller error scale and lower hole deviation scores during acquisition training and were better at finding the escape hole in the Barnes maze. The results are consistent with previous reports that choline supplementation during pregnancy attenuates cognitive deficits associated with early maternal separation, undernutrition[31]and alcohol exposure [16]. There was also an insignificant trend in attenuating body weight reductions by prenatal choline supplementation, which is consistent with previous reports by Monk [14]. Interestingly, offspring rats in the prenatal low-protein diet group did not exhibit impairment in latency in the Barnes maze, and prenatal choline-supplemented offspring rats had a shorter latency relative to their unsupplemented counterparts, similar to previous reports that choline-supplemented rats display shorter escape latencies [32, 33]. Taken together, these findings suggest that supplementing the maternal diet with additional choline during pregnancy substantially improved the performance of the prenatal protein-malnourished rats in the Barnes maze.

It has been reported that supplementing choline to dams resulted in significantly greater accumulation of choline and its metabolites in the fetal brain[34]. In the present study, we found that supplying additional choline to pregnant rats that were fed a low-protein diet dramatically increased the levels of choline, PE and PC in the brains of newborn rats. These effects could be explained by (1) the major function of choline is conversion to PC via the CDP-choline pathway [35], which accounts for ~70% of PC biosynthesis in rodents[36]. (2) When the level of choline increases, the alternative pathway for PC biosynthesis might be saved, through which PE is

converted to PC catalyzed by PEMT. This may explain why the newborn rat's brain PE, the substrate for the PEMT pathway, is increased.

PC serves as a primary lipid in cellular membranes and functions as a precursor for signaling molecules such as diacylglycerol, phosphatidic acid, lysophosphatidylcholine, and free fatty acids [37]. Neuronal differentiation is a complex phenomenon comprising a set of separate processes including neuritogenesis, neurite outgrowth, polarization, pathfinding, and synaptogenesis [38]. PC plays an important role during neuronal differentiation. First, an increased level of PC could have a structural function providing a platform for cytoskeletal or signaling proteins to activate differentiation. Second, PC and its metabolites could serve as signaling molecules to drive neuritogenesis [39]. Neuronal differentiation is important for the recovery of plasticity and regeneration after injuries or neuropathological conditions [39]. We find that the brain level of PC is increased in prenatal low-protein newborn rats whose mother received choline treatment. Consequently, an increased level of PC might result in changes in neuronal differentiation, which could subsequently influence the hippocampus structure and consequently contribute to the formation of enhanced cognitive performance. This might be the mechanism by which choline supplementation mitigates spatial learning deficits induced by prenatal protein malnutrition. In addition to being a precursor of the phospholipid components of membranes, choline is also a precursor to acetylcholine, an important ontogenetic signal. Moreover, choline also acts as a methyl donor by influencing DNA methylation and subsequent gene expression. Thus, the neuroprotection against spatial

learning deficits induced by prenatal protein malnutrition of choline may be multi-factorial.

In the present study, the measures for spatial learning were assessed 30 days after birth. Thus, it is reasonable to expect that the protective effects of prenatal choline supplementation on these measures might be long-lasting. Previous studies have demonstrated that prenatal choline supplementation has long-lasting potential effects on improving cognitive function in normal offspring and protecting against the cognitive neuro-impairment associated with aging [25, 40, 41], prenatal alcohol exposure[42], Down syndrome [18, 43], and seizure disorders [44, 45]. Collectively, these findings suggest that the current dietary guidelines for choline, which correspond to the level of intake deemed necessary to prevent maternal liver damage (Inst. Med., Natl. Acad. Sci. USA. 1998), may not be optimal for brain development and that higher levels of choline intake during pregnancy and lactation might enhance cognitive functioning of the offspring throughout their lives[46].

5. Conclusions

In summary, this study demonstrates that supplementing the maternal diet with additional choline during pregnancy substantially attenuates spatial learning deficits in prenatal protein-malnourished rats, likely by altering choline and its metabolites in the brain of the newborn rat, and further influences brain structure. This finding provides support for the theory that choline supplementation may effectively protect against the adverse effect of prenatal protein malnutrition.

Acknowledgements

This study was jointly supported by the Nutrition Research Fund of CNS-DSM (2013) and National Science Foundation of China (No. 81472966).

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Figure legends

Fig. 1 The body weights curves during the lactation period and the post-weaning period.

Data are shown as mean ($\pm$ SEM). The LPD group weighed less than the NPD group during the lactation period (A), and the LPD group continued to lag in growth during the post-weaning period compared to the NPD group (B). Body weights of the LPD-Cho group did not differ significantly from the LPD group at any time. (n = 12/group). * $p < 0.05$ compared with the NPD group by Bonferroni t-test.

Fig. 2 The four learning measures across the acquisition training days

Data are presented as mean ($\pm$ SEM) in latency (A), error scale (B), hole deviation scores (C) and mean speed (D) across six acquisition training days (n = 12/group).

Fig. 3 The averaged latency, error scale and hole deviation scores of the acquisition training days

Data are presented as mean ($\pm$ SEM) in averaged latency (A), error scale (B) and hole deviation scores (C) of acquisition training days (n = 12/group). * $p < 0.05$, ** $p < 0.01$ by Bonferroni t-test.

Fig. 4 The search search strategy measures across the evaluations

Data are presented as the mean ($\pm$ SEM) in strategy scores (A) and the percentage of trials that the spatial (B), serial (C) and random (D) search strategies were used (n = 12/group). * $p < 0.05$, ** $p < 0.01$ compared with the NPD group by Bonferroni t-test. # $p < 0.05$, ### $p < 0.01$ compared with the LPD-Cho group by Bonferroni t-test.

Fig. 5 Synaptic ultrastructure in the CA1 region of the hippocampus

The magnifications are 37,000 times the original size of NPD (A), LPD (B) and LPD-Cho (C) groups. The right side is an enlarged image of the box on the left side. The length of synaptic active zone and curvature of synaptic interface are measured between arrows Po and Pn; the thickness of postsynaptic density (PSD) is indicated by the thickest part of PSD; SC represents the synaptic cleft; SV represents synaptic vesicles.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5