

# Maternal marginal iodine deficiency affects the expression of relative proteins during brain development in rat offspring

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## Abstract

Marginal iodine deficiency is a major health problem in pregnant women, but its impact on nerve and intelligence development in offspring has been rarely reported. Our study aimed to investigate the effects of maternal marginal iodine deficiency on nerve and cognitive development in offspring and the related mechanisms. Marginal iodine-deficient rats were given 3 µg iodine per day, while normal control rats were given 4 µg iodine daily. Western blot was used to detect the amounts of brain-derived neurotropic factor (BDNF) and early growth response protein 1 (EGR1) in the hippocampus of each group. Immunohistochemistry was used to measure c-jun and c-fos expression in the hippocampal CA1 region. Finally, the water maze method was used to measure spatial performance. Free thyroxine (FT<sub>4</sub>) levels in marginal iodine-deficient rats decreased by about 30%. Seven days after birth, EGR1 and BDNF protein levels significantly decreased in the hippocampus of marginal iodine deficiency rats compared with the normal control group. In addition, c-jun and c-fos expression in the hippocampus of 40-day-old rats was decreased in marginal iodine-deficient rats, compared with control. The spatial learning and memory ability of 40-day-old marginal iodine-deficient rats had a downward trend compared with the normal control group. FT<sub>4</sub> significantly decreased after pregnancy in rats with marginal iodine deficiency, affecting the expression of related proteins in the brain of offspring.

**Key Words**

- ▶ pregnancy
- ▶ marginal iodine deficiency
- ▶ offspring
- ▶ brain development
- ▶ protein

*Journal of Endocrinology*  
(2013) 217, 21–29

## Introduction

It is well known that iodine is a trace element essential for the synthesis of triiodothyronine (T<sub>3</sub>) and thyroxine (T<sub>4</sub>), which play a crucial role in the process of early growth and development of most organs, especially the brain (Sethi & Kapil 2004), through genomic and nongenomic actions in neurons (Bernal 2005, Davis *et al.* 2008). During gestation, the mother is the only source of T<sub>4</sub> before midgestation

and the major source afterward for the developing brain as the fetal thyroid gland is not functional and is unable to produce thyroid hormone (TH) until the second trimester (Pop *et al.* 2003). So maternal inadequate intake of iodine leads to insufficient production of thyroid hormones, which is potentially damaging for neurodevelopment of the fetus throughout pregnancy (Morreale

*et al.* 2004) and results in permanent and profound effects on neurological functions that contribute to cognitive and neurological impairments (Bernal *et al.* 2003, Tremont *et al.* 2003). The hippocampus is a brain region involved in cognitive skills such as learning and memory (Gerges & Alkadhi 2004) and the CA1 area in the hippocampus is the most important region for spatial learning (Huang *et al.* 1995) where memory is encoded, consolidated, and stored (Vara *et al.* 2003). The maturation and function of the hippocampus are dependent on thyroid hormone (Gerges & Alkadhi 2004). Congenital hypothyroidism can reduce the expression of many proteins in the hippocampus of neonatal rats (Dong *et al.* 2005) and affect its function.

In 1996, China implemented the Universal Salt Iodization (USI) regulation. Since then, the iodine nutrition condition of Chinese people has been at an appropriate or excessive level. Marginal iodine deficiency is a major health problem for pregnant women. According to the evaluation criteria set for pregnant women by the World Health Organization (WHO) in 2007, marginal iodine deficiency is defined as a urinary iodine concentration between 100 and 150  $\mu\text{g}/\text{l}$  (WHO/UNICEF/ICC IDD 2007). From our survey in Shenyang, almost half of the pregnant women were in a state of marginal iodine deficiency (Shi *et al.* 2009), similar to survey results from other areas (Li *et al.* 2004, Zhang *et al.* 2005). It has been shown that the cognitive performance of offspring is severely affected by moderate-to-severe iodine deficiency in pregnant women. However, studies investigating the impacts of marginal iodine deficiency in pregnant women on nerve and cognitive performance of their offspring have been rarely reported. According to the characteristics of marginal iodine deficiency obtained from epidemiological surveys, we established an animal model of marginal iodine deficiency in Wistar rats that were relatively sensitive to iodine. With this established rat model, we investigated the influences and mechanisms of marginal iodine deficiency on nerve and cognitive performance in offspring.

## Materials and methods

### Experimental animals and grouping

For the first part, 80 female specific pathogen-free (SPF) Wistar rats that had been weaned for 1 month and weighed 100–110 g were purchased from Beijing Vital River Experimental Animal Technology LLC, Beijing, China. After adaptive feeding for 1 week, the rats were assigned to one of the five treatment groups and

counterbalanced for weight (mean weight, 107 g). The groups were divided as follows: i) low-iodine group (L group,  $n=20$ ), ii) marginal iodine deficiency group 1 (2  $\mu\text{g}$  iodine,  $n=10$ ), iii) marginal iodine deficiency group 2 (3  $\mu\text{g}$  iodine,  $n=20$ ), iv) normal control group (N group,  $n=20$ ), and v) normal control group 1 (N1 group,  $n=10$ ). Rats were fed for 3 months and then mated with normal male rats with a female-to-male ratio of 2:1. And after pregnancy, the above five groups were named LP, 2 $\mu\text{gP}$ , 3 $\mu\text{gP}$ , NP, and N1P group respectively.

Based on the findings of first part, for the second part, after adaptive feeding for 1 week, 90 female SPF Wistar rats were divided into three groups ( $n=30$  for each group) and counterbalanced for weight as follows: i) low-iodine group (L group), ii) marginal iodine deficiency group (M group), and iii) normal control group (N group). Rats were raised and mated according to the methods used in the first experiment and the offspring were fed the same diet as their mothers.

### Feeding

The L and N1 groups drank deionized water, while the marginal iodine deficiency and N groups drank deionized water with dissolved potassium iodate of different concentrations. The low-iodine chow was made by mixing low-iodine foods (corns, soybeans, and millets) supplemented with inorganic salts and trace elements necessary for animal survival. The iodine content in the chow was 60  $\mu\text{g}/\text{kg}$ , as measured by the Shenyang Academy of Agricultural Sciences. The N1 group was fed a normal rat diet (Institute of Endocrinology, China Medical University, Shenyang, China), but the food composition and the proportions were the same as the low-iodine diet. The average iodine content in the normal diet was 200  $\mu\text{g}/\text{kg}$ . According to the daily food intake (20 mg) and daily water intake (30 ml) for rats, the daily iodine intakes were 1.2  $\mu\text{g}/\text{day}$  (L group), 2  $\mu\text{g}/\text{day}$  (2 $\mu\text{g}$  group), 3  $\mu\text{g}/\text{day}$  (3 $\mu\text{g}$  group), 4  $\mu\text{g}/\text{day}$  (N group), and 4  $\mu\text{g}/\text{day}$  (N1 group) respectively.

### Collection and measurement of urinary iodine

Chemicals were purchased from John Chem Co. Ltd, Linyi, China for the measurement of urinary iodine excretion using ammonium persulfate–arsenic cerium catalytic spectrophotometry (WHO/UNICEF/ICCIDD 2007). Urine was collected with metabolic cages every 3 days after 2 months of feeding.

### Observation of fertilization and procreation conditions in pregnant rats

In the next morning after mating, the vaginal smears of the female rats were examined. A positive record of pessus and vaginal smears were defined as embryonic day 0 (E0). The pregnancy reaction, fertilization condition, and stillbirth condition were observed on a daily basis. Also, the total number and death number were observed and recorded. The following calculations were used for each group: Pregnancy rate = number of pregnant rats/number of female rats  $\times$  100%; mortality of pregnant rats = death number of pregnant rats/total number of pregnant rats  $\times$  100%; and newborn rat mortality = total death number of newborn rats/total number of newborn rats.

### Hormone determination

We used a solid-phase chemiluminescence enzyme immunoassay to measure TSH, total T<sub>4</sub> (TT<sub>4</sub>), free T<sub>4</sub> (FT<sub>4</sub>), total T<sub>3</sub> (TT<sub>3</sub>), and free T<sub>3</sub> (FT<sub>3</sub>) (Diagnostic Products Corporation, Los Angeles, CA, USA). The functional sensitivity of the TSH assay was 0.02 mIU/l. The intra-assay coefficients of variation (CV) of serum for TSH, TT<sub>4</sub>, FT<sub>4</sub>, TT<sub>3</sub>, and FT<sub>3</sub> were 1.57–4.12, 1.26–3.20, 2.24–6.33, 2.42–5.63, and 0.57–4.31% respectively. The inter-assay CV values were 1.26–5.76, 3.58–6.67, 4.53–8.23, 3.50–5.19, and 5.23–8.16% respectively.

### Measurement of hormone and iodine content in the thyroid

Thyroid tissues were precisely weighed, sheared into pieces, added in cold homogenate, and prepared into tissue homogenate, which was then centrifuged at 1000 g for 15 min. Supernatant was removed and diluted into 3 ml/mg for measurement. The hormone was measured using solid-phase chemiluminescence enzyme immunoassay, and iodine content in the thyroid was measured with the ammonium persulfate–arsenic cerium catalytic spectrophotometry (WHO/UNICEF/ICC IDD 2007).

### Immunohistochemistry

Rat brains were fixed in 4% paraformaldehyde and embedded in paraffin. Coronal sections (5  $\mu$ m) were collected on Super Frost glass slides (Hehyglass, Haimen, China). All sections were deparaffinized, rehydrated, and then treated for endogenous peroxidase with 3% H<sub>2</sub>O<sub>2</sub> for 10 min. After washing with PBS, all sections were

incubated with a rabbit anti-c-fos or anti-c-jun antibody (Santa Cruz Biotechnology, 1:100 dilution) at 37 °C for 30 min. Sections were then washed three times in PBS followed by additional incubation with biotinylated secondary antibodies (Maixin, Fuzhou, China) for 30 min at 37 °C. Then, the sections were incubated for 10 min in avidin–biotin–peroxidase (Maixin) and washed three times in PBS. Sections were then reacted with a solution of 3,3-diaminobenzidine (DAB, Sigma). Finally, the sections were counterstained with Mayer's hematoxylin, rinsed, and mounted in neutral gum (China National Medicines, Shanghai, China).

### Image analysis

The Microscopic Image Analysis System (MetaMorph/DP10/Bx41/UIC/OLYMPUS, US/JP) was used to analyze the c-fos and c-jun immunoreaction products in the bilateral dorsal hippocampal CA1 region (400 $\times$  magnification).

### Western blotting

On postnatal day 7 (PND7), pups in each group were deeply anesthetized and killed by ether. The brain was removed and the hippocampus was dissected on ice rapidly, frozen in liquid nitrogen, and stored at –70 °C until use.

Hippocampi dissected from pups at PND7 were homogenized in ice-cold solubilizing buffer containing protease inhibitors. Homogenate was centrifuged at 12 000 g for 15 min at 4 °C. The supernatant was collected for protein concentration measurement. Samples (50  $\mu$ g for early growth response protein 1 (Egr1) and 60  $\mu$ g for brain-derived neurotrophic factor (BDNF)) were separated by SDS–PAGE and then transferred onto PDVF membranes (Millipore, Billerica, MA, USA) by electrophoresis. Blots were blocked for 2 h at room temperature with 5% BSA and then incubated overnight at 4 °C with antibodies to Egr1 (1: Signal, Danvers, MA, USA) and BDNF (1:1000 Millipore). Blots were then incubated with HRP-conjugated secondary antibodies (Zhongshan Goldenbridge, Beijing, China). Finally, the blots were visualized with an ECL western blot detection system (Alphaview1.3, Proteinsimple Inc, CA, USA).

### Morris water maze

The Morris water maze experiments were conducted in 40-day-old rats. On the first day, the rats were put into a water maze for 120 s to swim freely and adapt to the

environment of the water maze. On the second day, a platform was set randomly in the middle of one quadrant. The rats were trained with two procedures per day – one in the morning and one in the afternoon. In each procedure, four separate training times were performed. In the first procedure, one quadrant was chosen. The rats were placed in the water at the center of the pool wall and the procedure was repeated in the other three quadrants. A computer was used to monitor and record the path, time (escape latency, EL), swimming length, swimming speed, and the strategy used to locate the transparent platform. The procedure was immediately stopped if a rat found the platform within 120 s. Rats were allowed to stay on the platform for 10 s for memory strengthening. If the rats did not find the platform within 120 s, then the EL was recorded as 120 s. At this point, the researchers subsequently guided the rats to the platform and allowed them stay on the platform for 10 s before removing the rats from the platform. After resting for 30–60 s, the rats proceeded to the next round of training. The interval of every training session was 10 s. The test environment and position stayed the same during training. The rats were trained eight times per day.

### Statistical analysis

Data were analyzed using SPSS 17.0 software and expressed as mean  $\pm$  s.d. A two-sample *t*-test was used to compare between two groups, and ANOVA was used for multigroup comparisons. A  $P < 0.05$  was considered statistically significant.

## Results

### Body weight, thyroid weight-to-body weight ratio, and urinary iodine level

After being fed for 3 months, the average weight of the 2 $\mu$ g group was 270.60  $\pm$  20.72 g ( $P < 0.05$  vs N group), which did

not meet the standards (Glinioer *et al.* 1995, Mun *et al.* 2006) for marginal iodine deficiency, and thus the following experiments were discontinued for the 2 $\mu$ g group. The average body weight of the L group was significantly decreased compared with the other three groups ( $P < 0.05$ ), but the average body weight between the 3 $\mu$ g iodine, N, and N1 groups was similar ( $P > 0.05$ ). The thyroid weight-to-body weight ratio increased significantly in the L group ( $P < 0.05$ ) than the 3 $\mu$ g, N, and N2 groups, while there was no difference among the 3 $\mu$ g, N, and N2 groups ( $P > 0.05$ ). The urinary iodine value in the L group (63.50  $\pm$  13.16  $\mu$ g/l) and the 3 $\mu$ g group (107.00  $\pm$  24.40  $\mu$ g/l) was significantly lower than the other two groups ( $P < 0.01$  and  $P < 0.05$  respectively), while the urinary iodine value in the N group (202.75  $\pm$  31.60  $\mu$ g/l) and the N1 group (210.30  $\pm$  23.19  $\mu$ g/l) did not change ( $P > 0.05$ ).

### The thyroid function of rats before pregnancy and on E17

All indexes of thyroid function between the N and N1 groups were similar before pregnancy ( $P > 0.05$ ; Table 1). Serum TSH levels in the L group were significantly higher than in the other groups ( $P < 0.01$ ), and no obvious difference was observed among the other groups ( $P > 0.05$ ). After pregnancy (E17: 17 days after their pregnancy), the TSH levels had a downward trend in all the groups. In addition, on E17, FT<sub>4</sub> had a downward trend in all the groups, and FT<sub>4</sub> in the L group was significantly lower than the 3 $\mu$ g and N groups ( $P < 0.05$  and  $P < 0.01$  respectively) after pregnancy for 17 days. The FT<sub>4</sub> of 3 $\mu$ g group decreased by 30%, while FT<sub>4</sub> in the N group only decreased by 4%. TT<sub>4</sub> in the L group was significantly lower when compared with the N group ( $P < 0.01$ ), while TT<sub>4</sub> in the 3 $\mu$ g group was decreased compared with the N group, but not statistically significant. FT<sub>3</sub> was not significantly different among all groups, but the 3 $\mu$ g group had slightly higher FT<sub>3</sub> levels than the N group and the increased trend continued after pregnancy. TT<sub>3</sub> decreased

**Table 1** The levels of various hormones reflecting the thyroid function of rats before pregnancy and on E17 (mean  $\pm$  s.e.m.). The P labeling represents the results for the groups in their 17-day pregnancy period.

Group	n	TT <sub>4</sub> ( $\mu$ g/dl)	FT <sub>4</sub> (pmol/l)	TT <sub>3</sub> (ng/dl)	FT <sub>3</sub> (pmol/l)	TSH (mIU/l)	FT <sub>3</sub> /FT <sub>4</sub>	TT <sub>3</sub> /TT <sub>4</sub>
L	7	2.41 $\pm$ 0.70*	18.82 $\pm$ 4.45*	89.34 $\pm$ 5.94	5.40 $\pm$ 0.33	0.92 $\pm$ 0.29*	0.32 $\pm$ 0.063	0.04 $\pm$ 0.012
LP	6	1.56 $\pm$ 0.55*	9.28 $\pm$ 0.12*	72.35 $\pm$ 5.96	4.71 $\pm$ 0.87	0.23 $\pm$ 0.03*	0.53 $\pm$ 0.08	0.075 $\pm$ 0.011
3 $\mu$ g	7	4.35 $\pm$ 0.60	31.03 $\pm$ 4.17	83.40 $\pm$ 10.48	5.67 $\pm$ 0.35	0.039 $\pm$ 0.04	0.215 $\pm$ 0.043	0.0210 $\pm$ 0.003
3 $\mu$ gP	7	2.50 $\pm$ 0.25	22.46 $\pm$ 1.51	64.36 $\pm$ 5.24	5.02 $\pm$ 0.55	0.016 $\pm$ 0.001	0.228 $\pm$ 0.016	0.0284 $\pm$ 0.004
N	7	4.59 $\pm$ 0.33	27.28 $\pm$ 2.77	90.82 $\pm$ 8.91	5.30 $\pm$ 0.35	0.032 $\pm$ 0.005	0.206 $\pm$ 0.023	0.0203 $\pm$ 0.002
NP	7	3.05 $\pm$ 0.53	27.12 $\pm$ 3.56	59.17 $\pm$ 5.88	4.43 $\pm$ 0.22	0.017 $\pm$ 0.03	0.213 $\pm$ 0.024	0.0271 $\pm$ 0.007
N1	7	4.61 $\pm$ 0.27	23.07 $\pm$ 1.86	91.00 $\pm$ 7.67	5.41 $\pm$ 0.43	0.033 $\pm$ 0.003		

\*Compared with N group,  $P < 0.05$ .

after pregnancy in all the groups.  $TT_3/TT_4$  and  $FT_3/FT_4$  levels in the L group were significantly lower than the low-iodine pregnancy group (LP) ( $P < 0.05$ ),  $3\mu\text{g}$ , and N groups. No obvious differences were observed among the other groups.  $TT_3/TT_4$  and  $FT_3/FT_4$  levels in the  $3\mu\text{g}$  and the marginal iodine deficiency pregnancy ( $3\mu\text{gP}$ ) groups were slightly higher than the N and normal iodine pregnancy (NP) groups, but the differences were not statistically significant ( $P > 0.05$ ).

### $T_3/T_4$ and iodine content in thyroid

In the L group, the levels of  $T_3$  and  $T_4$  were too low to be measured (Table 2). In addition, the iodine content in the thyroid of the L group was significantly lower than that in the other groups ( $P < 0.05$ ), and there was no obvious difference among other groups ( $P > 0.05$ ).  $T_3/T_4$  levels in the  $3\mu\text{g}$  group were slightly higher than the N and N1 groups but were not statistically significant ( $P > 0.05$ ). At E17, the iodine content in the thyroids of the  $3\mu\text{gP}$  and NP groups was lower than in the non-pregnancy groups. There was no significant difference between the above two groups ( $P > 0.05$ ).

### State of pregnancy, delivery, and thyroid function of rat offspring on P7

Fertilization rate, mortality of pregnant rats, and mortality of newborn rats in the low-iodine group ( $1.2\mu\text{g}$  iodine intake daily) were higher than those in the  $3\mu\text{g}$  and N groups ( $P < 0.05$ ), and the number of newborn rats in the low-iodine group was less than the other two groups ( $P < 0.05$ ) (Table 3). No difference was observed between the  $3\mu\text{g}$  and N groups. At P7, serum TSH levels in the offspring of the low-iodine group were significantly higher than those of the normal control group ( $P < 0.05$ ). Serum  $TT_4$

**Table 2**  $T_3/T_4$  and iodine content in thyroid (mean  $\pm$  S.E.M.).  $3\mu\text{gP}$  and NP groups measured on rats from M group and N group 17 days after their pregnancy

Group	n	$T_3/T_4$	Iodine content in thyroid ( $\mu\text{g}/100\text{ mg}$ )
L	7		$86.53 \pm 15.91^*$
$3\mu\text{g}$	7	$34.97 \pm 9.15$	$178.21 \pm 10.10$
N	7	$32.63 \pm 6.49$	$180.86 \pm 13.24$
N1	7	$32.94 \pm 5.45$	$178.00 \pm 15.07$
$3\mu\text{gP}$	7		$169.49 \pm 18.09$
NP	7		$169.13 \pm 12.81$

\*Compared with N group,  $P < 0.05$ .

**Table 3** Thyroid function of PND7 rats (mean  $\pm$  S.E.M.)

Group	n	$TT_4$ ( $\mu\text{g}/\text{dl}$ )	$FT_4$ (pmol/l)	TSH (mIU/l)
L	12	$1.12 \pm 0.10^*$	$6.62 \pm 0.45^*$	$0.138 \pm 0.02^*$
M	12	$2.45 \pm 0.38$	$10.22 \pm 0.58$	$0.077 \pm 0.048$
N	12	$2.44 \pm 0.07$	$10.47 \pm 0.60$	$0.089 \pm 0.004$

\*Compared with N group,  $P < 0.05$ .

and  $FT_4$  levels of the low-iodine group were also significantly lower than the N group. A significant difference was not observed between the  $3\mu\text{g}$  and N groups ( $P > 0.05$ ).

### Index analysis of Morris water maze behaviors of 40-day-old rats

On the first day of adaptive training, the time to find the platform was long and a difference in the average EL among the three groups was not obvious (Table 4). On the second day, rats in all groups adapted to the pool environment and most of the rats actively searched for the platform. The average EL of all groups continuously decreased, but the rate differed among groups, indicating that rats in each group had partial memory loss between training sessions. ANOVA indicated that a difference was not observed in EL between the N and M groups, while the EL of the N and M groups was significantly shorter than that for the L group. The results of the third day were similar to the second day. On the fourth day, the EL in the N group was still significantly shorter than the L group. In addition, the EL of the M group was longer than N group but was not statistically significant. The results of the fifth day were similar to the fourth day.

### The expression of c-fos and c-jun proteins

As observed from the results of immunohistochemistry, the expression levels of c-fos and c-jun in the M group were significantly lower than that in the N group ( $P < 0.05$ ) (Fig. 1). However, when compared with the low-iodine group, c-fos and c-jun expression in the M group was higher but not statistically significant.

### Hippocampus EGR1 and BDNF protein expression in PND7 rats

EGR1 and BDNF protein expressions were measured by western blot (Fig. 2). The results showed that the L and M groups had significantly lower Egr1 expression than the N group ( $P < 0.05$ ). Similarly, the BDNF protein

**Table 4** Escape latency (s) in the Morris water maze experiment (mean  $\pm$  s.e.m.)

Group	n	Second day	Third day	Fourth day	Fifth day
L	8	86.688 $\pm$ 8.02 <sup>*,†</sup>	64.566 $\pm$ 6.41 <sup>*,†</sup>	27.22 $\pm$ 5.06 <sup>*</sup>	28.725 $\pm$ 4.65 <sup>*</sup>
M	8	59.038 $\pm$ 7.86	37.656 $\pm$ 7.31	20.806 $\pm$ 5.13	19.819 $\pm$ 3.41
N	8	56.728 $\pm$ 7.19	37.434 $\pm$ 6.81	12.656 $\pm$ 2.03	10.970 $\pm$ 1.44

\*Compared with N group,  $P < 0.05$ , <sup>†</sup>compared with M group,  $P < 0.05$ .

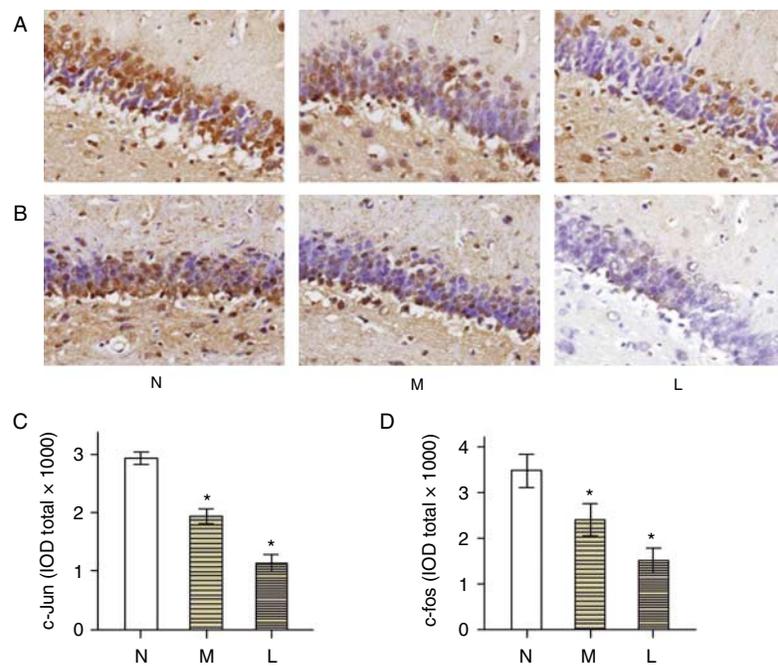
expression in the L and M groups were significantly lower than the N group ( $P < 0.05$ ).

## Discussion

The main focus of previous studies regarding iodine deficiency was to establish severe iodine deficiency models and investigate the impact of iodine deficiency on offspring development. Our study established a marginal iodine deficiency model in rats based on the following principles: in the normal physiological status, there were no symptoms of iodine deficiency, but during pregnancy, the requirement for iodine increased, resulting in iodine deficiency. This study also investigated the changes in iodine metabolism and thyroid function, as well as the influences of maternal marginal iodine deficiency on

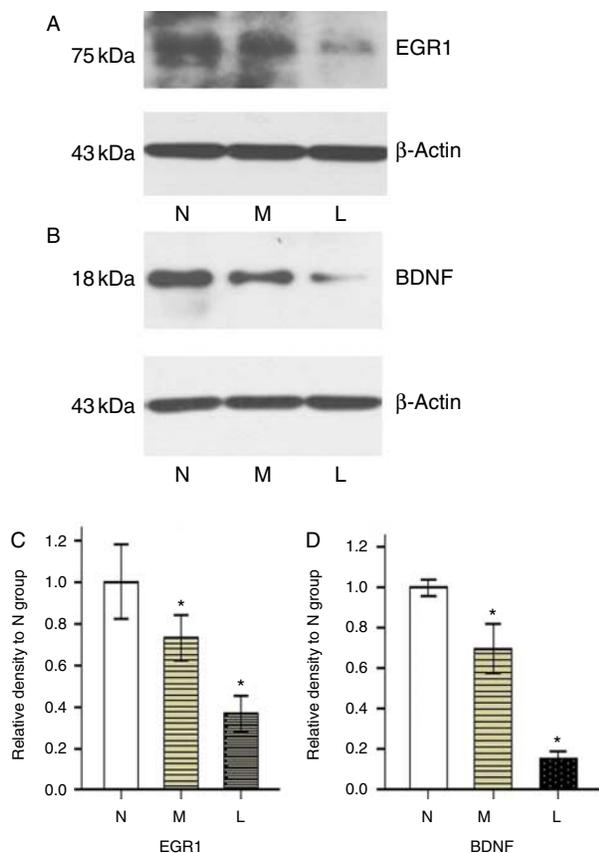
offspring neural intelligence development and the related molecular mechanisms.

The marginal iodine deficiency in our study was very mild, and therefore before pregnancy the thyroid was auto-regulated and had no functional changes. However, after pregnancy the iodine requirement increased significantly, which resulted in over stimulation of the thyroid and changes in thyroid function. We found that to synthesize 100 g thyroid hormone, which is physiologically required by a Wistar rat, 0.68  $\mu$ g iodine was needed. The average body weight of our experimental rats was  $< 300$  g, so a daily iodine intake of 2  $\mu$ g only met the physiological requirements (Escobar-Morreale *et al.* 1996). According to a literature report (Heninger & Albright 1975), when the daily iodine intake of a Wistar rat was 3  $\mu$ g, the  $T_4$  concentration reached most of the tissues.

**Figure 1**

Immunohistochemistry pictures of c-fos and c-jun in newborn rat hippocampi in each group (P40,  $\times 400$ ). (A) c-fos and (B) c-jun. (C and D) IHC IOD values of c-fos and c-jun respectively in newborn rat hippocampi in

each group on P40. \*Compared with N group,  $P < 0.05$ . Full colour version of this figure available via <http://dx.doi.org/10.1530/JOE-12-0410>.



**Figure 2**

Western blot of postnatal day 7 rat hippocampi of EGR1 and BDNF protein levels. (A) EGR1 and (B) BDNF. (C and D) Western blot gray values for EGR1 and BDNF respectively. \*Compared with normal control group (N),  $P < 0.05$ . Full colour version of this figure available via <http://dx.doi.org/10.1530/JOE-12-0410>.

Furthermore, if the daily iodine intake of a Wistar rat was 4  $\mu\text{g}$ , all the physiological requirements were satisfied. According to the ANI93 standard of animal diets, the iodine content of the diet used in our study was 200  $\mu\text{g}/\text{kg}$ . Assuming the daily food intake was 20 g, the daily iodine intake requirement was 4  $\mu\text{g}$ .

In our study, we chose daily iodine intakes of 2 and 3  $\mu\text{g}$  as the marginal iodine deficiency. We evaluated the model according to the results of epidemiological survey. The epidemiological characteristics of people in marginal iodine-deficient areas are prevalent before and after pregnancy: before pregnancy, the autoregulatory mechanism of the thyroid is active and serum TSH does not increase. Serum  $\text{FT}_4$  has a downward trend and  $\text{T}_3$  has an upward trend. Additionally, the urinary iodine is lower than the normal but has no influences on normal growth. After pregnancy, obvious symptoms of thyroid over stimulation appear, and TSH increases within the normal

range, the iodine content of the thyroid decreases, the  $\text{T}_3/\text{T}_4$  value further increases, and the number of newborn rats and their survival rate stays the same (Glinioer *et al.* 1995, Glinioer 1997a,b, Lavado-Autric *et al.* 2003, Morreale de Escobar & Escobar del Rey 2003, Mun *et al.* 2006, Pedraza *et al.* 2006).

In our study, growth, thyroid function, iodine content in the thyroid, and urinary iodine showed no difference between the N group (iodine intake was 4  $\mu\text{g}/\text{day}$ ) and the N1 group (normal diet), indicating that low-iodine foods with water containing iodine have no deficiency of nutritive elements and can be used as an alternative for a normal diet. After 3 months of feeding, the body weight of rats in the 2  $\mu\text{g}$  group (iodine intake was 2  $\mu\text{g}/\text{day}$ ) was significantly lower than the N group, suggesting that the iodine intake affected the growth of rats and thus did not meet our standard for marginal iodine deficiency. Before pregnancy, the urinary iodine of rats in the 3  $\mu\text{g}$  group (iodine intake was 3  $\mu\text{g}/\text{day}$ ) was significantly lower than the normal control groups (N group and N1 group), indicating a lower iodine excretion rate and iodine intake. However, the iodine content in the thyroid, hormone content in the thyroid, thyroid function, pregnancy status, and the procreation conditions were not different between these two groups, indicating that the rats in the 3  $\mu\text{g}$  group were not iodine deficient but only marginally deficient.

After pregnancy, the iodine content in the thyroid of the 3  $\mu\text{g}$  and N groups decreased, but the rate of decrease was similar. Before and after pregnancy, the  $\text{T}_3/\text{T}_4$  value of the 3  $\mu\text{g}$  group was slightly higher than the N group and the rate of increase after pregnancy was slightly higher than the N group. After pregnancy, the  $\text{FT}_4$  levels of the 3  $\mu\text{g}$  group decreased by about 30%, while the value in the N group only decreased by 4%, which was similar to the result of the epidemiological survey (Pedraza *et al.* 2006). In the survey, the decrease of  $\text{FT}_4$  did not exceed 15% after pregnancy in subjects with sufficient iodine. In our study, the 3  $\mu\text{g}$  group met the requirements for marginal iodine deficiency.

The hippocampus is the part of the brain that is related to cognitive ability, including learning and memory ability (Gerges & Alkadhi 2004). The CA1 area in the hippocampus is important for spatial learning. The memories are coded and incorporated and then stored plastically by synapses. The hippocampus is also a sensitive area regulated by the thyroid hormone. The abnormal expression of related proteins in the hippocampus affects brain intelligence. In this study, although there was no difference in the water maze test between the

M and N groups, there was increased EL in the M group, indicating impairment in learning and memory abilities.

BDNF expression can be directly regulated by thyroid hormone (Fujinoto *et al.* 2004). Some studies have shown that thyroid hormone deficiency results in a significant decrease in BDNF content in the brain of newborn rats (Lasley & Gilbert 2011). However, if the rats are supplied with thyroid hormone, the BDNF content will increase rapidly (Camboni *et al.* 2003). BDNF receptor binding with TrkB activates intracellular signal transduction. MARK plays an important role in the differentiation and migration of nerve cells, the reconstruction of synaptic plasticity, and the consolidation of learning and memory. Furthermore, MARK induces hippocampus granular cells to produce continuous long-term potentiation (LTP) (Kobayashi *et al.* 2006), which is currently recognized as the neural basis for learning and memory. The influences of BDNF on learning and memory are mainly due to the regulatory effects of BDNF on LTP (Jia *et al.* 2010). In our study, BDNF expression in the PND7 newborn rats in the M group was significantly lower than the N group, which suggests that the relative thyroid hormone deficiency in female rats with marginal iodine deficiency has a negative impact on BDNF and can further influence the brain development of offspring.

Egr1 belongs to the early growth reactive protein family and is also referred to as krox24 or Zif268. The expression of Egr1 is mainly regulated by thyroid hormone through TRE. Egr1 is related to the connectivity of regulatory nerves and the adaptability of synapses. Thyroid hormone deficiency can result in the decrease in Egr1 content in the cortex and hippocampus of the CNS (Kobayashi *et al.* 2009). Synaptic activation can induce the generation of EGR1 and regulate the synaptic plasticity related to learning and memory, which is critical for the maintenance of late LTP. The formation of long-term memory depends on the hippocampus and the consolidation of early constructed memory. During the process of LTP induction by the dentate gyrus of the hippocampus, the transcription of *Egr1* is quick and robust. Studies have shown that the expression of *Egr1* is a critical genetic mechanism for the maintenance of late LTP and the stability of long-term memory (Ranieri *et al.* 2012). In our study, the *Egr1* expression of PND7 newborn rats in the low-iodine group and the marginal iodine deficiency group were significantly lower than the normal control group, indicating that marginal iodine deficiency in female pregnant rats has a great influence on *Egr1* expression of offspring, which in turn affects learning and memory ability.

Immediate early genes (IEGs) can couple the short-term function of the extracellular signal and the long-term change in cell function, suggesting that IEGs are tightly related to learning and memory regulation (Herdegen *et al.* 1997, Guzowski 2002). *c-fos* and *c-jun* are the most important characteristic genes in the IEG families. Additionally, if cells have been stimulated by a series of physicochemical and biological factors, *c-fos* and *c-jun* can be rapidly expressed and encoded to generate the Fos and Jun proteins, which regulate neuronal plasticity. Neuronal plasticity is considered to play an important role in memory formation, especially long-term memory formation (French *et al.* 2001). In our study, the expression of *c-fos* and *c-jun* in the hippocampus CA1 area decreased significantly in the marginal iodine deficiency group. Immunoreaction products were mainly distributed in the cytomembrane and cytoplasm. In the nucleus, the expression of *c-fos* and *c-jun* was significantly lower than the N group but higher than the low iodine group, suggesting that the changes in *c-fos* and *c-jun* expression and their distribution during marginal iodine-deficient conditions play a role in the learning and memory abilities of newborn rats.

Expression of C-fos and c-jun protein in the M group was significantly lower than that of normal control group but higher than that of low-iodine group. The above result indicates that in MWM, the learning and memory capacity of the M group was on a downward trend compared with the normal control group, while it was still different from the low-iodine group, which has significantly decreased learning and memory capacity.

#### Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

#### Funding

This work was supported by Department of Education Innovation Team of Liaoning Provincial (Grant number LT2010108) and the Science and Technology Program of Liaoning Province (Grant number 2010225019); Doctoral Program Fund Higher Education (Fund for the Doctoral) (Grant number 20102104110005); special funds of clinical research of Chinese Medical Association – Endocrine disease research ([2010]60 Grant number 10020030225).

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Received in final form 15 January 2013

Accepted 17 January 2013

Accepted Preprint published online 17 January 2013