

Developmental toxic effects of chronic exposure to high doses of iodine in the mouse

Xue F. Yang, Jian Xu, Xiao H. Hou, Huai L. Guo, Li P. Hao,
Ping Yao, Lie G. Liu, Xiu F. Sun*

*Department of Nutrition and Food Hygiene, School of Public Health, Tongji Medical college,
Huazhong University of Science and Technology, Wuhan 430030, Hubei, China*

Received 11 November 2005; received in revised form 3 April 2006; accepted 24 May 2006
Available online 13 June 2006

Abstract

Chronic exposure to high doses of iodine induces thyroid dysfunction, but effects of chronic exposure to high amounts of iodine on pregnancy and fetal outcome are uncertain. In the present study, Balb/C mice were given different doses of iodine at the levels of 0 (sterile water), 1500, 3000, 6000, 12,000 and 24,000 $\mu\text{g/L}$ in drinking water for 4 months, then were mated and the developmental toxicity and teratogenicity were evaluated. An obvious colloid goiter was observed, and serum total thyroxine (TT_4) levels increased and serum total triiodothyronine (TT_3) levels decreased significantly in dams when iodine dose reached 3000 $\mu\text{g/L}$. Maternal effect was evident by the reduction of average daily food consumption in higher doses of iodine groups. Embryotoxicity and teratogenicity were mainly indicated by the reduced body weight in female fetuses, the decreased number of live fetuses, and the increased incidence of resorptions, and especially skeletal variations. These results suggest that exposure to maternally toxic doses of iodine may have a potential developmental toxic effect.

© 2006 Elsevier Inc. All rights reserved.

Keywords: High doses of iodine; Thyroid hormone; Developmental toxicity; Mouse

1. Introduction

The goal of eliminating iodine deficiency disease (IDD) has been achieved since Universal Salt Iodization (USI) policy has been widely carried out in many nations including China [1]. On the other hand, reports are increasingly appearing on the toxic effects caused by high amounts of iodine intake. Exposure to high amounts of iodine occurs via food [2], drinking water [3], medication [4] and iodized salt or iodinated oil [5]. Besides goiter, high amounts of iodine intake may increase the risk of hyperthyroidism, hypothyroidism, iodine-induced autoimmunity and thyroid cancer [6,7]; however, the effects of chronic exposure to high doses of iodine on pregnancy and fetal outcome are uncertain [8].

Thyroid hormone is essential for growth, development and cell differentiation. Before the fetal thyroid gland and pituitary–thyroid axis become functional (gestation day (GD)

17–18 in rats and GD 90 in humans), fetal thyroid hormones must come from the maternal circulation [9]. Maternal transfer of T_4 constitutes a major fraction of fetal serum T_4 , even after onset of fetal thyroid secretion, they may contribute to the maintenance of fetal development. Adequate iodine supply, fetal thyroid hormone production and circulating thyroid hormone concentrations are essential factors in development. Previous studies [10] revealed that iodine deficiency during pregnancy may result in stillbirths, abortions and congenital abnormalities such as cretinism, a grave, irreversible form of mental retardation; however, there is little information available in literature about whether high amounts of iodine also have an effect on embryonic development as iodine deficiency.

A retrospective study [11] reported that maternal hypothyroidism and hyperthyroidism have deleterious effects on the outcome of pregnancy, including miscarriage and birth defects such as sunken chest, extra fingers, cleft lip and palate, and ear deformities. The potential relationship between thyroid disease and birth defect needs attention. High amounts of iodine have a complex disruptive effect on the thyroid and may cause either

* Corresponding author. Tel.: +86 2783692711; fax: +86 2783693307.
E-mail address: sunxf@mails.tjmu.edu.cn (X.F. Sun).

hypothyroidism or hyperthyroidism [12–14], but do changes of maternal thyroid hormone status also induce developmental toxicity and teratogenicity? We therefore conducted this study to determine whether chronic exposure to high doses of iodine resulted in embryotoxicity and malformations.

2. Materials and methods

2.1. Animals

Young Balb/C mice obtained from Laboratory Animal Center of Hubei Provincial Center for Disease Control and Prevention (Wuhan, China) were maintained in constant temperature controlled rooms ($22 \pm 2^\circ\text{C}$) with controlled lighting (12 h light/12 h dark). Five animals were housed in groups of the same gender, with free access to commercial laboratory chow and sterile water. The content of iodine in the diet and water was $365 \mu\text{g}/\text{kg}$ and $8 \mu\text{g}/\text{L}$, respectively. The animals were cared for according to the *Guiding Principles in the Care and Use of Animals*. The experiments were approved by Tongji Medical College Council on Animal Care Committee.

2.2. Treatment with high doses of iodine

After 1-week acclimation to the laboratory environment animals were randomly assigned to six groups of 15 animals each (10 female and 5 male) according to body weight and given different doses of iodine at the levels of 0, 1500, 3000, 6000, 12,000 and 24,000 $\mu\text{g}/\text{L}$ by using sterile water as the vehicle. Four months later, female mice were placed into the metabolic cages of five mice each and urine samples over 3 h in the morning were collected for 3 days to measure urinary iodine concentration. Females were then paired with a male in a 2:1 ratio overnight and examined for a vaginal plug the following morning. The day on which a vaginal plug was observed was designated day 0 of gestation. The treatment with high doses of iodine continued through the period of gestation.

2.3. Maternal observations

Animals were examined daily throughout the experimental period for signs of toxicity. Maternal body weights as well as food and water consumption were recorded. Dams were euthanized by cervical dislocation on day 19 of gestation. The thyroid was separated and fixed in 10% neutral formalin, processed by the standard histological techniques, and stained with hematoxylin and eosin for light microscopic examination. Blood from each dam was collected. Aliquots of serum from these blood samples were stored at -20°C for thyroid hormone analysis.

2.4. Fetal observations

After pregnant mice were sacrificed, the gravid uterus of the pregnant mice was removed and weighed immediately, the numbers and positions of the live or dead fetuses, as well as resorptions, were recorded. Live fetuses were weighed individually, gender-determined, and examined for external abnormalities (exencephaly, cleft palate, abdominal hernia, polydactyly, open eyelid, and so forth). One-half or two-thirds of the fetuses were preserved in 95% ethanol and stained by Alizarin red for skeletal examination. The remaining fetuses were fixed in Bouin's solution for visceral evaluation.

2.5. Iodine concentration and thyroid hormone analysis

Iodine concentration in diet, water and urine was measured by Cer-Arsenite colorimetric method as modified by Fischer et al. [15]. Urinary creatinine concentration was determined by alkaline picrate method. The urinary iodine to creatinine ratio ($\mu\text{g}/\text{g Cr}$) was used to estimate urinary iodine concentration. Serum total thyroxine (TT_4) and total triiodothyronine (TT_3) were measured by RIA kits obtained from the Chinese Academy of Atomic Energy in Beijing.

2.6. Statistical analysis

Because of its skewed distribution, the median was used to describe the central tendency of urinary iodine concentration. The Kruskal–Wallis method was used to test the differences in ranking of urinary iodine concentration. One-way analysis of variance (ANOVA) was used to analyze thyroid hormone levels, maternal food and water consumption, maternal body weight gain, placental weight. Two-way ANOVA with factors = treatment and sex was performed to analysis the gender difference in fetal weight. When significant effects were observed by ANOVA, the data for the treated groups were compared statistically with the control group by Duncan's multiple range test. The ratios of live, resorbed and dead fetuses were analyzed by variance and Duncan's test after arcsine transformation. The incidence of skeletal anomalies was analyzed by χ^2 -test. Significance was set at the 0.05 probability level.

3. Results

3.1. Biological indicators of chronic high doses of iodine exposure in female mice

The concentration of iodine in urine is currently the most widely used as biochemical marker of iodine intake. After exposure to high doses of iodine for 4 months, urinary iodine concentration of female mice increased in a dose-dependent manner ($r=0.96$, $p<0.01$) (Fig. 1). An obvious colloid goiter was observed in thyroid of dams exposed to high doses of iodine (Fig. 2). With iodine dose increasing, follicular epithelial cells gradually became flattened, and the follicles gradually became distended with colloid. Compared to control group, serum TT_4 level increased and serum TT_3 level decreased significantly in dams when iodine dose reached 3000 $\mu\text{g}/\text{L}$, whereas exposure to 1500 $\mu\text{g}/\text{L}$ iodine had no obvious effect (Fig. 3).

3.2. Pregnancy outcome following chronic high doses of iodine exposure

The effects of chronic high doses of iodine exposure on pregnancy outcome are summarized in Table 1. There was no significant increase in the number of non-pregnant or abortion females in high doses of iodine exposure groups. Compared to control group, average daily food consumption of maternal mice was markedly reduced in 12,000 and 24,000 $\mu\text{g}/\text{L}$ doses

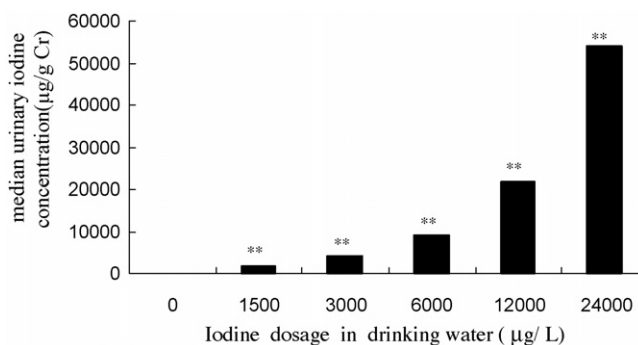


Fig. 1. Effect of chronic excessive iodine exposure on urinary iodine level in female mice. The urinary iodine to creatinine ratio ($\mu\text{g}/\text{g Cr}$) was used to estimate iodine concentration in urine and data were expressed as median, each bar represented the median of a group of six samples. ** $p<0.01$ compared with controls by Kruskal–Wallis method.

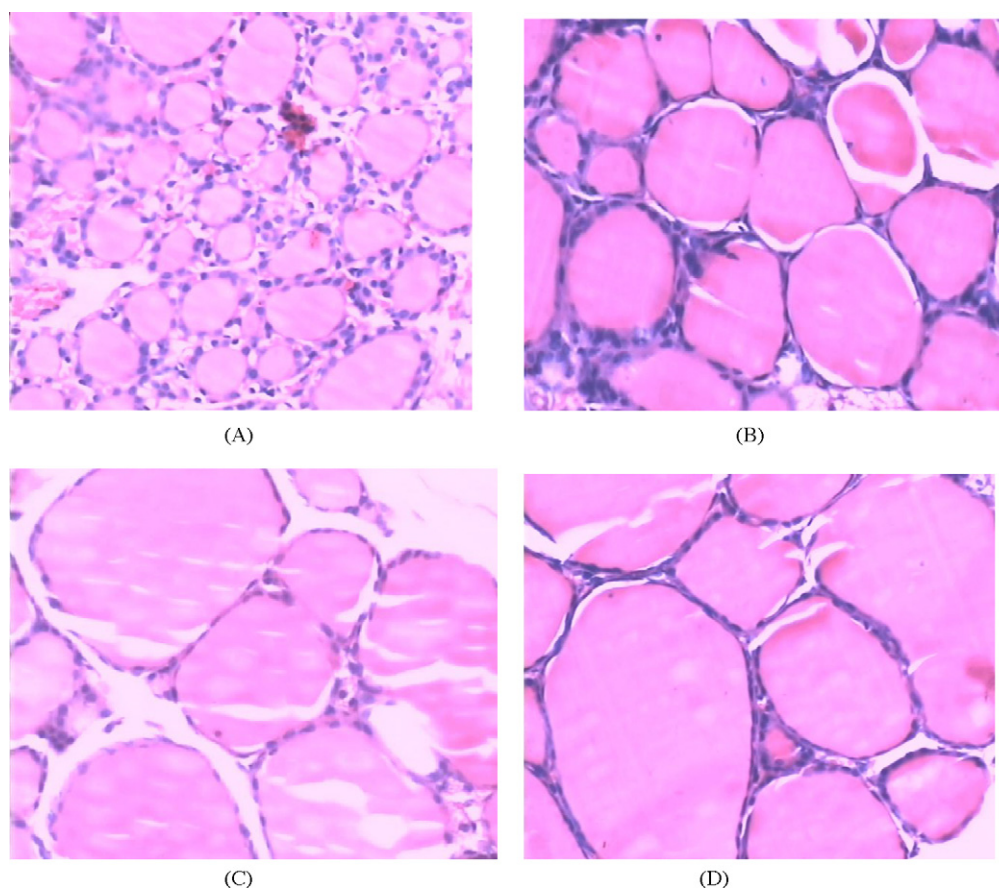


Fig. 2. Morphology of maternal thyroid gland (HE, magnification 100 \times). Thyroid glands obtained from control dams (A) or dams exposed to 3000 $\mu\text{g/L}$ (B), 12,000 $\mu\text{g/L}$ (C) and 24,000 $\mu\text{g/L}$ (D) iodine. As shown in (B–D), chronic excessive iodine exposure induced colloid goiter, follicular epithelial cells gradually become flattened, and the follicles gradually became distended with colloid.

Table 1
Pregnancy outcome following chronic high doses of iodine exposure

Dose groups ($\mu\text{g/L}$)	0	1500	3000	6000	12000	24000
Dams						
Number of dams pregnant (%)	10 (100)	9 (90)	9 (90)	8 (80)	9 (90)	9 (90)
Number of examined litters	7	8	8	7	7	8
Implants/litter	5.6 \pm 2.4	6.6 \pm 1.3	7.3 \pm 2.1	5.7 \pm 1.3	7.6 \pm 1.3	7.6 \pm 1.5
Average daily food consumption (g)	4.8 \pm 0.4	4.6 \pm 0.3	4.7 \pm 0.3	4.5 \pm 0.5	4.3 \pm 0.4*	4.3 \pm 0.3*
Average daily water consumption (ml)	4.9 \pm 0.8	4.8 \pm 0.9	4.8 \pm 1.2	4.5 \pm 1.3	4.2 \pm 0.7	4.2 \pm 1.2
Body weight gain (g)	11.386 \pm 3.339	10.762 \pm 2.058	11.363 \pm 3.531	10.840 \pm 1.705	11.243 \pm 2.651	11.138 \pm 3.673
Mean placenta weight (g)	0.126 \pm 0.019	0.113 \pm 0.014	0.106 \pm 0.009*	0.104 \pm 0.011*	0.109 \pm 0.019*	0.102 \pm 0.012*
Fetuses						
Live fetuses/implants (%) ^a	92.3 \pm 7.2	72.5 \pm 9.4*	69.8 \pm 14.6*	67.9 \pm 17.5*	64.4 \pm 10.0*	63.2 \pm 17.4*
Resorbed fetuses/implants (%) ^a	7.7 \pm 7.2	27.5 \pm 9.4*	26.2 \pm 10.8*	28.1 \pm 13.5*	28.6 \pm 13.1*	27.0 \pm 15.3*
Dead fetuses/implants (%) ^a	0 \pm 0	1.6 \pm 4.4	4.1 \pm 5.7	4.1 \pm 7.0	7.0 \pm 9.0	9.9 \pm 15.1
Fetal body weight (g)						
Males	1.21 \pm 0.12	1.13 \pm 0.29	1.14 \pm 0.11	1.21 \pm 0.12	1.21 \pm 0.06	1.18 \pm 0.11
Females	1.20 \pm 0.13	1.09 \pm 0.16	1.11 \pm 0.11	1.05 \pm 0.09*	1.05 \pm 0.11*	1.05 \pm 0.18*
Ratio of males/total	0.48	0.47	0.50	0.46	0.44	0.46

Data represented mean \pm S.D.

^a Data were analyzed after arcsine transformation.

* $p < 0.05$ compared with the control group by ANOVA and Duncan's test.

Table 2
Skeletal variations induced by chronic high doses of iodine exposure

Dose group ($\mu\text{g/L}$)	0	1500	3000	6000	12000	24000
Number of examined litters	7	8	8	7	7	8
Number of fetuses examined ^a	22	23	19	18	22	28
Number of skeletal malformation (%) ^b	2 (9.1)	6 (26.1)	8 (42.1)*	8 (44.4)*	11 (50.0)*	15 (53.6)*
Supernumerary ribs	2	5	6	6	8	11
Agenesis of sternbrae	0	1	0	1	2	0
Poor ossification of metacarpals and metatarsals	0	0	0	0	0	1
Abnormal fusion of supraoccipital	0	0	0	1	1	2
Malaligned vertebral centra	0	0	2	0	0	1

^a One-half or two-thirds of the live fetuses were used for skeletal examination.

^b No multiple defects found in the same fetus.

* $p < 0.05$ compared with the control group by χ^2 -test.

groups and average placental weight was also reduced in high doses of iodine exposure groups except 1500 $\mu\text{g/L}$ group. No obvious changes in average daily water consumption and maternal body weight gain were noted among all of the doses groups throughout the gestation. The results of fetal observation showed that high doses of iodine exposure induced a decrease in the incidence of live fetuses and an increase in the incidence of reabsorbed fetuses (Table 1). Excess iodine-associated reduction in fetal body weight was observed only in female fetuses of 6000, 12,000 or 24,000 $\mu\text{g/L}$ groups. Neither obvious external alternations nor visceral malformations were found in fetuses in any treated groups.

3.3. Skeletal variations induced by chronic high doses of iodine exposure

Exposure to high doses of iodine resulted in an increase in the incidence of skeletal variations dose-dependently ($r = 0.80$,

$p < 0.05$), and this increase was statistically significant in the high doses of iodine groups except 1500 $\mu\text{g/L}$ group compared to control group (Table 2). Skeletal alterations included supernumerary ribs, agenesis of sternbrae, poor ossification of metacarpals and metatarsals, abnormal fusion of supraoccipital and malaligned vertebral centra. No multiple defects found in the same fetus. The form of ectopic or supernumerary ribs (SNR) was commonly observed (Table 2).

4. Discussion

To our knowledge this is the first study to examine the reproductive toxicity induced by maternal chronic high amounts of iodine exposure before and during pregnancy. At what level should iodine intake be considered excessive? Suzuki defined iodine excess as the amount of iodide beyond the physiological requirement for adequate thyroid hormone synthesis [16]. For most adults, 150 μg iodine is daily required for thyroid hormone synthesis and up to 200 μg per day for pregnant women. Zhao et al. [17] suggested that maximum allowable iodine concentrations may be set at 800 $\mu\text{g/L}$ in the urine of adults or 300 $\mu\text{g/L}$ in drinking water if a 5% goiter prevalence is defined as a public health problem. Under certain conditions, the consumption of iodine may be much higher. One study [18] found that the inhabitants of the Japanese island of Hokkaido who consume large quantities of seaweed called kombu take in more than 200 mg (200,000 μg) of iodine per day, a 1000 times the recommended daily requirement. Another study reported that the iodine concentration in the well water is above 1000 $\mu\text{g/L}$, up to 2800 $\mu\text{g/L}$ in some areas of China [19]. Generally, thyroid dysfunction was found to occur when iodine was administered at levels 10 times the physiologic requirement; however, effect of excess iodine on thyroid function varies in different species. The previous studies showed that mice are more sensitive to excess iodine exposure than rats [20].

In the present study, exposure to 1500, 3000, 6000, 12,000 and 24,000 $\mu\text{g/L}$ iodine in drinking water for 4 months, which corresponded to 5-, 10-, 20-, 40-, and 80-fold of the adequate iodine intake for mice, resulted in significant increase in urinary iodine concentration. Additionally, an obvious colloid goiter was also induced. In the thyroid of dams exposed to high doses of iodine, the follicles were filled with colloid and the epithelial

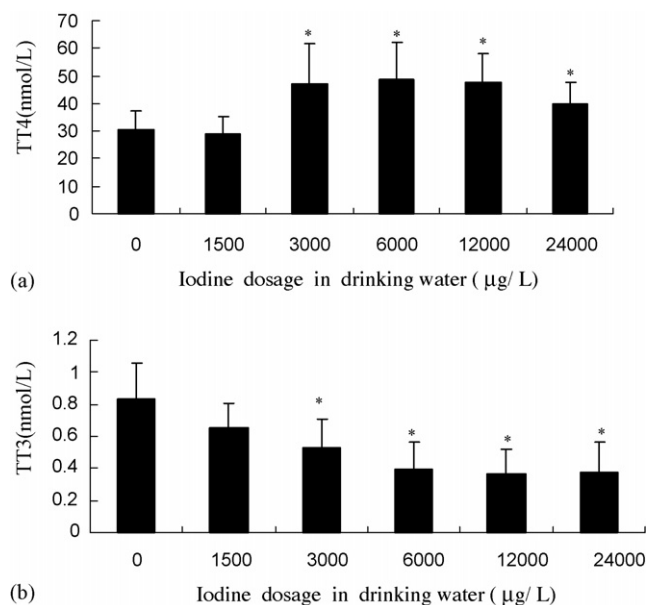


Fig. 3. Effects of chronic excessive iodine exposure on serum thyroid hormone level in maternal mice at term. Each bar represented the mean and S.D. of duplicate determination from seven to eight dams. * $p < 0.05$ compared with the control group by ANOVA and Duncan's test.

cells were flattened, which was different from the situation in iodine deficiency. Similar results have been reported in other studies on the animals or human subjects exposed to high doses of iodine. This indicated that the model of chronic high doses of iodine exposure was established.

Iodine excess reduced maternal food consumption in higher dose of iodine group (12,000 and 24,000 $\mu\text{g/L}$), but no change of maternal weight gain in these groups was observed. This might be related to an altered metabolic condition associated with a high dose iodine exposure. Reduction in placental weight might be possibly a consequence of maternal toxicity. Chronic high doses of iodine exposure produced embryotoxicity as indicated by the decrease in the number of live fetuses and the increase in the rate of resorptions and skeletal variations. Interestingly, lower body weight was only found in female fetuses. This suggested that females may be more susceptible to iodine excess than males. Recently, several studies [21–23] were carried out to observe the effect of food restriction on embryo development in the rabbit and rat. The results showed that feed restriction to feed levels that produce substantial reductions (50% compared to *ab libitum* fed animals) in maternal body weight gain might result in developmental toxicity expressed by abortion, reduced fetal weight, and minor alterations in bone development. In present study, reduction in maternal food consumption (about 10% reduction) was only observed in 12,000 and 24,000 $\mu\text{g/L}$ groups. But the developmental toxic effect such as increased resorption, reduction in fetal body weight and reduction in placental weight had already been found in the lower dose groups. Therefore, adverse pregnancy outcome induced by excess iodine at lower doses might not result from maternal toxicity. Whether adverse pregnancy outcome induced by excess iodine at higher doses might partly be the consequence of reduced maternal food intake or not still need further study.

The mechanisms involved in the maternal and embryo toxicity induced by high doses of iodine exposure have not been clarified. The changes of thyroid hormone level may play an important role. Depending on the dose of iodine, susceptibility variances of species and on the previous conditions of the gland, iodine excess can induce hypothyroidism or hyperthyroidism. In the present study, high doses of iodine treatment resulted in an increase of TT_4 and a decrease of TT_3 , consistent with previous animal studies [24,25]. This change may be mainly related to the inhibition of 5'-deiodinase activity, resulting in a decrease in the generation of T_3 from T_4 [26]. Maternal thyroid hormone status affects fetal thyroid hormone level via the placenta, which modulate the transfer of iodine and small but important amounts of thyroid hormone (especially T_4) from mother to fetus [27]. Recent findings from University of Chicago research [28] represented the first evidence in humans that an excess of thyroid hormone during pregnancy could impair embryogenesis of growing fetuses through transplacental passage of maternal thyroid hormone, and also indicated that hormonal replacement must be assessed and fine-tuned so as not to exceed the normal requirements. Several clinical studies [29,30] reported that chronic maternal exposure to medications containing iodine (such as amiodarone and potassium iodide) may induce hypothyroidism and goiter in the offspring. One study [31] carried out

in Denmark, where mild to moderate iodine deficiency is prevalent, found that supplementation of moderate iodine (150 μg per day) to healthy pregnant women with no previous history of thyroid disease could inhibit thyroid function in the neonates. So, besides the influence on maternal thyroid hormone, excessive iodine transferred by placenta has a direct inhibition on the development and function of fetal thyroid. Although the detailed mechanism need further research, both effects might have led to hypothyroidism in fetuses [32], which induced embryotoxicity as indicated mainly by the fetal growth retardation in female fetuses and the increase in the incidence of resorptions and skeletal variations. These toxic effects were obviously observed in the groups with doses starting at 3000 $\mu\text{g/L}$, which corresponded to 10-fold of the adequate iodine intake for mice. Under certain conditions, the amount of iodide ingested by people is beyond the physiological requirement tens or hundreds times [18,19]. Therefore, the potential developmental toxic effects of chronic exposure to high doses of iodine need attention.

Thyroid hormone plays a key role in normal skeletal development, linear growth and the maintenance of adult bone mass [33]. The delayed appearance of ossification centers is a frequent finding in newborns with congenital hypothyroidism. The same phenomenon was observed in the present study. Other forms of skeletal variations including agenesis of sternbrae, abnormal fusion of supraoccipital and malaligned vertebral centra, especially supernumerary ribs (SNR) also found in fetuses of mice exposed to high doses of iodine. Thyroid hormone receptor (TR) and retinoic acid receptor (RAR) are members of the steroid hormone receptor superfamily, as well as have been shown to share an identical P-box sequence, which implicates that they can bind the same DNA sequences and can interact physically. Supernumerary ribs in mouse fetuses induced by retinoic acid were also reported [34]. The molecular mechanisms of thyroid hormone and retinoic acid might overlap. Several studies [35–37] have found that axial skeletal defects (such as extra ribs) correlated with the abnormal expression of developmental control genes-*Hox* genes, which is now confirmed to be regulated by retinoic acid. A thyroid hormone responsive element (TRE) was also found in the regulatory regions of some *Hox* genes [38]. However, there is little evidence that *Hox* genes expression is regulated directly by thyroid hormone. So, the molecular mechanisms of skeletal variations induced by excessive iodine need further investigation.

The current findings may have potentially important implications for pregnant women who lived in iodine excess areas or supplemented with high amounts of iodine during pregnancy. Whether such changes in our exposures to high amounts of iodine could be having an adverse impact on pregnancy and fetal outcome is a significant issue that needs further attention.

Acknowledgements

This work was supported by the National Natural Science Foundation of China, No. 30230330. We wish to thank Zu P. Chen and Yu Q. Yan for their instruction on experiment design.

References

- [1] Delange F, Lecomte P. Iodine supplementation: benefits outweigh risks. *Drug Saf* 2000;22:89–95.
- [2] Konno N, Makita H, Yuri K, Iizuka N, Kawasaki K, Norimichi K, et al. Association between dietary iodine intake and prevalence of subclinical hypothyroidism in the coastal regions of Japan. *J Clin Endocrinol Metab* 1994;78:393–7.
- [3] Zhao JK, Chen ZP, Maberly GF. Iodine-rich drinking water of natural origin in China. *Lancet* 1998;352:2024.
- [4] Martino E, Bartalena L, Bogazzi F, Braverman LE. The effects of amiodarone on the thyroid. *Endocr Rev* 2001;22:240–54.
- [5] Wolff J. Physiology and pharmacology of iodized oil in goiter prophylaxis. *Medicine* 2001;80:20–36.
- [6] Prakash R. High thyroid volume in children with excess dietary iodine intakes. *Am J Clin Nutr* 2005;82:708–9.
- [7] Koutras DA. Control of efficiency and results, and adverse effects of excess iodine administration on thyroid function. *Ann Endocrinol* 1996;57:463–9.
- [8] Zimmermann M, Delange F. Iodine supplementation of pregnant women in Europe: a review and recommendations. *Eur J Clin Nutr* 2004;58:979–84.
- [9] Calvo RM, Jauniaux E, Gulbis B, Contempre B, Morreale de EG. Fetal tissues are exposed to biologically relevant free thyroxine concentrations during early phases of development. *J Clin Endocrinol Metab* 2002;87:1768–77.
- [10] Hetzel BS, Mano MT. A review of experimental studies of iodine deficiency during fetal development. *J Nutr* 1989;119:145–51.
- [11] Wolfberg AJ, Nagey DA. Thyroid disease during pregnancy and subsequent congenital anomalies. In: Paper presented at the annual meeting of the Society for Maternal–Fetal Medicine in New Orleans. 2002.
- [12] Markou K, Georgopoulos N, Kyriazopoulou V, Vagenakis AG. Iodine-induced hypothyroidism. *Thyroid* 2001;11:501–10.
- [13] Roti E, Uberti ED. Iodine excessive and hyperthyroidism. *Thyroid* 2001;11:493–500.
- [14] Nishiyama S, Makeda T, Okada T, Nakamura K, Kotani T, Hishinuma A. Transient hypothyroidism or persistent hyperthyrotropinemia in neonates born to mothers with excessive iodine intake. *Thyroid* 2004;14:1077–83.
- [15] Fischer PWF, L'Abbé MR, Giroux A. Colorimetric determination of total iodine in foods by iodide-catalyzed reduction of Ce^{4+} . *Anal Chem* 1986;69:687–9.
- [16] Beckers C, Delange F, Gaintan E, Suzuki H, Koutras DA, Medeiros-Neto GA. Etiology of endemic goiter. In: Stanbury JB, Heztel BS, editors. *Endemic goiter and endemic cretinism: iodine nutrition in health and disease*. New York, NY: John Wiley & Sons; 1980. p. 237–53.
- [17] Zhao J, Wang P, Shang L, Sullivan KM, van der Haar F, Maberly G. Endemic goiter associated with high iodine intake. *Am J Public Health* 2000;90:1633–5.
- [18] Suzuki H, Higuchi T, Sawa K, Ohtaki S, Horiuchi Y. Endemic coast goiter in Hokkaido. *Acta Endocrinol* 1965;50:151–76.
- [19] Zhao J, Wang P, Zhang Q, Wang H, Hu X. Ecology of high iodine intake and endemic goiter in three counties of Jiangsu Province, China. *Chin Med J* 2002;115:850–4.
- [20] Chen XY, Sun XF, Pang H, Yang XF, Yu D, Hou XH, et al. Acute toxicity and mutagenicity of KIO_3 . *J Toxicol* 2005;19:129–31.
- [21] Terry KK, Foley GL, Kadyszewski E, Fleeman TL, Hurtt ME, Chapin RE. Effects of feed restriction on fertility in female rats. *Birth Defects Res B* 2005;74:431–41.
- [22] Fleeman TL, Cappon GD, Chapin RE, Hurtt ME. Effects of feed restriction during organogenesis on embryo-fetal development in the rat. *Birth Defects Res B* 2005;74:442–9.
- [23] Cappon GD, Fleeman TL, Chapin RE, Hurtt ME. Effects of feed restriction during organogenesis on embryo-fetal development in rabbit. *Birth Defects Res B* 2005;74:424–30.
- [24] Xiang JM, Chen ZP, Di HJ, Yan YQ, Chen QY. Effects of iodine excess on antioxidizing ability of mice. *Chin J Endem* 1999;4:245–8.
- [25] Harjai KJ, Licata AA. Effects of amiodarone on thyroid function. *Ann Intern Med* 1997;126:63–73.
- [26] Bednarczyk T, Pietrzykowski A, Slon M, Nauman A. Pharmacologic effect of excess iodine on type I thyroxine 5'-deiodinase activity in rat thyroid. *Endokrynol Pol* 1993;44:405–12.
- [27] Calvo RM, Jauniaux E, Gulbis B, Contempre B, Asuncion M, Gervy C, et al. Fetal tissues are exposed to biologically relevant free thyroxine concentrations during early phases of development. *J Clin Endocrinol Metab* 2002;87:1768–77.
- [28] Anselmo J, Cao D, Karrison T, Weiss RE, Refetoff S. Fetal loss associated with excess thyroid hormone exposure. *JAMA* 2004;292:691–5.
- [29] Bartalena L, Bogazzi F, Braverman LE. Effects of amiodarone administration during pregnancy on neonatal thyroid function and subsequent neurodevelopment. *J Endocrinol Invest* 2001;24:275–83.
- [30] Serreau R, Polack M, Leger J. Fetal thyroid goiter after massive iodine exposure. *Prenat Diagn* 2004;24:745–54.
- [31] Nohr S, Laurberg P. Opposite variations in maternal and neonatal thyroid function induced by iodine supplementation during pregnancy. *J Clin Endocrinol Metab* 2000;85:623–7.
- [32] Nishiyama S, Makeda T, Okada T, Nakamura K, Kotani T, Hishinuma A. Transient hypothyroidism or persistent hyperthyrotropinemia in neonates born to mothers with excessive iodine intake. *Thyroid* 2004;14:1077–83.
- [33] Bassette JHD, Williams GR. The molecular actions of thyroid hormone in bone. *Trends Endocrinol Metab* 2003;14:356–65.
- [34] Rengasamy P, Padmanabhan RR. Experimental studies on cervical and lumbar ribs in mouse embryos. *Cong Anom* 2004;44:156–71.
- [35] Branch S, Rogers JM, Brownie CF, Chernoff N. Supernumerary lumbar rib: manifestation of basic alteration in embryonic development of ribs. *J Appl Toxicol* 1996;16:115–9.
- [36] Akker E, Fromental-Ramain C, Graaff W. Axial skeletal patterning in mice lacking all paralogous group 8 Hox genes. *Development* 2001;128:1911–21.
- [37] Juan AH, Ruddle FH. Enhancer timing of Hox gene expression: deletion of the endogenous Hoxc8 early enhancer. *Development* 2003;130:4823–34.
- [38] Awgulewitsch A, Bieberich C, Bogarad L, Shashikant C, Ruddle FH. Structural analysis of the Hox-3.1 transcription unit and the Hox-3.2–Hox-3.1 intergenic region. *Proc Natl Acad Sci USA* 1990;87:6428–32.