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Thyroid function alterations attributed to high iodide supplementation in maternal rats and their offspring

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ABSTRACT

Objective: Our aim was to investigate thyroid function alterations attributed to high iodide supplementation in maternal rats and their offspring.

Methods: Depending on their iodide intake, the pregnant rats were randomly divided into three groups: normal iodide intake (NI), 10 times high iodide intake (10 HI) and 100 times high iodide intake (100 HI) groups. Iodine concentration in the urine and maternal milk; iodine content and mitochondrial superoxide production; expression of TRα1, TRβ1, NIS and Dio1 in both the thyroid and mammary glands were all measured. The offspring were exposed to different iodide-containing water (NI, 10 HI and 100 HI) from weaning to postnatal day 180 (PN180). Serum thyroid hormone levels were measured in both maternal rats and their offspring.

Results: Iodine concentration in the urine and maternal milk, as well as iodine content in the thyroid and mammary glands was significantly increased in both the 10 HI and 100 HI groups ($p < .05$). In the 100 HI group of maternal rats, low FT3 levels, high FT4, TPOAb and TgAb levels were detected. In addition, an increased mitochondrial superoxide production and decreased expression of TRα1, TRβ1, NIS and Dio1 in the thyroid and mammary glands was found ($p < .05$). A positive staining of $CD4^+$ that co-localized with TRβ1 in the infiltrated cells within the thyroid follicles was observed. At PN180 in the offspring, the FT3 and FT4 levels showed a significant decrease, while the levels of serum TSH, TPOAb and TgAb were significantly increased in both 10 HI and 100 HI groups ($p < .05$).

Conclusion: In maternal rats, although normal thyroid function can be maintained following 10 HI, thyroiditis can be induced following 100 HI on lactation days 7, 14, and 21. In the offspring at PN180, hypothyroidism complicated with thyroiditis can occur in both the 10 HI and 100 HI groups.

1. Introduction

Moderate to severe iodine deficiency during pregnancy may lead to insufficient maternal thyroid hormone production, causing irreversible adverse effects on the offspring [1]. Populations sensitive to iodide deficiency include pregnant women and their fetuses, as there is a high demand for iodide during gestation to support the growth and development of the fetus. Iodine is a critical constituent of thyroid hormones which is essential for intrauterine and postnatal development. WHO/ICCIDD/UNICEF has recommended that the daily intake of iodide for lactating women to be 250 μg/d. The supplementation of iodine has been shown to improve the maternal thyroid function index. However, further experimental research needs to be conducted to determine the effects and safe upper limits for iodine supplementation during pregnancy and lactation periods [1]. Additionally, the underlying molecular mechanisms at play following iodide supplementation during pregnancy and lactation periods have not been fully characterized. The aim of this study was to investigate thyroid function alterations attributed to high iodide supplementation in maternal rats and their offspring.

2. Methods and materials

2.1. Animals feeding and groups

Seven-week-old female Wistar rats were mated with fertile males (1:1). The presence of a vaginal plug was indicative of pregnancy (Day

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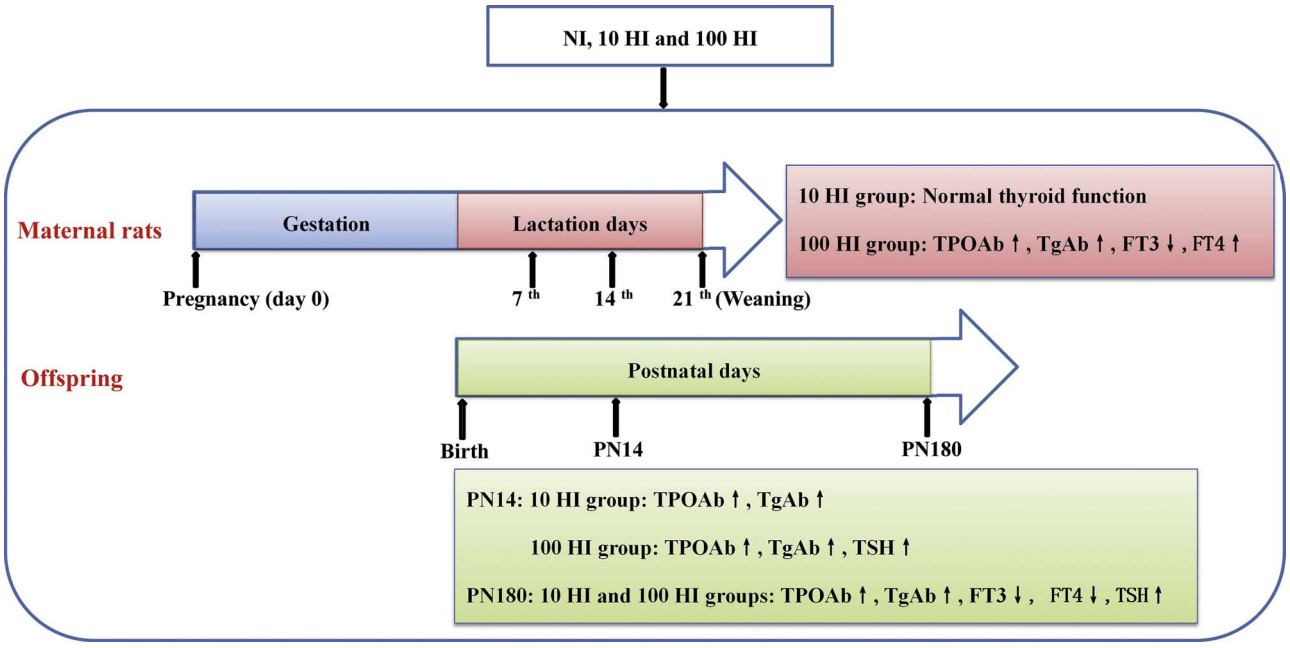

Fig. 1. Study design. The maternal rats were fed with a normal diet and iodine-containing drinking water from Day 0 of pregnancy (appearance of a vaginal plug) until weaning, and were sacrificed on the lactation days 7, 14, 21. The offspring were exposed to different iodide-containing water (NI, 10 HI and 100 HI) from weaning to PN180, and were sacrificed on PN14 and PN180.

0 of pregnancy). The pregnant rats were randomly divided into normal iodide intake (NI), 10 times high iodide intake (10 HI) and 100 times high iodide intake (100 HI) groups. Eight pregnant rats were used in each group. The NI group consumed deionized water and a normal diet, while the 10 HI and 100 HI groups received different dosages of potassium iodide diluted in the deionized water with a normal diet, resulting in daily iodide intake of 7.5 μg/d, 75 μg/d and 750 μg/d respectively. The maternal rats were sacrificed on lactation days 7, 14 and 21. Their offspring were continuously exposed to different iodide-containing water (NI, 10 HI, 100 HI) from weaning to postnatal day 180 (PN180), and were sacrificed on postnatal day 14 (PN14) and 180 (PN180) respectively (Fig. 1). Animal procedures were approved by the Institutional Animal Care and Use Committee of Tianjin Medical University (No. TMUaMEC2016054).

2.2. Reagent

Anti-Thyroid Hormone Receptor alpha antibody (ab53729), Anti-Thyroid Hormone Receptor beta antibody (ab2744), Anti-Sodium Iodide Symporter antibody (ab17795), Goat Anti-Mouse IgG H&L (HRP) (ab6789) and Goat Anti-Rabbit IgG H&L (HRP) (ab7090) were purchased from Abcam (Abcam, Cambridge, MA, USA). Goat Anti-Rabbit IgG-PE: sc-3739 and Goat Anti-Mouse IgG-FITC: sc-2010 were bought from Santa Cruz (Santa Cruz Biotechnology, Inc., CA, USA). Rabbit Anti-CD4 was purchased from Bioss (BIOSYNTHESIS BIOTECHNOLOGY CO, LTD, Beijing, China). MitoSOX Red (3,8-phenanthridinediamine, 5-(6′-triphenylphosphoniumhexyl) −5,6-dihydro-6-phenyl), mitochondrial superoxide indicator (M36008) and TRIzol® Reagent were purchased from Invitrogen (Invitrogen Life Technologies, CA, USA). GAPDH (14C10) Rabbit mAb was purchased from CST (Cell Signaling Technology Inc, MA, USA). Transcriptor First Strand cDNA Synthesis Kit was purchased from Roche (Roche, Shanghai, China). GoTaq® qPCR Master Mix was purchased from Promega (Promega, Madison, USA). All the other chemicals made in China were analytic grade.

2.3. Measurement of iodine concentration in the urine and maternal milk, iodine content in the thyroid and mammary glands

Iodine concentration in the urine and maternal milk, as well as iodine content in the thyroid and mammary glands was measured by As-Ce catalytic spectrophotometry [2] in the Key Lab of Hormones and Development Ministry of Health, Institute of Endocrinology, Tianjin Medical University.

2.4. Measurement of serum thyroid hormones

Blood samples were drawn from the carotid artery, centrifuged, and stored at −80 °C. Levels of free triiodothyronine (FT3), free thyroxine (FT4), thyrotropin (TSH), thyroid peroxidase antibody (TPOAb) and thyroglobulin antibody (TgAb) were determined by Rat Specific ELISA kits (Baoman Biological Technology, Shanghai, China) according to the manufacturer's instruction.

2.5. Flow cytometry

MitoSOX Red was used to measure mitochondrial superoxide production by flow cytometry. 5 μM of MitoSOX Red was added to the thyroid cell suspension and incubated for 10 min at 37 °C in the dark. Flow cytometry was carried out using a FACSCalibur (BD Bioscience, San Jose, CA). FL2 channel forward scattering (forward scatter, FSC) and lateral scattering (side scatter, SSC) data was collected; 10000 cells were collected for each sample. The control group without MitoSOX was regarded as the blank zero group for standardization.

2.6. Western blot analysis

50 μg proteins were separated by SDS-PAGE and transferred to the PVDF membrane. The membrane was incubated overnight at 4 °C with primary antibodies followed by secondary antibodies. GAPDH was used as a loading control. All blot intensities were normalized with that of the loading control GAPDH.