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Iron deficiency without anemia causes maternal hypothyroxinemia in pregnant rats

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ABSTRACT

Because of increased total red blood cell mass and the demands of the fetus, iron requirements are greater during pregnancy than at most other times. Previous experiments in nonpregnant women have shown that iron deficiency (ID) can reduce circulating thyroxine and triiodothyronine levels; therefore, we hypothesized that ID before pregnancy can reduce thyroid hormone levels in maternal circulation and in the thyroid gland during pregnancy. In the present study, 2 types of rat models with ID were established using diets with different iron concentrations. Levels of thyroid hormone, hemoglobin, serum iron, liver iron, serum ferritin, serum transferrin receptor, and serum thyroid-stimulating hormone as well as thyroid peroxidase activity were measured throughout pregnancy, and thyroid structure was analyzed. Both mild ID with anemia and ID without anemia resulted in maternal hypothyroxinemia from midgestation to the end of the pregnancy. Thyroid peroxidase activity significantly decreased, even before the reduction of liver iron concentrations in ID groups. Iron deficiency reduced the size of follicular cavities but did not destroy the follicular structure. Linear regressions were performed to compare total levels of maternal serum thyroxine to indices of iron status for individual dams. This is the first rat study to report our results stating that ID can cause maternal hypothyroxinemia during early pregnancy.

1. Introduction

Maternal thyroid dysfunction during pregnancy is associated with abnormal neural and motor development in children [1]. In 2007, 4800 pregnant women who were living in an iodine-sufficient area in China were screened during the first half of pregnancy. The prevalence of maternal hypothyroxinemia was 2.1% [2]. Among these pregnant women with hypothyroxinemia, only 7.65% were positive for thyroid antibody, but the etiology of the rest was uncertain [2]. In a study of pregnant women who were living in an area in which borderline iodine deficiency is common, Zimmermann et al [3] reported that iron deficiency (ID) was predictive of lower total thyroxine (TT_4) during pregnancy. In previous rodent experiments, severe iron deficiency with anemia (ID + A) was found to be associated with decreased serum thyroxine (T_4) and triiodothyronine (T_3) concentrations through decreasing iron-dependent enzyme thyroid peroxidase (TPO) activity [4-9]. Iron deficiency with

Abbreviations: ANOVA, analysis of variance; BSA, bovin serum albumin; G, gestational day; ID, iron deficiency; ID + A, iron deficiency with anemia; ID − A, iron deficiency without anemia; OD, optical density; P, postnatal day; T_3, triiodothyronine; T_4, thyroxine; TfR, transferrin receptor; TPO, thyroid peroxidase; TSH, thyroid-stimulating hormone; TT_3, total triiodothyronine; TT_4, total thyroxine.

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anemia also affects deiodinase activity [6,10], alters the central nervous system's control of thyroid metabolism [10], and changes nuclear T_3 binding [11].

Iron deficiency is the most common nutritional deficiency in the world [12], affecting an estimated 2 billion people [13]. Children and pregnant women are the most vulnerable [3,12]. There are 2 main types of ID, iron deficiency without anemia (ID – A) and ID + A [14]. The first is more common, as the frequency is approximately 2.5 times that of ID + A [15]. In a nationwide epidemiological survey of China in 2000, the prevalence of ID and ID + A was 42.6% and 19.1%, respectively, in pregnant women [16]. According to the National Health and Nutrition Examination Survey, from 1999 to 2006, the prevalence of ID in pregnant women in the United States was 6.96%, 14.36%, and 29.56% in the first, second, and third trimesters, respectively [17]. These statistics emphasize that ID is prevalent in pregnant woman. Iron requirements are greater during pregnancy than at other times, especially from the second trimester onward. This is due to the expansion of total red blood cell mass and the requirements of the fetus [18].

Based on previous human and rodent studies, we hypothesized that ID could reduce maternal thyroid hormone levels in circulation and in the thyroid gland during pregnancy. Currently, few studies have reported any association between ID – A and thyroid function during pregnancy. In the present study, 2 rat models were established with each imitating 1 of the 2 types of ID found in pregnant women, and the effects of ID on maternal thyroid function and thyroid morphology were explored throughout pregnancy. Results showed that both ID – A and ID + A could cause maternal hypothyroxinemia during early pregnancy. This is speculated to have been caused by the reduced activity of TPO, an iron-containing enzyme crucial to the synthesis of thyroid hormones.

2. Methods and materials

2.1. Diets and rats

Female Sprague-Dawley rats (n = 90), weighing approximately 200 g, were obtained from SLAC Laboratory Animal (Shanghai, China). They were randomly assigned to 1 of 3 groups: control, ID + A, or ID – A (n = 10 for each group at each time point). They were fed diets with different iron concentrations (10, 30, and 70 ppm) from 2 weeks before to mating until they were killed at gestational days (G) 0, G13, and postnatal day (P) 0. Iron concentrations of 10, 30, and 70 ppm were given to rats in the ID + A, ID – A, and control groups, respectively. The diets were prepared in the laboratory by adding ferrous sulfate heptahydrate in place of ferric citrate, to the formula for the AIN-93G diet [19]. Iodine, selenium, copper, and zinc contents were measured using inductively coupled plasma mass spectrometry (Series 7500A; Agilent Technologies, Inc), and no difference in content of any of these elements was found between the diets (data not shown). As shown in Table 1, the composition of experimental diets and daily intake of protein, fat, fiber, vitamins, and other minerals were the same in all groups. Rats were housed in a temperature-controlled animal facility with a reversed 12:12-hour light/dark cycle. The temperature was maintained at 24°C, and relative humidity was approximately 45%. Food and demineralized water were administered freely to all groups. All procedures were approved by the China Medical University Ethics Committee.

Table 1 – Ingredient composition of the diets fed to rats

Ingredients (g/kg diet)	Control	ID – A	ID + A
Cornstarch	529.5	529.5	529.5
Casein (>85% protein)	200.0	200.0	200.0
Sucrose	100.0	100.0	100.0
Soybean oil (no additives) [a]	70.0	70.0	70.0
Fiber	50.0	50.0	50.0
Mineral mix (AIN-93G-MX) [b]	35.0	35.0	35.0
Vitamin mix (AIN-93-VX)	10.0	10.0	10.0
L-Cysteine	3.0	3.0	3.0
Choline bitartrate	2.5	2.5	2.5

[a] Jinlongyu edible oil (Yi Hai Kerry Food Marketing Co, Ltd, Shenzhen, China) was obtained from BHG Market Place (Shenyang, China).
[b] Modified from a mineral mixture (AIN-93G-MX). Control, ID – A, and ID + A diets contained 70, 30, and 10 ppm iron, respectively.

2.2. Tissue collection

The day that the vaginal plug was detected was considered G0. Dams were weighed, placed under deep anesthesia, and then euthanized with intraperitoneal injection by chloral hydrate on G0, G13, and P0. Blood samples (approximately 14 mL) were obtained from the hearts for measurement of hemoglobin and thyroid hormone levels. Rats were perfused with physiological saline through the left ventricle. At each time point, thyroid lobes (n = 20 for each group at each time point) were dissected, washed in cold physiological saline, blotted with filter paper, and weighed. Five of them were fixed in 4% paraformaldehyde at each point in time. Ten of them were stored at –80°C for measurement of thyroid hormone, and another 5 were stored for measurement of TPO activity. A small portion of liver (approximately 0.100 g) from each rat was removed, washed, weighed, and digested in 5-mL nitric acid for 24 hours.

2.3. Iron status of ID dams

A pregnant rat model of ID + A was established. This condition was defined as levels of liver iron, serum ferritin, and hemoglobin that were lower than in the control group at P0. A pregnant rat model of ID – A was also established. This condition was defined as levels of liver iron and serum ferritin that were lower than in the control group but hemoglobin levels that were the same as in the control group at P0.

Whole blood from each rat heart was collected into anticoagulation tubes (BD Vacutainer K2 EDTA) and coagulation tubes (BD Vacutainer SST II Advance). Then hemoglobin (2-mL anticoagulation blood) was immediately analyzed with an automated blood coagulation analyzer (K4500; Sysmex, Co, Japan).

The wet digestion method was used to digest the iron solution of liver tissues. Iron concentration was determined using graphite furnace atomic absorption spectroscopy (atomic absorption spectrophotometer 180-80; Hitachi High-Technologies, Co, Japan), as described in a previous study [20].