

## Research Article

# Effect of Preeclampsia on Ultrastructure of Thyroid Gland, Hepatic Type 1 Iodothyronine Deiodinase, and Thyroid Hormone Levels in Rats

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**Background.** Although hypothyroidism during pregnancy may develop grave outcomes for both mothers and offspring, management of which is still a challenge due to the insufficient understanding of this disease. The close correlation between hypothyroidism and preeclampsia is well documented, suggesting that preeclampsia is a potential risk factor for the development of maternal hypothyroidism. However, the exact role of preeclampsia in gestational hypothyroidism is still obscure. **Objective.** In this study, we explored the possible mechanisms of the effect of preeclampsia on thyroid function of maternal rats. **Methods.** Thirty pregnant rats were randomly divided into normal pregnancy control (NOP), preeclampsia (PE), and preeclampsia supplemented with amlodipine besylate (PEAml). NG-Nitro-L-arginine-methyl ester was used to induce preeclamptic symptoms. On gestational day 21, rats were sacrificed, and then, the ultrastructure of the thyroid gland, type 1 iodothyronine deiodinase (Dio1) expression, and serum-free thyroxine ( $FT_4$ ), free triiodothyronine ( $FT_3$ ), and thyroid stimulation hormones (TSH) were assessed. **Results.** Compared to NOP rats, results of PE rats showed that thyroid follicular cells' ultrastructure was damaged; both hepatic Dio1 mRNA and protein levels were decreased. Interestingly, these changes were ameliorated in PEAml rats. Additionally,  $FT_4$ ,  $FT_3$ , and TSH levels have no significant differences among groups. **Conclusion.** These findings indicated that preeclampsia could disrupt synthesis, secretion, and metabolism function of thyroid hormones by damaging thyroid follicular cells and interfering Dio1 expression.

## 1. Introduction

The stability of thyroid function is essential for normal growth, development, and metabolism, particularly in the gestation period and growing fetus [1]. Thyroid disorders, particularly hypothyroidism, are a common syndrome in women of childbearing age and feature frequently in preg-

nancy [2, 3]. It is well established that pregnant women with hypothyroidism will result in substantially greater risks of detrimental pregnancy and offspring outcomes such as miscarriage, stillbirth, neonatal respiratory distress syndrome, and fetal central nervous development defect [4–6]; consequently, serious burdens can be brought to the family and the society. However, evaluation and management of

pregnant women with hypothyroidism are challenging due to the lack of sufficient cognition on the occurrence and development of disease [7].

Preeclampsia is one of the most common severe complications of pregnancy. Significantly increased frequency of hypothyroidism can be detected in preeclamptic women [8–11] and was positively correlated with the severity and medical history of preeclampsia [12–14]. Furthermore, preeclampsia may also predispose to the development of hypothyroidism in later years after delivery [15]. It also has been suggested that attention should be paid to the monitoring of thyroid function in pregnant women with preeclampsia because of the higher risk of hypothyroidism [16]. From these considerable clinical evidences, the obviously close association between hypothyroidism and preeclampsia is established. The exact mechanism of hypothyroidism in preeclamptic women, however, remains obscure.

Adequate thyroid function largely depends on the ability of thyroid hormone synthesis, secretion, and metabolism [17–19]. Thyroid follicular cells are the basic structure of the thyroid gland and essential for principal function of the thyroid gland: the site for synthesis and secretion of the thyroid hormones [20]. In thyroid follicular cells, endoplasmic reticulum, mitochondria, and microvilli are major organelles involved in thyroid hormone synthesis; lysosomes provide degradation pathways for thyroid hormone secretion [21]. Thyroxine ( $T_4$ ) and triiodothyronine ( $T_3$ ) are hormones synthesized and secreted from the thyroid gland;  $T_3$  is the bioactive form of the thyroid hormone [22]. About 80% of circulating  $T_3$  is derived from the metabolic transformation: the extrathyroidal peripheral conversion of  $T_4$  to  $T_3$  [23]. The peripheral conversion is mainly catalyzed by type 1 iodothyronine deiodinase (Dio1) [24], which is predominantly expressed in the liver [25].

With the background information above, we sought to identify how thyroid function is influenced by preeclampsia. Therefore, we evaluated the ultrastructure of the thyroid gland, the hepatic Dio1 expression, and circulating thyroid hormone levels in pregnant rats with preeclampsia induced by NG-nitro-L-arginine-methyl ester (L-NAME), to provide reliable experimental evidences for revealing the underlying mechanisms of hypothyroidism in preeclampsia; we further observed whether these changes were improved after administration of amlodipine besylate, a commonly used antihypertensive agent.

## 2. Materials and Methods

**2.1. Equipment.** In this study, the following major equipment was used: BP-6A tail-cuff system (Taimeng Technology, Chengdu, China), Sorvall ST8 centrifuge (Thermo Fisher Scientific, MA, USA), KHBST-360 microplate reader (Kehua Bio-engineering, Shanghai, China), CL-2000i microparticle chemiluminescence analyzer (Mindray, Shenzhen, China), EMUC6 Ultramicrotome (Leica, Wetzlar, Germany), H-600IV transmission electron microscope (Hitachi, Tokyo, Japan), KZ-II high-flux tissue grinder (Servicebio, Wuhan, China), SLAN®-96s qPCR system (Shanghai Hongshi Medical Technology Co., Ltd, Shanghai, China), MS-PB magnetic

stirrer (Servicebio, Wuhan, China), DYY-6C electrophoresis apparatus (Liuyi, Beijing, China), and photographic film (Kodak, NY, USA).

**2.2. Chemicals.** In this study, the following major chemicals were used: L-NAME was obtained from Meilun Biotechnology Co., Ltd, Dalian, China, while amlodipine besylate was purchased from Pfizer, Dalian, China.

**2.3. Animals.** Sprague-Dawley rats (280–300 g) were included in this study, purchased from the Laboratory Animal Centre of Chongqing Medical University, Chongqing, China. Rats were housed in SPF barrier animal facility (temperature, 20–24°C; humidity, 50–60%; 12 h light/dark cycle) and had access to the standard food and water ad libitum. All the experimental procedures followed the *Guide for the Care and Use of Laboratory Animals* and were approved by the Welfare and Ethics of Experimental Animals Committee of Institute of Laboratory Animal Sciences, Sichuan Academy of Medical Sciences & Sichuan Provincial People's Hospital (No. Lunshen2019-001). And the SPF animal facility was accredited by the Sichuan Provincial Laboratory Animal Management Committee.

**2.4. Experimental Protocol.** Each female rat was mated overnight, and spermatozoa were found in the vaginal smear which was defined as the first day of pregnancy.

On day 13 of pregnancy, pregnant rats were divided randomly into three groups: pregnant rats that received daily subcutaneous injection of normal saline and gavage of 0.5% CMC-Na (normal pregnancy control group, NOP,  $n = 10$ ), pregnant rats that received daily subcutaneous injection of 250 mg/kg L-NAME and gavage of 0.5% CMC-Na (preeclampsia group, PE,  $n = 10$ ), and pregnant rats that received daily subcutaneous injection of 250 mg/kg L-NAME and gavage of 0.5 mg/kg amlodipine besylate (preeclampsia +amlodipine besylate group, PEAmI,  $n = 10$ ). L-NAME was dissolved in sterile normal saline. Amlodipine besylate was suspended in 0.5% CMC-Na, and the human equivalent dosage of amlodipine besylate for rats was determined based on clinical usage and body surface area conversion. Subcutaneous injection of L-NAME and gavage of 0.5% CMC-Na or amlodipine besylate were lasted from gestational day 13 to 20.

Preeclamptic symptoms were detected on gestational day 20. Systolic blood pressure was measured by the tail-cuff noninvasive method [26]. Urine protein was measured by a semiquantitative (negative, +, ++, +++, and +++) urine dipstick test for morning urine samples [27, 28].

On day 21 of pregnancy, all rats were anesthetized using intraperitoneal injection of 50 mg/kg pentobarbital sodium, and blood samples were obtained through abdominal aortic puncture, and serum was separated for measurement of thyroid hormones. After anesthesia and euthanasia, tissue samples from the thyroid gland were resected and fixed in 3% glutaraldehyde for ultrastructural analyses. Tissue samples from the liver were dissected and stored at -80°C until measurement of Dio1 expression.

TABLE 1: The prime information of GAPDH and Dio1.

| Genes | Primer    | Sequences (5'-3')      | Product size (bp) |
|-------|-----------|------------------------|-------------------|
| GAPDH | R-GAPDH-S | GGTGCTGAGTATGTCGTGGAGT | 105               |
|       | R-GAPDH-A | GGAAGGGCGGAGATGA       |                   |
| Dio1  | R-Dio1-S  | GGTGGACACAATGCAGAACCA  | 114               |
|       | R-Dio1-A  | TAGTTCCAAGGGCCAGGTTT   |                   |

**2.5. Observation of Ultrastructure of Thyroid Gland.** After being fixed with 3% glutaraldehyde, the thyroid gland tissue was immersed in 1% osmium tetroxide for postfixation, dehydrated in graded acetone (30, 50, 70, 80, 90, 95, and 100%), successively infiltrated in a graded series of buffers that contain the dehydrating agent and epoxy resin osmotic solution at a ratio of 3:1, 1:1, and 1:3, for 0.5-1 h each permeation, and then embedded in Epon 812 resin, cut into semithin cross-sections with optical localization, and proceeded into ultrathin sections, doubly stained with uranium acetate and lead citrate. The ultrastructure changes were observed and photographed using a transmission electron microscope (TEM) [29]. Blinding of the independent observer was achieved by consecutive numbering of each sample.

**2.6. Detection of Dio1 Expression.** qPCR and western blot were used to assess the Dio1 mRNA expression and protein expression, respectively.

Dio1 mRNA expression was detected by qPCR as follows: (1) Total RNA was extracted from the rats' liver samples using TRIzol (Invitrogen Life Technology, CA, USA). The quantity and quality of RNA were verified by NanoDrop 2000 (Thermo Scientific, MA, USA). (2) 1  $\mu$ g of extracted RNA was used to synthesize cDNA using ReverTra Ace® qPCR RT Master Mix (TOYOBO, Osaka, Japan), and the reverse transcription reaction was carried out at 37°C for 15 min, 98°C for 5 min, and 4°C using a thermocycler. (3) Quantitative PCR reaction was performed using SYBR® Green Real-time PCR Master Mix (TOYOBO, Osaka, Japan), sense primer, antisense primer, template cDNA, and PCR grade water. The reaction conditions were predenaturing condition of 95°C for 2 min, 95°C reaction for 15 s, and 58°C annealing and extension for 30 s with a total of 40 cycles. The primers are showed in Table 1. (4) Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal standard, and all results were normalized to GAPDH, and relative mRNA expression was calculated by the 2<sup>-ΔΔCt</sup> method [30].

Dio1 protein expression was detected by western blot analysis as follows: The rat liver tissue was washed twice with ice-cold PBS to remove the blood stain, and 20 mg of the tissue was lysed using RIPA lysis buffer (Servicebio, Wuhan, China) supplemented with 1 mM of PMSF (Servicebio, Wuhan, China) to isolate the total proteins. Protein concentration was determined using a BCA kit (Servicebio, Wuhan, China). According to the total protein concentration, protein samples (50  $\mu$ g) were mixed with the loading buffer and denatured at 100°C for 5 min and then were separated by SDS-PAGE (Servicebio, Wuhan, China) and transferred onto

a PVDF membrane at 220 mA for 60 min. Membranes were blocked with 5% skimmed milk for 1 hour, then incubated with Dio1 antibody (1:500 dilutions, Proteintech, Wuhan, China) and internal loading control  $\beta$ -actin (1:5000 dilutions, Servicebio, Wuhan, China) at 4°C overnight. After washing with TBST for 3 times, incubation followed with secondary antibody HRP labelled goat anti-rabbit IgG (1:5000 dilutions, Multisciences, Hangzhou, China) at room temperature for 30 minutes. After chemiluminescence (Servicebio, Wuhan, China), the gel imaging films were scanned, and the Alpha Image Software (Alpha Innotech Co., CA, USA) was used to analyze the optical density of the target bands.

**2.7. Measurement of Serum Thyroid Hormones.** TSH was measured by the ELISA assay. Both free T<sub>4</sub> (FT<sub>4</sub>) and free T<sub>3</sub> (FT<sub>3</sub>) were measured by chemiluminescence assay technique.

For the measurement of TSH, the Rat TSH ELISA Kit (Elabscience, Wuhan, China) was applied, which adopts the Sandwich-ELISA principle and contains the micro-ELISA plate precoated with an antibody specific to rat TSH. The detailed procedure is as follows: (1) Add 100  $\mu$ L standard working solution or rat serum sample to each micro-ELISA plate well and then incubate for 90 min at 37°C. (2) Remove the liquid out of each well, immediately add 100  $\mu$ L working solution of biotinylated detection antibody specific for rat TSH to each well, mix gently, and then incubate for 1 hour at 37°C. (3) Aspirate the solution from each well, add 350  $\mu$ L wash buffer to each well, and soak for 1~2 min, then aspirate the solution from each well and pat it dry against clean absorbent paper. Repeat this step 3 times. (4) Add 100  $\mu$ L of HRP conjugate working solution to each well and incubate for 30 min at 37°C. (5) Aspirate the solution from each well and repeat the wash process for 5 times as conducted in step (3). (6) Add 90  $\mu$ L of the substrate reagent to each well, incubate for about 15 min at 37°C, and protect the plate from light. (7) Add 50  $\mu$ L of stop solution to each well and determine the optical density (OD) of each well at once with a microplate reader set to 450 nm. The OD value is proportional to the concentration of rat TSH. And the concentration of rat TSH in the serum samples was calculated by comparing the OD values of samples to the standard curve.

Serum FT<sub>4</sub> and FT<sub>3</sub> were measured as follows: Put the test tubes containing serum samples to the sample placement position of the full-automated Mindray CL-2000i system. A biotin-streptavidin competitive chemiluminescence immunoassay was adopted in the system for the measurement of FT<sub>4</sub> and FT<sub>3</sub>, with the apparatus and reagents used in this assay coming from Mindray (Mindray, Shenzhen, China).

**2.8. Statistical Analysis.** Data were expressed as mean values  $\pm$  standard deviations. Statistical analyses were performed with SPSS statistical software (version 18.0 for Windows, IBM, USA). Data of groups were compared using a one-way analysis of variance (ANOVA) test or Wilcoxon rank sum test. The significance level of  $P < 0.05$  was considered to indicate statistical significance.

### 3. Results

#### 3.1. Systolic Blood Pressure and Urine Protein in Each Group.

Changes in systolic blood pressure and urine protein were measured on day 20 of pregnancy (Table 2).

PE rats were determined by an extremely significantly higher systolic blood pressure and urine protein compared to those in NOP rats ( $P < 0.01$ ), while systolic blood pressure and urine protein from PEAmI rats were significantly ameliorated compared with PE ( $P < 0.01$ ) and returned towards normal levels.

#### 3.2. Effect of Preeclampsia on Ultrastructure of Thyroid Gland.

The thyroid follicular cells of NOP were observed under an electron microscope. The nucleus was oval, and the chromatin was evenly distributed in the nucleus. Abundant rough endoplasmic reticulum, mitochondrion, ribosome, colloidal granule, lysosome, and so on can be seen in the cytoplasm with a clear structure and tight junctions. A few microvilli were neatly arranged on the cell surface (Figures 1(a) and 1(b)).

The thyroid follicular cells of PE were observed under an electron microscope. The nucleus was irregular, and the chromatin was aggregated in the nucleus. Many markedly dilated rough endoplasmic reticula and some swollen, cristae broken mitochondria were showed in the cytoplasm. Lysosome decreased. Ribosome, colloidal granules, and so on were also noticed. Large vacuoles and short, blunt, sparse microvilli were depicted in some cells (Figures 1(c) and 1(d)).

The thyroid follicular cells of PEAmI were observed under an electron microscope. The nucleus was oval, and the chromatin in the nucleus was mildly aggregated. Slightly expanded rough endoplasmic reticulum and slightly swollen mitochondria were observed in the cytoplasm. Lysosomes and ribosomes with intact structures were also revealed in the cytoplasm. A few microvilli with well-developed borders were exhibited on the cell surface (Figures 1(e) and 1(f)).

#### 3.3. Effect of Preeclampsia on Dio1 Expression in the Liver.

Changes in hepatic Dio1 mRNA expression were analyzed (Figure 2(a)). The obtained results indicated that Dio1 mRNA expression levels were significantly decreased by 49.1% in PE rats than that in NOP rats ( $P < 0.01$ ), while PEAmI rats did increase the Dio1 mRNA expression level by 62.1% relative to that of PE animals ( $P < 0.01$ ).

Changes in hepatic Dio1 protein expression were detected (Figures 2(b) and 2(c)). Consistent with the decrease of Dio1 mRNA expression, the expression of the encoding protein Dio1 in rats' livers was significantly downregulated after L-NAME exposure (PE) compared with NOP rats ( $P < 0.01$ ): grey value analysis showed that in NOP, the pro-

TABLE 2: Systolic blood pressure (SBP) and urine protein.

| Parameters             | NOP           | PE                  | PEAmI                      |
|------------------------|---------------|---------------------|----------------------------|
| SBP (mm Hg)            | $89 \pm 11.6$ | $143 \pm 14.8^{**}$ | $97 \pm 6.3^{\#}$          |
| Urine protein (grades) | Negative      | + to ++++**         | Negative to + <sup>#</sup> |

Data are mean values and standard deviations. \*\* vs. NOP,  $P < 0.01$ ; <sup>#</sup> vs. PE,  $P < 0.01$ .  $n = 10$  per group.

tein Dio1 was  $0.65 \pm 0.19$ -fold of reference protein ACTIN, while in PE, the ratio of Dio1/ACTIN was  $0.34 \pm 0.13$ , indicating that Dio1 protein expression reduced by 47.7%. Compared with the PE, Dio1 in PEAmI rats were significantly elevated by 32.1% ( $P < 0.05$ ), which was  $0.5 \pm 0.19$ -fold of reference protein ACTIN.

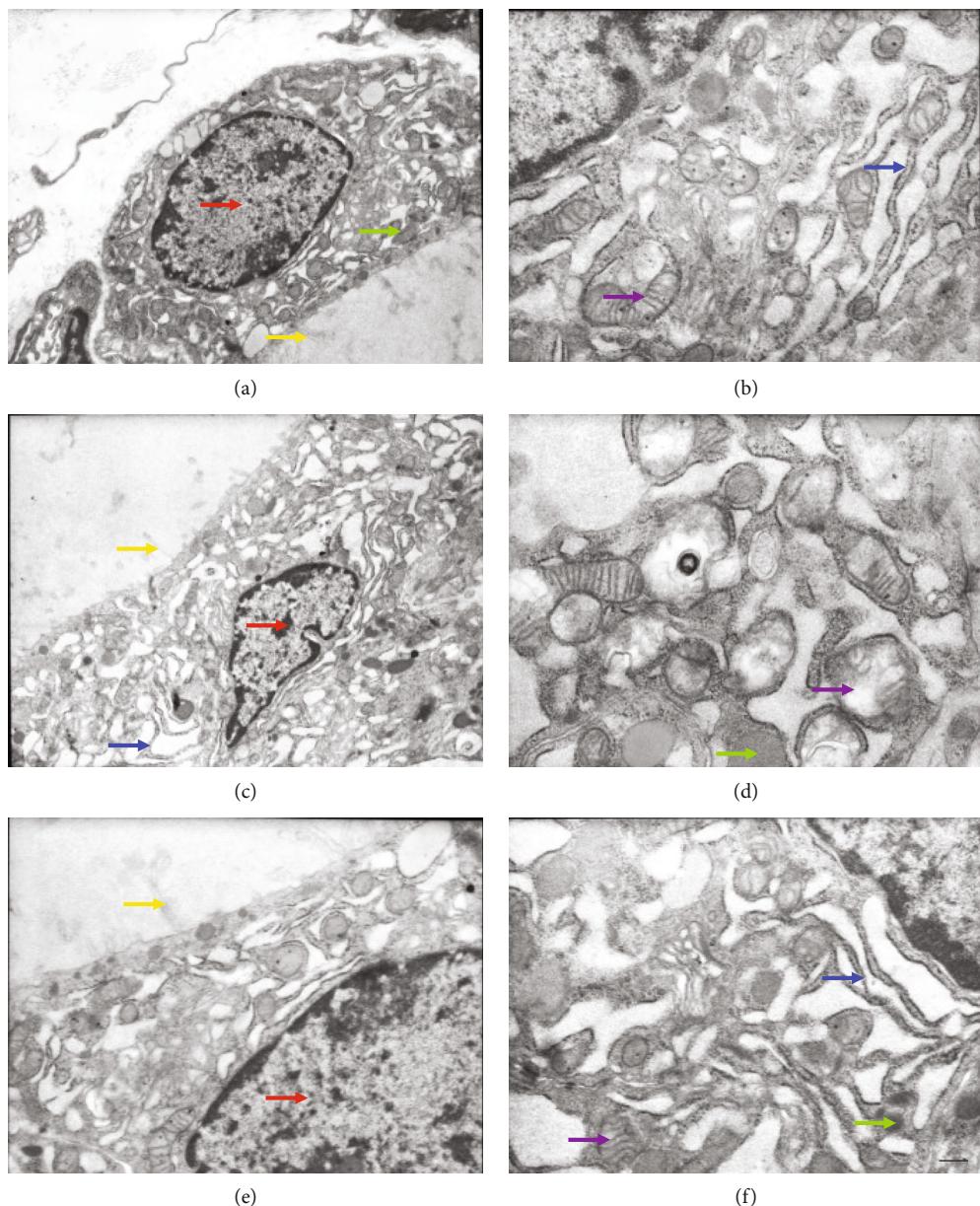
#### 3.4. Effect of Preeclampsia on Serum Levels of Thyroid Hormones.

Changes in TSH, FT<sub>4</sub>, and FT<sub>3</sub> were measured, and no significant difference between groups was observed ( $P > 0.05$ ) (Table 3).

### 4. Discussion

Many previous clinical studies have demonstrated that preeclampsia can result in thyroid dysfunction in pregnant women and had a significant adverse impact on maternal and fetal outcomes [31]. However, to the best of our knowledge, the specific mechanism of the effect of preeclampsia on hypothyroidism still needs to be elucidated. In this study, the significantly increased systolic blood pressure and urine protein in PE group rats induced by L-NAME are in accordance with the preeclamptic symptoms in the clinic [28]. According to the successful established animal model of preeclampsia, we investigate the underlying mechanism of the effect of preeclampsia on thyroid function.

Thyroid follicular cells are important functional units of the thyroid gland and the place of thyroid hormone synthesis and secretion; the stability of its structure directly affects the ability of thyroid hormone synthesis and secretion [32, 33]. We, therefore, investigated the ultrastructure changes in thyroid follicular cells under the preeclamptic status. By using a transmission electron microscope, findings showed that preeclampsia may damage the nuclei and various organelles. As the preeclamptic syndrome occurred, the nucleus of follicular cells started to be irregular and dilation of rough endoplasmic reticulum appeared; therefore, the synthesis of thyroglobulin (Tg) is reduced. And it is acknowledged that thyroid hormones were formed by iodinating and coupling the tyrosyl residues of Tg mediated by thyroid peroxidase [21]. Moreover, swollen mitochondria can reduce ATP production by seriously affecting the ability of mitochondrial respiration and oxidative phosphorylation [34]. Microvilli are not only the place where oxidization of iodide occurs but also the main structure to assist the iodized colloidal particles in the follicular lumen to reabsorb into the follicular epithelium and form the colloid vesicle (i.e., Tg); the abnormal microvilli meant the decreased function of iodination and transportation and further affect the synthesis of thyroid hormones [35, 36]. On the other side, the decreased lysosomes in the follicular epithelium can adversely affect degradation ability



**FIGURE 1:** Effect of preeclampsia on the ultrastructure of thyroid follicular cells in rats. Measured by TEM analysis. (a, b) Represent NOP group; (c, d) represent the PE group; (e, f) represent the PEAmI group, scale bar = 1  $\mu$ m. Red arrow: nucleus; blue arrow: rough endoplasmic reticulum; purple arrow: mitochondria; green arrow: lysosome; yellow arrow: microvilli.  $n = 4-6$ .

of which to the colloid vesicle, which leads to the fewer amount of  $T_3$  and  $T_4$  secreted into blood [37]. We can clearly see that these microscopic changes could result in deleterious effect on the thyroid hormone synthesis and secretion ability, and interestingly, all these ultrastructural damages were relieved after the antihypertensive treatment of preeclampsia.

$T_3$  is the bioactive form of thyroid hormone, which exerts powerful and rapid intracellular action. The metabolism of extrathyroidal  $T_3$  is critical to the homeostasis of thyroid hormones in the body's environment [38]. Approximately 80% peripheral  $T_3$  are derived from  $T_4$  outer ring deiodination [39]. Hepatic Dio1 is a major enzyme with outer ring deiodinase activities, which is essential in the tissue-specific regulation of thyroid hormone bioactivity [3]. According to this, to

fully investigate how preeclampsia disturbed thyroid function at the metabolic level, we detected the hepatic Dio1. Results showed that both mRNA expression and protein expression of Dio1 were significantly decreased under pre-eclamptic conditions in rats' livers and remarkably upregulated after treatment with the antihypertensive drug, suggesting that the downregulated Dio1 expression was caused by preeclampsia; as a result, the metabolism of thyroid hormones can be affected which may lead to decreased hepatic  $T_3$  production.

Most of the  $T_4$  and  $T_3$  are carried by thyroxine-binding globulin (TBG) in blood, resulting in less than 0.05% of them remaining free [33]. FT<sub>4</sub> and FT<sub>3</sub> are hormones that enter target cells and bind to receptors to elicit biological effect.

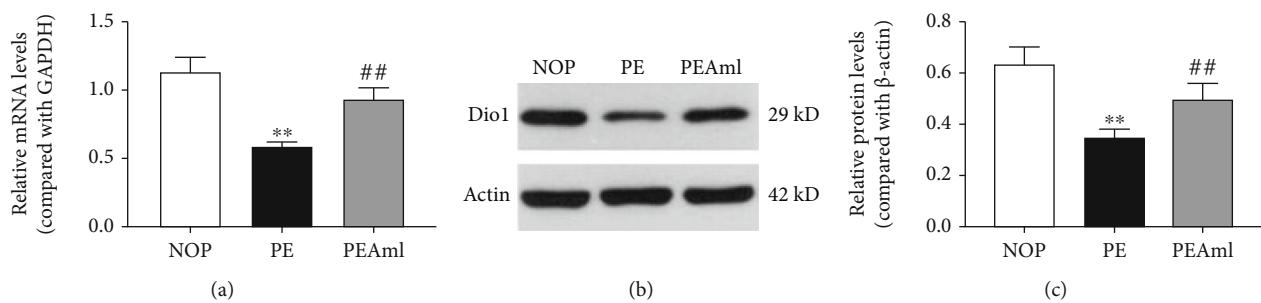


FIGURE 2: Effect of preeclampsia on mRNA and protein levels of hepatic Dio1 in rats. Measured by qPCR and western blot, respectively. (a) The mRNA expression of hepatic Dio1 in the NOP, PE, and PEAmI groups. (b) The typical protein bands of hepatic Dio1 in the NOP, PE, and PEAmI groups. (c) The protein expression of hepatic Dio1 in the NOP, PE, and PEAmI groups. \*\*vs. NOP,  $P < 0.01$ ; #vs. PE,  $P < 0.05$ ; ##vs. PE,  $P < 0.01$ .  $n = 10$  per group.

TABLE 3: Serum levels of TSH, FT<sub>4</sub>, and FT<sub>3</sub>.

| Parameters               | NOP        | PE         | PEAmI      |
|--------------------------|------------|------------|------------|
| TSH (ng/mL)              | 5.5 ± 1.1  | 5.3 ± 1.5  | 5.0 ± 2.1  |
| FT <sub>4</sub> (pmol/L) | 29.2 ± 2.0 | 29.3 ± 2.6 | 29.9 ± 2.1 |
| FT <sub>3</sub> (pmol/L) | 2.8 ± 1.9  | 2.8 ± 1.3  | 3.3 ± 1.2  |

Data are mean values and standard deviations.  $n = 10$  per group.

Moreover, in pregnant status, the TBG is stimulated by human chorionic gonadotropin (HCG), which can affect the levels of total T<sub>4</sub> and total T<sub>3</sub>; however, serum FT<sub>4</sub> and FT<sub>3</sub> are less affected [33, 40]. In addition, TSH is a hormone that can promote the release and synthesis of T<sub>4</sub> and T<sub>3</sub>, also a key role in the thyroid function negative-feedback loop [41]. Thus, it was necessary for us to measure serum FT<sub>4</sub>, FT<sub>3</sub>, and TSH, which were valuable indicators with high sensitivity for reflecting overall thyroid function [42]. It was observed that serum TSH, FT<sub>4</sub>, and FT<sub>3</sub> levels had no significant changes in preeclamptic rats. We speculated that the reason why these changes were not statistically significant may involve TBG, a combined protein produced in the liver and having high affinity with thyroid hormones [43]: firstly, the gestational period of rats is short, only about 21 days [44], and the modelling was started on day 13 of pregnancy. It was reported that TBG had a half-life of more than 5 days [45]. Therefore, the remaining uncleared circulating TBG still continues to produce free thyroid hormones in the late stage of modelling. In addition, preeclampsia could deleteriously affect the liver [46]; consequently, both the liver TBG production and binding ability of TBG might be interfered [47], therefore raising the fraction of free thyroid hormones in serum, thus, overall, counteracting the decrease of the thyroid hormone caused by the impairment of thyroid synthesis, secretion, and metabolic transformation ability in the preeclamptic model. Unfortunately, we did not evaluate TBG to confirm this hypothesis.

To objectively assess the effect of preeclampsia on thyroid function, we especially designed the PEAmI group, and amlo-dipine besylate (Aml), as an antihypertensive drug, was used to treat preeclampsia in this group. Results showed that compared with the PE group, the preeclamptic symptoms, the thyroid ultrastructure damage, and the decreased Dio1 expression were ameliorated in the PEAmI group. Aml has

already demonstrated that the therapeutic efficacy of which was exerted through potentiating vascular endothelial growth factor (VEGF) signaling pathways [48–50]. The main function of VEGF is to regulate vascular tone, trigger angiogenesis, accelerate proliferation, and repair epithelial cells [51], consequently playing an important role in regulating and maintaining vasculature in normal organs [52]. Previous studies have demonstrated that the occurrence of preeclampsia was tightly linked with the decreased bioavailability of VEGF [53–55]. Moreover, results of our study and others [56, 57] have also showed that the preeclamptic symptom could be relieved after the treatment of Aml. Additionally, in the vascular endothelium of the thyroid gland and liver, VEGF has been found to be highly expressed; the suppression of VEGF cellular signaling pathways can result in vascular disturbances and even regression in these organs [58–60]. We therefore speculate that preeclampsia could develop blood vessel injury of the thyroid gland and liver via disturbing VEGF signaling pathways, leading to impairment of the thyroid ultrastructure and expression of hepatic Dio1, further interfering the synthesis, secretion, and metabolism of thyroid hormones; the results of the PEAmI group prove this speculation, which reminds us that special attention should be paid to the protection of the thyroid gland and liver in preeclamptic women, to avoid the onset of hypothyroidism. However, more detailed analysis should be involved to identify the exact role of VEGF during the pathologic process of hypothyroidism caused by preeclampsia.

## 5. Conclusions

Taken together, by investigating the multiple aspects of thyroid functional status in L-NNAME-induced preeclamptic rats, our results demonstrated for the first time that the ultrastructure of thyroid follicular cell and hepatic Dio1 expression could be significantly impaired by preeclampsia, and all the damages were ameliorated after antihypertensive therapy, which provides the reliable evidence for understanding the underlying mechanism of hypothyroidism in preeclamptic condition.

## Data Availability

The initial data used to support the findings of this study are available from the corresponding author upon request.

## Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

## Authors' Contributions

Yunlu Liu and Zhuping Xu contributed equally to this work.

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