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# Roles and potential mechanisms of selenium in countering thyrotoxicity of DEHP

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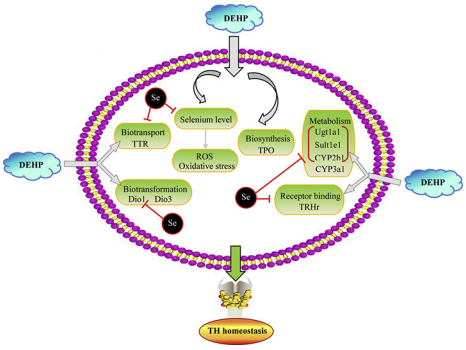
## HIGHLIGHTS

- Thyroid hormones reduced by DEHP were elevated after selenium supplementation.
- Selenium countered thyrotoxicity of DEHP via recovering the redox equilibrium.
- Selenium countered thyrotoxicity of DEHP via upregulating Dio1 and TTR levels.
- Selenium countered thyrotoxicity of DEHP via downregulating TRHr and hepatic enzymes.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Di-(2-ethylhexyl) phthalate (DEHP) as a ubiquitous environmental contaminant could disturb thyroid hormone (TH) homeostasis. Selenium as an essential trace element has protective effects on thyroids. To verify roles of selenium in countering thyrotoxicity of DEHP and elucidate potential mechanisms, Sprague-Dawley rats and Nthy-ori 3-1 cells were treated with DEHP or/and selenomethionine (SeMet). Results showed that selenium supplementation elevated plasma free thyroxine (FT4) that was decreased by DEHP, and free triiodothyronine (FT3) and thyroid stimulating hormone (TSH) levels were also partially recovered. DEHP-caused histopathologic changes were ameliorated after selenium supplementation, as indicated by recovered thyroid follicular epithelial cell numbers and cavity diameters. DEHP disrupted the redox equilibrium, causing depletions of SOD, GPx1, GPx3, and TxnRd, and accumulations of MDA. Nevertheless, selenium supplementation effectively improved the redox status. DEHP affected biosynthesis, biotransformation, biotransport, and metabolism of THs, as well as thyrotropin releasing hormone receptor (TRHr) levels. Plasma selenium, thyroid peroxidase (TPO), deiodinase 1 (Dio1), and transthyretin (TTR) were downregulated, while Dio3, Ugt1a1, Sult1e1, CYP2b1, CYP3a1, and TRHr were upregulated by DEHP. However, selenium supplementation led to elevations of selenium, Dio1 and TTR, and reductions of Ugt1a1, Sult1e1, CYP2b1, and TRHr. TPO, Dio3, and CYP3a1 were not significantly affected by selenium supplementation. Taken together, selenium could ameliorate DEHP-caused TH dyshomeostasis via modulations of the redox status, Dio1, TTR, TRHr, and hepatic enzymes.



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## 1. Introduction

Di-(2-ethylhexyl) phthalate (DEHP) is the most common phthalate and extensively used in various products such as flexible plastic, toys, medical devices, and personal care products. As the non-covalent binding to the plastic matrix, DEHP can be easily released from substrates into the environment. As a ubiquitous environmental contaminant and an endocrine disruptor, DEHP has been paid great concern for its potential toxic effects such as reproductive toxicity (Ha et al., 2016a), developmental toxicity (Lyche et al., 2009), hepatotoxicity (Ha et al., 2016b), and carcinogenicity (Chang et al., 2017). In addition, some studies and our previous work have found that DEHP has thyroid-disrupting effects on humans and animals, suggesting that the thyroid is vulnerable to DEHP (Kim et al., 2017; Liu et al., 2015; Ye et al., 2017).

Thyroid hormones (THs) that are synthesized and released by thyroids play vital roles in multiple physiological processes such as energy homeostasis, metabolism, and brain development. TH dyshomeostasis would lead to a variety of adverse subclinical or clinical conditions. Therefore, the mechanism by which DEHP disturbs TH homeostasis has been the subject of intensive research. The study observed that DEHP exposure could affect the iodide uptake of thyroid follicular cells via changing the transcriptional activity of the sodium/iodide symporter (NIS) (Wenzel et al., 2005). Another study reported that MEHP (the main metabolite of DEHP) decreased TT3 and TT4 levels though inducing gene expressions of Dio1, Dio2, NIS, thyroglobulin (Tg), and Ugt1ab in zebrafish embryos (Zhai et al., 2014). Another mechanism proposed is associated with TH related receptors. Phthalates could bind to hormonal receptors (TRα and TRβ) to disturb the signaling of THs and then affect TH production (Zoeller, 2005). Our recent study observed similar results and indicated that DEHP could cause hypothyroxinemia via activating the Ras/Akt/TRHr pathway and inducing hepatic enzymes such as Ugt1a1, CYP2b1, and Sult1e1 (Ye et al., 2017). In addition, some studies including our previous work suggested that the competitive suppression of TTR by DEHP should be considered as a potential mode of action to lead to the decline of THs (Ishihara et al., 2003; Liu et al., 2015).

As relatively more attention has been paid to the disrupting effect of DEHP on thyroid functions and TH homeostasis, some potential mechanisms have been gradually proposed. However, studies on how to effectively ameliorate or antagonize thyrotoxicity of DEHP are still limited. Though the study by Erkekoglu et al. (2012) mentioned the potential protective effect of selenium against DEHP-caused disturbance of TH homeostasis, the exact mechanisms are still unclear.

Selenium is an essential trace element of fundamental biological importance. The thyroid has the highest selenium content that is covalently incorporated into several selenoproteins such as families of GPx, thioredoxin reductases (TxnRd), and Dios, which contribute to TH biosynthesis, antioxidative defense, and redox control of thyrocytes as well as to TH metabolism. Epidemiological studies have also demonstrated that adequate selenium supplementation is beneficial for thyroid functions, can prevent the negative impact of excess iodide load, and may be preventive for thyroid cancer (Köhrle and Gärtner, 2009). In women, an inverse association between selenium status and thyroid volume was observed, indicating the protective effect of selenium against goiter and thyroid tissue damages (Derumeaux et al., 2003).

Therefore, given our previous studies and important roles of selenium in thyroid physiology, we hypothesized that selenium could effectively ameliorate thyrotoxicity of DEHP via improving TH biosynthesis, biotransformation, biotransport, and metabolism. To validate the hypothesis, SD rats and Nthy-ori 3-1 cells (human thyroid follicular epithelial cell line) were used in the current study. Our present findings indicate that selenium supplementation could partially but significantly ameliorate DEHP-caused TH dyshomeostasis through modulations of the redox status, Dio1, TTR, TRHr, and hepatic enzymes.

## 2. Materials and methods

### 2.1. Animals and treatments

Thirty-two male Sprague-Dawley rats purchased from Daping Hospital Animal Laboratory (Chongqing, China) were divided randomly into four groups ($n = 8$/dose group) by counterbalancing the body weight. The four groups were as follows: (I) Control (corn oil), (II) DEHP treatment, (III) Selenomethionine (SeMet) treatment, and (IV) Co-treatment with DEHP and SeMet. The corn oil, DEHP, and SeMet were all purchased from Sigma-Aldrich, USA. Animals were housed in plastic cages that were placed in a room with a controlled ambient temperature (22 ± 1 °C), a relative humidity (50 ± 5%), and a 12-h light-dark cycle. Feed and drinking water were supplied ad libitum. Animals were dosed by gavage for 31 consecutive days. Doses used of DEHP and SeMet were 500 mg/kg/day and 1 mg/kg/day, respectively. Experimental protocols used, including the age of rats, duration, and route of exposure were chosen according to the recommendations of the US EPA Endocrine Disrupter Screening and Testing Advisory Committee. All animal procedures were approved by the Research Ethics Committee of Chongqing Population and Family Planning Science and Technology Research Institute.

### 2.2. Cells and treatments

The human thyroid follicular epithelial cell line (Nthy-ori 3-1) was purchased from the European Collection of Cell Cultures (Salisbury, UK). Cells were cultured in RPMI 1640 medium with 10% fetal bovine serum in a humidified incubator (37 °C, 5% $CO_2$). DEHP was prepared in DMSO and diluted to 200 μM. SeMet and sodium selenite (SS; Sigma-Aldrich, USA) were dissolved in sterile water and diluted to 10 μM and 1 μM, respectively.

### 2.3. Cell viability assay

Nthy-ori 3-1 cells were treated with DEHP with/without SeMet or SS in 96-well plates for 24 h, followed by incubation with 10 μl of Cell Counting Kit-8 solution (Dojindo Laboratories, Japan) at 37 °C for 1 h. The absorbance was determined at 450 nm. Cell viability was compared with the vehicle control.

### 2.4. Hormone assay

FT3, FT4, TSH, and TRH in rat plasma were determined using enzyme-linked immunoabsorbent assay kits (Assay Designs, Inc., USA) following manufacturer's instructions. No significant cross-reactivity or interference was observed. Each sample was measured in duplicate.

### 2.5. Histopathologic evaluation

Histopathologic changes of rat thyroids were detected by hematoxylin and eosin (HE) staining analysis and transmission electron microscope (TEM) analysis. Briefly, thyroids were fixed in 4% paraformaldehyde for 24 h. Fixed thyroids were embedded, sliced and stained with HE. Then, histological alterations including numbers of follicular epithelial cells and diameters of thyroid follicular cavities were quantitatively assessed using the image analysis software, Image-Pro Plus 6.0. Ultrastructural changes in thyroids were assessed by TEM. Thyroids were diced after treatment in 2.5% glutaraldehyde at 4 °C for 24 h. Then, slices of thyroids were postfixed in 1% osmium tetroxide. FEI Tecnai 12G2 transmission electron microscope was used to examine sections.

### 2.6. Selenium determination

Plasma samples for ICP/MS analysis were collected in trace element-free polyethylene specimen tubes. Plasma selenium was determined