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Gestational di-(2-ethylhexyl) phthalate exposure causes fetal intrauterine growth restriction through disturbing placental thyroid hormone receptor signaling

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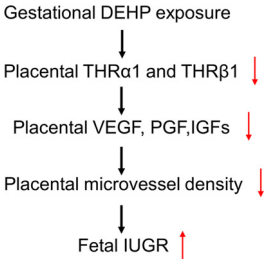
GRAPHICAL ABSTRACT



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ABSTRACT

Previous study reported that gestational Di-(2-ethylhexyl) phthalate (DEHP) exposure caused fetal intrauterine growth restriction (IUGR). We aimed to investigate the role of placental thyroid hormone receptor (THR) signaling in DEHP-induced IUGR. Dams were treated with DEHP (50 or 200 mg/kg) by gavage daily throughout pregnancy. As expected, gestational DEHP exposure dose-dependently caused fetal IUGR. The mRNA levels of placental *Thrα1* and *Thrβ1* were reduced and nuclear translocation of placental THRα1 and THRβ1 were suppressed in DEHP-exposed mice even though thyroid hormones in maternal and fetal sera were unaffected. Correspondingly, *Vegf*, *Pgf*, *Igf1* and *Igf2*, several THR downstream genes essential for placental angiogenesis, were down-regulated in placenta of DEHP-exposed mice. Histopathology showed that vascular space in the labyrinthine region was shrunken in placenta of DEHP-treated mice. The microvessel density in labyrinthine region was reduced in DEHP-treated mice. A nested case-control study based on MABC suggested that microvessel density was decreased in placenta of SGA cases. Moreover, protein abundance of placental THRα1 and THRβ1 were lower in SGA cases. In conclusion, gestational DEHP exposure increases fetal IUGR incidence

Abbreviations: DEHP, di-(2-ethylhexyl) phthalate; IUGR, intrauterine growth restriction; THR, thyroid hormone receptor; VEGF, vascular endothelial growth factor; PGF, placenta growth factor; IGF, insulin-like growth factor; PAEs, phthalate diesters; TDS, testicular dysgenesis syndrome; LBW, low birth weight; CD34, cluster designation 34; GD, gestation day; SGA, small for gestational age; AGA, appropriate for gestational age; H&E, hematoxylin and eosin; TT_3, total triiodothyronine; TT_4, total thyroxine; FATP, fatty acid transporter; GLUT, glucose transporter

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through disturbing placental THR signaling. The present study, at least partially, elucidate the underlying mechanism of DEHP-induced fetal IUGR.

1. Introduction

Phthalate diesters (PAEs), known as plasticizers imparting softness and flexibility to plastics made of PVC, are extensively used worldwide. DEHP is the most commonly used PAEs, which accounts for 50% of the annual gross product (Singh and Li, 2012). Varieties of products contain PAEs, ranging from structural adhesives, pesticides, pharmaceuticals, personal care products, food packaging to even children's toys (Romero-Franco et al., 2011; Seckin et al., 2009). Because PAEs are lipophilic and not stably attached to the plastic matrix, they can easily release into surrounding environment (Silva et al., 2004), and come into human body through skin (Janjua et al., 2008), digestion (Frederiksen et al., 2007; Wormuth et al., 2006), and respiration (Heudorf et al., 2007; Huang et al., 2011). Recent years, the use of DEHP increased rapidly in Asian countries, especially China (Chao et al., 2015). High concentrations of PAEs were detected in soils and vegetables samples in East China (Ma et al., 2015). PAEs are environmental endocrine disruptors and disturb reproductive function of males. Results from experimental study have demonstrated that DEHP exposure disturbed the hypothalamic-pituitary-testis axis, induced the reduction of serum testosterone and decreased the sex organs' weight and coefficient (Ha et al., 2016). Epidemiological studies investigated the impact of PAEs exposure on male reproductive system. The decreased sperm concentration and motility and increased sperm DNA fragmentation index were reported in men with a professional exposure to PAEs (Huang et al., 2011, 2014). Surprisingly, concentrations of PAEs metabolites in urine were linked to the decreased serum testosterone level and sperm quality in entire men population (Wang et al., 2015, 2016).

Currently, PAEs have attracted more attentions for developmental toxicities. Researches on rodents suggested that gestational PAEs exposures detrimentally affected reproductive development in males with the reduced anogenital distance and abnormal Leydig cell aggregation in developmental testis, just like testicular dysgenesis syndrome (TDS) in humans (Aydogan Ahbab and Barlas et al., 2015; Li et al., 2016; Veeramachaneni and Klinefelter, 2014). Further studies found that gestational PAEs exposure impaired neurodevelopment in rodent animals (Smith and Holahan, 2014; Xu et al., 2015). In previous study, we found that gestational DEHP exposure caused fetal growth restriction in mice (Shen et al., 2017), but the underlying mechanism how gestational DEHP exposure induces fetal IUGR is not yet elucidated.

Thyroid hormones are crucial for placental and fetal development, but thyroid dysfunction is common among pregnant women with a high prevalence worldwide (Krassas et al., 2010). Many contemporary studies have demonstrated that maternal hypothyroidism caused fetal IUGR, low birth weight (LBW) and preterm births (Aggarawal et al., 2014; Saki et al., 2014). Interestingly, similar adverse birth outcomes were found among pregnant women with hyperthyroidism (Chen et al., 2014; Saki et al., 2014). Thyroid hormone actions are mainly mediated by THRs encoded by the THRA and THRB genes (Brent, 2012; Ortiga-Carvalho et al., 2014). Rodent and human placenta highly express THRs and the expression of THRs increase with gestational age (Chan et al., 2004; Leonard et al., 2001). However, whether gestational DEHP exposure disturbs placental THR signaling is still unknown.

Gestational DEHP exposure caused fetal IUGR had been proved. The present study was designed to examine whether gestational DEHP exposure disturbs placental THR signaling. Our findings showed that gestational DEHP exposure not only down-regulated the expression of placental *Thrα1* and *Thrβ1* but also repressed nuclear translocation of placental THRα1 and THRβ1.

Table 1
The sequence and length of gene-specific primers.

Genes	Sequence	Length
18S	Forward:5'-GTAACCCGTTGAAGGGATT-3' Reverse:5'-CCATCCAATCGGTAGTAGAG-3	151
THRα1	Forward:5'-GACAAGGCCACCGGTTATCA-3' Reverse:5'-CTTGTCGATGACACAGCAGC-3'	132
THRβ1	Forward:5'-CTGATCCGTGTTTTCCCTCTC-3' Reverse:5'-TCTGTACTGGCATTCCCTCTG-3'	101
Pgf	Forward:5'- ACTTGGGAACACAAGAAGCCT-3' Reverse:5'- CGACCCCACACTTCGTTGAA-3'	131
Vegf	Forward:5'-TATTCAGCGGACTCACCAGC-3' Reverse:5'-AACCAACCTCCTCAAACCGT-3	156
Vegfr	Forward:5'-TCAAGCTAGAGGTGTCCCCG-3' Reverse:5'-CTCGGCACCTATAGACACCC-3'	132
Igf1	Forward:5'- AAGGCAGTTTACCCAGGCTC-3' Reverse:5'- GGCCGAGGTGAACACAAAAC-3'	125
Igf2	Forward:5'- CTTCAGCAGCGTCCACTTCA-3' Reverse:5'- TTGGTACCACAAGGCCGAAG-3'	105
Igfr1	Forward:5'- CCAAGCTCACCGTCATCACT-3' Reverse:5'- GAAGAGTTTCCAGCCACGGA-3'	110
Glut1	Forward:5'-ACCATCTTGGAGCTGTTCCG-3' Reverse:5'-GCCTTCTCGAAGATGCTCGT-3'	131
Fatp1	Forward:5'-CGCCGATGTGCTCTATGACT-3' Reverse:5'-ACACAGTCATCCCAGAAGCG-3'	138
Fatp4	Forward:5'-GGCTCAGGGGCCAATAAACT-3' Reverse:5'-TCCCAAGGGCTAAGCGAAAG-3'	102

2. Materials & methods

2.1. Chemicals and reagents

Di-(2-ethylhexyl) phthalate (DEHP) was provided by Sigma Chemical Co. Anti-THR α1, β1 and anti-cluster designation 34 (CD34) antibodies were bought from Abcam. PVDF membrane was purchased from Merck Millipore. Chemiluminescence detection kit was obtained from Pierce Biotechnology. Trizol was bought from Molecular Research Center, In. RNase-free DNase and Reverse Transcription System was supplied by Promega Corporation. All others laboratory reagents needed were bought from Sigma or as indicated in the specified methods.

2.2. Animals

Hygeian and adult ICR mice were introduced from Beijing Vital River. Animals were fed ad libitum and housed in a standard condition for two weeks before use. For copulation purpose, females were mated with males at the ratio of 2:1 starting from overnight 9:00 P.M. to next morning 7:00 A.M. All females were checked for vaginal plug and the presence was defined as gestation day (GD) 0. To investigate DEHP exposure induced fetal IUGR, dams were treated with DEHP (50 or 200 mg/kg) daily by gavage throughout pregnancy. The doses of DEHP used referred to our previous study (Shen et al., 2017). Half of dams were sacrificed on GD15 morning 9:00 A.M. All placentae were separated aseptically for real time RT-PCR and Western Blotting. Maternal sera were collected for measurement of thyroid hormones. The remaining dams were sacrificed on GD18 morning 9:00 A.M. The