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Review

Fetal origin of endocrine dysfunction in the adult: The phthalate model

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ABSTRACT

Di-(2-ethylhexyl) phthalate (DEHP) is a plasticizer with endocrine disrupting properties that is found ubiquitously in the environment as well as in human amniotic fluid, umbilical cord blood, human milk, semen, and saliva. It is used in the industry to add flexibility to polyvinyl chloride-derived plastics and its wide spread use and presence has resulted in constant human exposure through fetal development and postnatal life. Epidemiological studies have suggested an association between phthalate exposures and human reproductive effects in infant and adult populations. The effects of fetal exposure to phthalates on the male reproductive system were unequivocally shown on animal models, principally rodents, in which short term deleterious reproductive effects are well established. By contrast, information on the long term effects of DEHP *in utero* exposure on gonadal function are scarce, while its potential effects on other organs are just starting to emerge. The present review focuses on these novel findings, which suggest that DEHP exerts more complex and broader disruptive effects on the endocrine system and metabolism than previously thought.

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Contents

Abbreviations: 22R-OHC, 22R-hydroxycholesterol; ACTH, adrenocorticotropic hormone; ALC, adult Leydig cells; ATIIR, angiotensin receptor; DEHP, di-(2-ethylhexyl) phthalate; DHEA, dehydroepiandrosterone; DHEAS, sulfated dehydroepiandrosterone; EHXA, 2-ethylhexanoic acid; FSH, follicle-stimulating hormone; GD, gestational day; GnRH, gonadotropin-releasing hormone; LH, luteinizing hormone; MEHP, mono(2-ethylhexyl) phthalate; MIS, Müllerian inhibitory substance; MR, mineralocorticoid receptor; MSRF, methylation-sensitive restriction finger printing; PND, postnatal day; PPAR, peroxisome proliferator activated receptor; PVC, polyvinyl chloride; ROS, reactive oxygen species; SRY, sex-determining region of the Y chromosome; STAR, steroidogenic acute regulatory protein; T3, triiodothyronine; T4, thyroxine; TDS, testicular dysgenesis syndrome; TSH, thyroid stimulating hormone; TSPO, translocator protein (18-kDa); ZG, zona glomerulosa; ZF, zona fasciculata; ZR, zona reticularis.

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1. Introduction

Phthalate esters are used in industry as plasticizers to add softness and flexibility to polyvinyl chloride (PVC) plastics. Phthalate-free PVC is white and rigid, but with the addition of phthalates it can be molded and used for a wide variety of products, including personal care products, toys, food wrappings, medical devices, lubricants, waxes, insecticides, and building and household products among many others [1,2]. Interestingly, medical devices have been identified as a potential risk to young children due to their high content of phthalates, and account for some of the highest exposures [3,4]. Studies measuring phthalate exposure in neonatal medical intensive care units found levels as high as 50 times greater than those in children between the ages of 6 and 10 years old [5–7]. Di-(2-ethylhexyl) phthalate (DEHP) is the most commonly used phthalate in industry, with three million metric tons produced each year [8]. In some instances, DEHP can account for 40% of the total weight of the product [9]. Because DEHP is not covalently bound to the PVC polymer, it leaches into the environment and comes into contact with humans mainly through dermal exposure [10], oral ingestion [9,11], and inhalation [12,13]. In humans, maternal exposure to phthalates [14–17] provides the first source of fetal exposure, and has been identified amniotic fluid [18], umbilical cord blood [16], and other bodily fluids [19]. This exposure to phthalates continues after birth through breast feeding [20–22], baby and infant food sources [11,23,24], and contact to the environment [2]. These abundant man-made sources of phthalates result in human exposure estimated at 1.7–52.1 μg/kg/day [4,25–27], and under specific circumstances, infants can be exposed to up to 3 times as much [1]. This is a public health concern since exposure to DEHP in rodents was found to be an endocrine disruptor, and in humans there are correlations between phthalate exposure and decreased anogenital distance [17], reduced testosterone levels [28], and poor semen quality [29–31]. These clinical findings, together with cryptorchidism, hypospadias, and testicular cancer [32], were grouped under the term testicular dysgenesis syndrome (TDS), which is thought to originate from an insult to Sertoli or Leydig cell function during development [33,34]. This has led to a focus on the possible involvement of phthalates in TDS [35], in agreement with the reported increase in cryptorchidism, hypospadias [36–39], decreased semen quality [40–42], and increased testicular cancer [35]. In the female, DEHP also targets the reproductive system, but data on the mechanisms have lagged compared to the male. New data from studies of female offspring exposed *in utero* to DEHP is suggesting sex-specific effects of phthalates [43], and that DEHP has a multiorgan effect that depends on the time of exposure.

We will review herein the data on the long-term effects of *in utero* exposure to DEHP in the male and female offspring.

2. Steroidogenesis

Systemic steroid hormones are primarily formed by the gonads, adrenal glands, and during *in utero* development by the placenta. The brain [44–46] and heart [47–49] have also been identified as steroid-producing tissues forming limited amounts of steroid hormones mainly for local needs.

Cholesterol is the building block of steroid hormone biosynthesis and the import of cholesterol into the mitochondria is the rate-limiting step of steroidogenesis (Fig. 1) [50,51]. This process is facilitated by the steroidogenic acute regulatory protein (STAR), a hormone-induced short lived protein which acts at the mitochondria to induce cholesterol transport. Steroidogenic cholesterol at the outer mitochondria membrane binds to the translator protein (18-kDa) TSPO part of a complex of proteins which mediate cholesterol movement to the inner mitochondrial membrane where CYP11A1 cleaves the side chain of cholesterol to form pregnenolone. Production of progesterone from pregnenolone is catalyzed by 3βHSD. Tissue specificity of steroids formed is provided by the tissue-specific expression of enzymes downstream of pregnenolone and progesterone [52]. Expression of CYP17A1 drives the production of testosterone by the Leydig cells of the testes, and in the ovary, granulosa and theca cells form estradiol from androgen. The adrenal gland is composed of three distinct layers, the outermost zona glomerulosa (ZG) which synthesizes aldosterone, the zona fasciculate (ZF) which forms cortisol, and the zona reticularis (ZR) which produces dehydroepiandrosterone (DHEA) and DHEA sulfate (DHEAS). Is important to note, that rodents do not express CYP17A1 in the adrenal gland and as a consequence corticosterone is the predominant glucocorticoid [53–55].

3. Development

3.1. Testes

During early sexual development in the mouse, male and female gonads are indistinguishable and dependent on the expression of the sex-determining region of the Y chromosome (SRY) to commit to male phenotype. The initial commitment of few embryonic stem cells to become primordial germ cells occurs similarly in both sexes around GD7 [56], followed by their migration into the genital ridge between GD9 and 10.5, where they become residents in the undifferentiated gonad [57]. SRY expression results in upregulation of SOX9, a key promoter of Sertoli cell differentiation, which drives the regression of the female Mullerian ducts by producing Mullerian inhibitory substance (MIS) [58]. Fetal Sertoli cells regulate the development of the primordial germ cells into the testis from day 11.5 to 13.5 post coitus [59], and regulate the formation of the seminiferous cords by controlling the spatial organization of