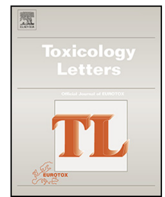
Toxicology Letters 238 (2015) 117–125

Contents lists available at ScienceDirect

Toxicology Letters

journal homepage: www.elsevier.com/locate/toxlet



# Prenatal ethanol exposure induces the osteoarthritis-like phenotype in female adult offspring rats with a post-weaning high-fat diet and its intrauterine programming mechanisms of cholesterol metabolism

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## HIGHLIGHTS

- Ethanol induced osteoarthritis-like phenotype in female adult offspring.
- Ethanol induced cholesterol accumulation in cartilage.
- Ethanol induced intrauterine programming of cartilage cholesterol metabolism.

## ARTICLE INFO

*Article history:*
Received 6 May 2015
Received in revised form 10 July 2015
Accepted 21 July 2015
Available online 26 July 2015

*Keywords:*
Ethanol
Intrauterine growth retardation
Osteoarthritis
Serum cholesterol
Intrauterine programming alteration

## ABSTRACT

Osteoarthritis (OA) development is associated with hypercholesterolemia in adults. Our previous study demonstrated that offspring with intrauterine growth retardation (IUGR) due to prenatal ethanol exposure (PEE) had a high risk of developing hypercholesterolemia and metabolic syndrome when fed a post-weaning high-fat diet (HFD). In this study, we examined the changes in articular chondrocytes of IUGR offspring induced by PEE and explored its intrauterine programming mechanisms related to cholesterol metabolism. Using the PEE/IUGR model, serum and tibias from female fetuses and adult female offspring fed a post-weaning HFD were collected and examined for cholesterol metabolism and histology. The results showed that PEE adult offspring manifested significant catch-up growth. Their serum total cholesterol (TCH) and low-density lipoprotein-cholesterol increased and high-density lipoprotein-cholesterol decreased; the osteoarthritis-like phenotype and an increased TCH content were observed in articular cartilage; and the expression of insulin-like growth factor1 (IGF1) and cholesterol efflux pathway, including ATP-binding-cassette transporter A1 and liver X receptor, was reduced. The expression of IGF1 and cholesterol efflux pathway was also lower in the PEE fetuses. This study showed PEE could induce an enhanced susceptibility to HFD-induced OA in adult female IUGR offspring. The underlying mechanism related to cholesterol accumulation in cartilage mediated by intrauterine programming.

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## 1. Introduction

Osteoarthritis (OA), the main reason for the elderly dyskinesia, is a chronic joint disease characterized by a major pathological feature of articular cartilage degeneration and osteophyte formation (Lawrence et al., 2008). Currently, the prevalence of OA increases each year. Specifically, obese individuals tend to have a high morbidity. The conventional view suggests that obesity increases the load and aggravates the degeneration of articular cartilage. However, local biomechanical factors associated with

*Abbreviations:* IUGR, intrauterine growth retardation; PEE, prenatal ethanol exposure; HFD, high-fat diet; TCH, total cholesterol; IGF1, insulin-like growth factor1.

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http://dx.doi.org/10.1016/j.toxlet.2015.07.017
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changes in the onset and progression of knee OA in the obese population cannot explain the relationship between obesity and OA in non-load bearing joints (Cicuttini et al., 1996; Grotle et al., 2008; Sayer et al., 2003). An epidemiological investigation showed that obesity is associated with a 5–8-fold increased risk of hand OA (Oliveria et al., 1999). Some researchers have proposed that obese individuals with systemic lipid metabolic disorders will have cholesterol accumulation in their cartilage because the efflux function of cartilage is impaired, which could induce OA (Al-Arfaj, 2003; Tsezou et al., 2010). These studies suggest that the occurrence of OA is closely related to chondrocyte cholesterol metabolic disorder.

Intracellular cholesterol in peripheral tissues are mainly converted through ATP-binding-cassette transporter A1 (ABCA1) to apolipoprotein A1 (ApoA1), a form of high-density lipoprotein-cholesterol (HDL-C), which then enters the blood. This process is mediated by transcriptional factor peroxisome proliferator-activated receptor (PPAR) and liver X receptor (LXR) (Zanotti et al., 2012). Some studies have found that, similar to atherosclerosis, the expression of LXR, which regulates cholesterol efflux, was significantly lower in OA chondrocytes (Gkretsi et al., 2011). Human osteoarthritic chondrocytes are often presented with intracellular lipids deposits. Cholesterol efflux gene expression (such as ABCA1, LXRα and β) was significantly lower in osteoarthritic cartilage compared to normal cartilage. Treatment of osteoarthritic chondrocytes with the LXR agonist TO-901317 significantly increased the ApoA1 and ABCA1 expression levels, as well as cholesterol efflux, and eliminated deposits in OA chondrocytes (Tsezou et al., 2010). Insulin-like growth factor 1 (IGF1) increases the efflux of cholesterol by transcriptionally activating peroxisome proliferator-activated receptor-gamma (PPARγ), and promotes the combination of intracellular cholesterol and extracellular HDL (Fitzgerald et al., 2002; Kim et al., 2010). Accordingly, the IGF1-mediated cholesterol efflux system (PPARγ/LXRα/ABCA1) may be involved in structural and functional changes of articular cartilage induced by high cholesterol levels.

Intrauterine growth retardation (IUGR) is mainly characterized by low birth weight, which refers to those with a birth weight of less than 2500 g at normal gestational age or two standard deviations less than the mean body weight of normal babies at the same gestational age (Valsamakis et al., 2006). Prenatal exposure to ethanol is a clear risk factor for IUGR (Endres et al., 2005; Simpson et al., 2005). Under condition of postnatal over-nutrition, IUGR offspring have a catch-up growth trend and an increased susceptibility to metabolic syndrome (MS), including dyslipidemia and hyperglycemia (Morrison et al., 2010; Tosh et al., 2010). Our previous studies showed that prenatal ethanol exposure (PEE) gives rise to the mice or rat fetus being over-exposed to maternal glucocorticoid (GC) and results in IUGR (Liang et al., 2011; Shen et al., 2014; Xia et al., 2014). Furthermore, the prevalence of OA was substantially higher in women than that in men (Boyan et al., 2012; Price and Herndon, 2009). As mention above, the occurrence of OA is closely related to metabolic disorders (chondrocyte cholesterol metabolic disorder) (Katz et al., 2010), and other studies and our results indicated that female rat offspring with IUGR were more susceptible to diet-induced metabolic syndrome (Carbone et al., 2012; Wang et al., 2014; Xia et al., 2014). Additionally, we recently found that adult female rat IUGR offspring by PEE have increased OA susceptibility when subjected to strenuous running (Unpublished). Therefore, in the present study, we used female rats as the study subject to illustrate the structural and functional changes of articular chondrocytes in PEE adult offspring fed a post-weaning HFD and explore its intrauterine programming mechanisms on cholesterol metabolism in local articular cartilage.

## 2. Materials and methods

### 2.1. Materials

Ethanol (analytical pure grade) was obtained from Zhen Xin Co, Ltd. (Shanghai, China). Isoflurane was purchased from Baxter Healthcare Co. (Deerfield, IL, USA). Total cholesterol (TCH), low density lipoprotein-cholesterol (LDL-C) and HDL-C assay kits were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). Trizol reagent kits were obtained from Omega Bio-Tek (Doraville, USA). GeXP multiplex gene expression analysis kits were purchased from Beckman-Coulter, Inc. (Fullerton, CA, USA). The oligonucleotide primers for rat Q-PCR genes (PAGE purification) and GeXP multiplex gene expression analysis (HPLC purification) were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). Polyclonal antibodies for collagen type II a1 (Col2a1), IGF1, PPARγ, LXR, ABCA1 and matrix metalloproteinase (MMPs) were obtained from SantaCruz Biotechnology, Inc. (Santa Cruz, CA, USA). Other chemicals and agents were of analytical grade.

### 2.2. Animals and treatment

The protocol was approved by the Committee on the Ethics of Animal Experiments of Wuhan University School of Medicine (Permit Number: 14016). Specific pathogen free (SPF) Wistar rats (female: 180–220 g, male: 260–300 g) were obtained from the Experimental Center of Hubei Medical Scientific Academy (No. 2010-2012, Hubei, China). This experiment was performed in the Center for Animal Experimentation of Wuhan University (Wuhan, China), which has been accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International).

Animals were housed (room temperature: 18–22°C; humidity: 40–60%), acclimated, and mated. Upon confirmation of mating by the appearance of sperm in a vaginal smear, the day of confirmation was considered to be gestational day (GD) 0. Pregnant rats were transferred to individual cages and randomly divided into control and PEE groups. From GD11 to GD20, the PEE group was given ethanol 4 g/kg body weight per day by oral gavage as described before (Chen and Nyomba, 2003), and the control group was given the same volume of distilled water.

For experiments with fetal rats, 8 randomly selected pregnant rats with 10–14 live fetuses from each group were anesthetized with isoflurane and euthanized on GD20. The female fetuses were quickly removed and weighed, and IUGR was diagnosed if the body weight of a fetus was two standard deviations less than the mean body weight of the fetuses in the control group (Engelbregt et al., 2001). Fetal femurs were separated under a dissecting microscope; the right femurs were collected and immediately frozen in liquid nitrogen, followed by storage at −80 °C for subsequent PCR analyses; the left knee joints were randomly selected (one per litter) and fixed in 4% paraformaldehyde for 24 h before being embedded in paraffin for further analysis.

The other pregnant rats ($n = 8$ for each group) were kept until normal delivery on GD21. On postnatal day 1 (PD1), the numbers of pups were normalized to 8 pups per litter to assure adequate and standardized nutrition until weaning (postnatal week 4, PW4). After weaning, 8 female pups from eight different pregnant rats were randomly selected for each group, and all of the female pups were fed an HFD until being sacrificed on PW24. The HFD was previously described by our laboratory and provided 18.9% of its energy content as protein, 61.7% as carbohydrate, and 19.4% as fat (Shen et al., 2014). The body weights of the offspring rats were measured weekly until PW24, and the corresponding growth rates were calculated as described previously (Shen et al., 2014). At PW24, the offspring were anesthetized with isoflurane and decapitated in a room separate from where the other animals were kept. The blood samples collected from each littermate were pooled together and centrifuged, and the sera were isolated, followed by storage at −80 °C for subsequent analyses. The right femurs were dissected

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