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Chronic exposure of mice to environmental endocrine-disrupting chemicals disturbs their energy metabolism

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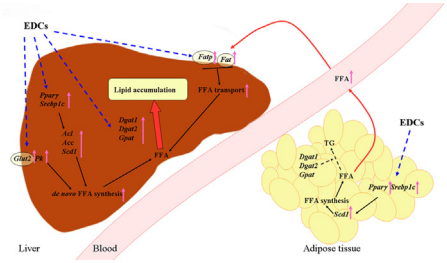
HIGHLIGHTS

- Effects of chronic EDCs exposure on energy metabolism in male mice were evaluated.
- Chronic exposure to atrazine and cypermethrin enhanced of FFA synthesis.
- The transport of FFA from blood into hepatocytes increased after chronic EDCs exposure.
- Exposure to atrazine and cypermethrin increased lipid droplets and TG levels in their livers.

GRAPHICAL ABSTRACT



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ABSTRACT

We evaluated the effects of a 20-week chronic exposure of mice to a low dose of cypermethrin (CYP), atrazine (ATZ) and 17α-ethynyestradiol (EE2) on energy metabolism. Here, male mice were exposed to 50 μg/kg BW/day CYP, 100 μg/kg BW/day ATZ or 1 μg/kg BW/day EE2 supplied in their drinking water for 20 weeks. During the exposure, mice were fed a high energy diet (HD). The bodyweights were not significantly affected by chronic exposure to EDCs, while the serum-free fatty acids (FFA) levels, hepatic lipid accumulation and triacylglycerol (TG) contents increased significantly in the ATZ- and CYP-HD groups. To determine the mechanism involved, we determined the expression levels of the genes in the glucose and fat metabolism pathways in the liver and adipose tissue. The results showed that chronic exposure to ATZ and CYP increased the mRNA levels of a number of key genes involved in both the *de novo* FFA synthesis pathway and the transport of FFA from blood. The increased amount of FFA was partially consumed as energy through β-oxidation in the mitochondria. Some of the FFA was used to synthesize TG in the liver by up-regulating primary genes, which resulted in increased TG levels and lipid accumulation. The results indicate that chronic exposure to EDCs has the potential to cause energy metabolic dysregulation and hepatotoxicity in mice.

1. Introduction

Environmental endocrine-disrupting chemicals (EDCs) include a wide range of chemicals that mainly originated from pesticides, detergents and surfactants, phthalates, alkylphenols and different types of natural or synthetic estrogens. As reported, EDCs have the potential to alter the function of the endocrine system and consequently cause adverse health effects in humans

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and wildlife (Vos et al., 2000). It is clear that EDCs exert their effects by mimicking endogenous hormones, antagonizing normal hormones, and altering the natural pattern of hormone synthesis or metabolism through the activation or repression of nuclear hormone receptors, including estrogen receptors (ERs), androgen receptors (ARs), thyroid receptors (TRs), retinoid receptors and others (Sonnenschein and Soto, 1998; Diamanti-Kandarakis et al., 2009). There is growing interest in possible EDC-induced health threats, including population declines, increased incidence of cancer, inhibition of reproductive function and disruption of the immune and nervous systems (Vos et al., 2000; Schantz and Widholm, 2001; Kortenkamp, 2007; McKinlay et al., 2008; Jin et al., 2012; Gregoraszczuk and Ptak, 2013).

In recent years, the incidence of obesity and associated metabolic syndrome diseases has increased dramatically worldwide (Guh et al., 2009; Slotkin, 2011). There is growing evidence of the increased impact of environmental pollutants on fat metabolism and obesity. For example, Miyawaki et al. (2007) reported that perinatal and postnatal exposure of mice to low doses of bisphenol A (BPA) increased the body adipose tissue mass, serum total cholesterol (TC) and triacylglycerol (TG) levels. Lee et al. (2010) reported that low doses of a number of persistent organic pollutants (POPs) may increase the risk of diabetes, possibly through endocrine disruption. Additionally, there is no doubt that some environmental EDCs function as environmental obesogens through the disruption of normal developmental and homeostatic controls of adipogenesis and energy balance (Grün and Blumberg, 2006; Chen et al., 2009). For example, BPA was found to enhance basal glucose uptake in mouse 3T3-F443A adipocytes due to increased GLUT 4 protein levels (Sakurai et al., 2004). BPA was also shown to increase the gene expression of adipogenic transcription factors in 3T3-L1 pre-adipocytes (Phrakonkham et al., 2008). Today, epidemiological investigations have revealed that EDC exposure is tightly related to bodyweight increase and obesity occurrence. Stahlhut et al. (2007) reported that the concentrations of several prevalent phthalate metabolites showed statistically significant correlations with abdominal obesity and insulin resistance in a national cross-section of men in the U.S. Wang et al. (2012) demonstrated that urine BPA levels were tightly related to the body mass index (BMI) in school children in Shanghai City, China. In sum, the large amount of different types of EDCs affect lipid metabolism and contribute to the obesity epidemic.

Cypermethrin (CYP), atrazine (ATZ) and 17α-ethynyestradiol (EE2), three different types of environmental EDCs, have been widely used as pesticides, herbicides and drugs (Van den Belt et al., 2003; Spanò et al., 2004; Nakamura et al., 2008; Victor-Costa et al., 2010; Wang et al., 2010; Jin et al., 2013). These chemicals have been reported to disturb different endpoints related to the endocrine and reproductive systems (Scholz and Gutzeit, 2000; Kirigaya et al., 2006; Wang et al., 2010; Jin et al., 2013). In the environment, the concentration of CYP in surface water can reach 2.8 μg/L (Jaensson et al., 2007). ATZ is commonly monitored in groundwater and surface water, and the concentration has reached as high as 1.6 μg/L in the Liao-He River in China (Gfrerer et al., 2002). As for EE2, the concentration in environmental aquatic systems ranges from undetected to more than 30 ng/L (Cui et al., 2006). Thus, the high levels of these chemicals present in the environment indicate that the misuses of EDCs can have effects on non-target organisms. In the last decade, significant contributions have been made to our understanding of the relationship between EDCs and health. However, there are still only limited data on the possible roles of EDCs in energy metabolism. In this study, for better understanding the risk of chronic exposure to different types of EDCs on energy metabolism in mice, the selected dose of CYP, ATZ and EE2 was a litter higher than environmental levels. We observed that the chronic exposure to CYP, ATZ or EE2 has the potential to cause energy metabolic dysregulation and hepatotoxicity in mice. These data are intended to provide new insights into the chronic toxicity of environmental EDCs in humans and wildlife.

2. Materials and methods

2.1. Chemicals

CYP (CAS No.: 52315-07-8; purity: >95%) and EE2 (CAS No: 21221-29-4, purity: ≥98%) were purchased from Sigma-Aldrich. ATZ (CAS No.: 1912-24-9; purity: >97%) was purchased from TCI. All chemicals were used as received. These chemicals were dissolved in alcohol to prepare stock solutions.

2.2. Animals and experimental design

Male 3-week-old pubertal C57BL/6J mice ($n = 45$) were purchased from the China National Laboratory Animal Resource Center (Shanghai, China). The mice were kept in our animal facilities (illuminated with strip lights, 200 lux at cage level with a photoperiod of 12 h light to 12 h dark; 22 ± 1 °C) for 1 week prior to the experiments. Water and food were available *ad libitum*. The mice were randomly divided into 5 groups ($n = 9$). Four groups were fed a high-energy diet (HD) and treated either with vehicle, 50 μg/kg BW/day CYP, 100 μg/kg BW/day ATZ or 1 μg/kg BW/day EE2 supplied in the drinking water and named HD, CYP-HD, ATZ-HD and EE2-HD, respectively. Exposure to the treatments continued for 20 weeks. The last group was fed with a regular basal diet (BD) for the entire period. The composition of the BD was described previously (Bieri, 1979; Endo et al., 2002). The HD was prepared by adding 10% sucrose (wt/wt), 25% lard (wt/wt) and 1% cholesterol (wt/wt) to the BD. During chemical administration, the body weight and food and water consumption amounts of each mouse were measured every week. The dose of ATZ, CYP and EE2 were calculated by multiplying the concentrations by volume of water consumed. After 20 weeks, all of the mice were sacrificed. Liver and epididymal fat tissues were quickly removed, weighed and stored at −80 °C for further use. Partial samples of the livers were collected from 3 randomly selected mice from each group, were embedded in an O.C.T. compound (Sakura Finetek, USA) and were frozen at −80 °C. Additionally, partial samples of the livers and epididymal fat were fixed directly in a 4% paraformaldehyde solution for histological analysis. Blood was collected, and the plasma was separated via centrifugation at 4 °C and stored at −20 °C until use. Every effort was made to minimize animal suffering during each experiment. All experiments were performed in accordance with the Guiding Principles in the Use of Animals in Toxicology of Zhejiang University of Technology.

2.3. Hepatic lipids and histopathological analysis

Cryosections of liver samples were cut at 10 μm thickness on a cryostat (Microtome Cryostat HM 550, Walldorf, Germany) and stained with Oil Red O to determine the presence of hepatic lipids. Livers and epididymal fats fixed in 4% paraformaldehyde were processed sequentially in ethanol, xylene and paraffin. Then, the tissues were embedded in paraffin wax, sectioned (4 μm) using a Leica RM2235 (Germany) and mounted on slides. Finally, the sections were stained with hematoxylin and eosin (H&E) prior to examination with a microscope (Olympus, Japan).

2.4. Determination of serum TG, TC, FFA, HDL, LDL and hepatic TG and pyruvate levels

The serum TG, TC, free fatty acids (FFA), high density lipoprotein (HDL) and low density lipoprotein (LDL), and hepatic TG and pyruvate contents were determined using their respective kits purchased from the Nanjing Jianchen Institute of Biotechnology (Nanjing, China), according to the manufacturer's instructions. For determining hepatic TG levels, the liver tissue was first homogenized with 3 volumes of methanol, and then, 6 volumes of chloroform were added and extracted for 16 h at room temperature. The chloroform layer was then collected by centrifugation at 3000*g* for 10 min. A total of 10 μL of each sample were used to determine TG levels. For determining hepatic pyruvate contents, the livers were defrosted and homogenized with 10 volumes of cold saline. The homogenate was centrifuged at 2500 × *g* at 4 °C for 10 min, and the supernatant was collected to determine the pyruvate content. In both experiments, the protein content was determined using a commercial BCA protein kit provided by Sangon Company (Shanghai, China).

2.5. Quantification of mRNA

Total RNA was isolated from the liver using TRIzol reagent (Takara Biochemicals, Dalian, China), and cDNA was synthesized using a reverse transcriptase kit (Toyobo, Japan). Real-time quantitative polymerase chain reaction (RT-qPCR) was performed in an Eppendorf MasterCycler® ep RealPlex^2 (Wesseling-Berzdorf, Germany). All oligonucleotide primers that were used are listed in Table s1. The levels of *18SRNA* transcripts were determined for reference as a housekeeping gene. The following PCR protocol was adopted: denaturation for 1 min at 95 °C, followed by 40 cycles of 15 s at 95 °C and 1 min at 60 °C. The PCR protocol and the relative quantification of gene expression among the treatment groups were performed as previously described (Jin et al., 2008, 2012).