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The metabolic fingerprint of p,p′-DDE and HCB exposure in humans

Samira Salihovic [a,e,*], Andrea Ganna [b,c], Tove Fall [a], Corey D. Broeckling [d], Jessica E. Prenni [d], Bert van Bavel [e], P. Monica Lind [f], Erik Ingelsson [a,g], Lars Lind [h]

[a] Department of Medical Sciences, Molecular Epidemiology and Science for Life Laboratory, Uppsala University, Uppsala, Sweden
[b] Analytic and Translational Genetics Unit, Department of Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114, USA
[c] Program in Medical and Population Genetics, Broad Institute of Harvard and MIT, 7 Cambridge Center, Cambridge, MA 02142, USA
[d] Proteomics and Metabolomics Facility, Colorado State University, Fort Collins, CO, USA
[e] MTM Research Centre, School of Science and Technology, Örebro University, Örebro, Sweden
[f] Department of Medical Sciences, Occupational and Environmental Medicine, Uppsala University Hospital, Uppsala, Sweden
[g] Department of Medicine, Division of Cardiovascular Medicine, Stanford University School of Medicine, Stanford, CA 94305, USA
[h] Department of Medical Sciences, Cardiovascular Epidemiology, Uppsala University, Uppsala, Sweden

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ABSTRACT

Background: Dichlorodiphenyldichloroethylene (p,p′-DDE) and hexachlorobenzene (HCB) are organochlorine pesticides with well-known endocrine disrupting properties. Exposure to p,p′-DDE and HCB concerns human populations worldwide and has been linked to metabolic disorders such as obesity and type 2 diabetes, but details about these associations in humans from the general population are largely unknown.
Objectives: We investigated the associations between p,p′-DDE and HCB exposure and global metabolomic profiles in serum samples from 1016 participants from the Swedish population-based Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study.
Methods: HCB and p,p′-DDE levels were determined using gas chromatography coupled to high-resolution mass spectrometry (GC–HRMS). Metabolite levels were determined by using a non-targeted metabolomics approach with ultra-performance liquid chromatography coupled to time-of- flight mass spectrometry (UPLC–TOFMS). Association analyses were performed using multivariate linear regression.
Results: We found circulating levels of p,p-DDE and HCB to be significantly associated with circulating levels of 16 metabolites following adjustment for age, sex, education level, exercise habits, smoking, energy intake, and alcohol intake. The majority of the 16 metabolites belong to lipid metabolism pathways and include fatty acids, glycerophospholipids, sphingolipids, and glycerolipids. Overall, p,p′-DDE and HCB levels were found to be correlated to different metabolites, which suggests that different metabolic fingerprints may be related to circulating levels of these two pesticides.
Conclusions: Our findings establish a link between human exposure to organochlorine pesticides and metabolites of key metabolic processes mainly related to human lipid metabolism.

1. Introduction

Organochlorine pesticides are a collective name for a large group of compounds that have been used as agrochemicals. Their toxicity and capability to control insects, such as termites, root worms, grasshoppers, and disease vectors has led to their ubiquitous environmental distribution. DDT (1,1,1-trichloro-2, 2-bis (p-chlorophenyl) ethane) is one of the most well-known among the organochlorine pesticides and large quantities of DDT have been used to control insects on agricultural crops. HCB on the other hand has been used to a lesser extent as an agrochemical, but is still formed as a by-product during incomplete waste combustion and chlorination processes. Moreover, DDT is still being dispersed to the environment as it is used in selected equatorial countries to control insects that carry diseases such as malaria (van den Berg, 2009). In humans, DDT is metabolized into two major breakdown products including p,p′-DDE (1, 1-dichloro-2, 2-bis (p-chlorophenyl) ethylene) and p,p′-DDD (1, 1-dichloro-2, 2-bis (p-chlorophenyl) ethane). While DDT is quickly metabolized, p,p′-DDE is extremely persistent and is typically used as a biomarker of DDT exposure in biological tissues (UNEP, 2015).

The national restrictions and global regulation through the Stockholm Convention have resulted in a decline in the environmental concentrations of HCB and p,p′-DDE. However, due to their persistence and high bioaccumulation potentials they are still found in biological matrices from the general population in countries all over the world.

* Corresponding author at: Department of Medical Sciences, Molecular Epidemiology and Science for Life Laboratory, Uppsala University, SE-751 41 Uppsala, Sweden.
E-mail address: samira.salihovic@medsci.uu.se (S. Salihovic).

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Among the organochlorine pesticides, p,p′-DDE is frequently detected in the highest concentrations (Airaksinen et al., 2011; Salihovic et al., 2012a). The main exposure pathway to p,p′-DDE and HCB in the general population comes from ingestion of contaminated food, particularly fish, meat, and dairy products with high lipid content (Darnerud et al., 2006).

Over the years, numerous experimental studies performed on animals have linked HCB and p,p′-DDE to negative health effects, including immunological, neurological, developmental, endocrine, reproductive, and cancerogenic effects (IARC, 2015; WHO, 2012). Their mechanisms of action have been suggested to be mediated by their ability to mimic the effects of hormones, leading to disruption of multiple metabolic pathways. In humans, negative health effects have been reported in both occupationally and accidentally exposed populations (Hayden et al., 2010; Starling et al., 2014). However, in recent years, evidence is accumulating that even the low-dose background exposure typically observed in general populations might be related to negative health effects in humans. For example, exposure to p,p′-DDE and HCB has been found related to metabolic diseases such as type 2 diabetes (Airaksinen et al., 2011; Lee et al., 2011; Lee et al., 2010; Son et al., 2010; Taylor et al., 2013; Turyk et al., 2009) and obesity (Lee et al., 2012; Legler et al., 2015), suggesting that persistent organochlorine pesticides such as these cannot be ruled out as possible causative factors and/or mediators in the development of metabolic disease. Despite these findings, the molecular mechanisms and pathogenetical processes underlying these associations remain unclear.

From this perspective, metabolomics, which refers to global profiling and quantification of a large number of metabolites in an individual biological sample, has become an important tool to identify metabolic biomarkers and metabolic pathways that might be involved in the development of metabolic disease (Ganna et al., 2014; Wang et al., 2011). Metabolomics can also be applied to reveal how biochemical processes are affected by exposure to various environmental pollutants. Recent studies have detected changes in levels of specific metabolites in response to exposure to different environmental pollutants (Bonvallot et al., 2013; Cabaton et al., 2013; Ellis et al., 2012; Lu et al., 2014). However, most of this research has been performed in animals; only a few studies were performed in humans, and notably, none of these dealt with endocrine disrupting organochlorine pesticides, such as p,p′-DDE and HCB.

In this study, we applied, for the first time, mass spectrometry-based metabolomics profiling in a large population-based setting to analyze the effects of p,p′-DDE and HCB exposure on global metabolic profiles with the objective to improve knowledge about the relationships between exposure to organochlorine pesticides and the intermediary metabolism in humans.

2. Materials and methods

2.1. Study population

The PIVUS study was initiated in 2001 with the primary aim to investigate causes of cardiovascular disease in an elderly population. As one of the secondary aims, this study examined possible associations between levels of persistent organic pollutants and various health effects such as cardiometabolic diseases. In short, eligible participants were all 70 years of age and resided in the community of Uppsala, Sweden at baseline. The participants were randomly chosen from the register of community living and were invited between April 2001 and June 2004. The participants received an invitation by letter within 2 months of their 70th birthday. Of the 2, 025 subjects invited, 1, 016 participants (50.1% females) were investigated, giving a participation rate of 50.2%. The study was approved by the Ethics Committee of the University of Uppsala and the participants gave written informed consent (Lind et al., 2005).

2.2. Sample collection

All participants were asked to answer a questionnaire about their medical history, regular medication, education level, exercise habits, dietary, alcohol and smoking habits. All study participants were investigated in the morning after an overnight fast. No medication or smoking was allowed after midnight. After the serum and plasma samples were collected, they were placed in freezers (−80 °C) until analysis. We excluded individuals that could not have their environmental pollutants analyzed due to low sample volume and those whose metabolomics data did not pass our strict quality control. Hence, the eligible study sample for the present study was 965 individuals.

2.3. Organochlorine pesticide analysis

2.3.1. Sample preparation

Selected organochlorine pesticides were measured in stored plasma samples collected at baseline, all details on the analyses have been described by Salihovic et al. (2012b). Briefly, labeled ^{13}C-internal standards were added to the sample prior to extraction by solid phase extraction using Oasis® HLB (Waters, Milford, MA, USA) cartridges. Clean-up of sample lipids and interferences was performed by multilayer chromatography using acid silica. Labeled ^{13}C-labeled recovery standards were added to all samples extracts prior to subsequent instrumental analyses.

2.3.2. Instrumental analysis

Sample extracts were injected onto a 6890 N gas chromatograph (GC) (Agilent Technologies, Atlanta, GA, USA) equipped with a 30 m × 0.25 i.d. × 0.25 μm DB-5 capillary column (SGE Analytical Science, Victoria, AUS) and measurements were performed on a Micromass Autospec Ultima (Waters, Mildford, MA, USA) mass spectrometer (MS) operating at ≥10,000 resolving power using EI ionization at 35 eV. Measurements were performed in selected ion monitoring (SIM) and quantification was performed according to the isotope dilution method using ^{13}C-labeled standards.

2.3.3. Quality control

For quality control (QC), concentrations of well characterized QC plasma sample routinely used in the laboratory was included in each batch of authentic samples and used during the entire analytical investigation. For laboratory and method contamination, procedural water blank samples were incorporated in each batch of authentic samples. The limit of detection (LOD) for p,p′-DDE was 2.13 ng/g lipid and for HCB 14.1 ng/g lipid. The analytical method employed to all plasma samples was successfully validated in terms of recovery, precision, and reproducibility (Salihovic et al., 2012b).

2.4. Metabolomics analysis

2.4.1. Sample preparation

Non-targeted metabolite profiling was performed using the method previously described by Broeckling et al. (2013). Briefly, 96-well plate protein precipitation and extraction of serum samples was performed using methanol. The supernatant was aliquoted to three separate 96-well plates and stored at −20 °C until analysis.

2.4.2. Instrumental analysis

Separation and data acquisition was performed on Acquity UPLC coupled to a Xevo G2 Q-TOFMS (Waters Corporation, Milford, USA) with an atmospheric electrospray interface operating in positive ion mode. Non-consecutive duplicate sample aliquots were injected onto an Acquity UPLC BEH C8 (1.8 μM, 1.0 × 100 mm) analytical column. Mass analysis was performed alternatively in MS mode at collision energy of 6 V and in idMS/MS mode using a collision energy ramp (15–30 V).