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Urinary levels of endocrine-disrupting chemicals, including bisphenols, bisphenol A diglycidyl ethers, benzophenones, parabens, and triclosan in obese and non-obese Indian children

Jingchuan Xue [a], Qian Wu [a], Sivasubramanian Sakthivel [b], Praveen V. Pavithran [b], Jayakumar R. Vasukutty [b], Kurunthachalam Kannan [a,c,*]

[a] Wadsworth Center, New York State Department of Health, and Department of Environmental Health Sciences, School of Public Health, State University of New York at Albany, NY, USA
[b] Department of Endocrinology, Amrita School of Medicine, Amrita Institute of Medical Sciences & Research Centre, Kochi, Kerala, India
[c] Biochemistry Department, Faculty of Science and Experimental Biochemistry Unit, King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia

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ABSTRACT

Obesity has been recognized as a major global public health concern. In particular, childhood obesity is a major risk factor for other health issues, such as type 2 diabetes, in later stages of life. A few earlier studies have associated exposure to endocrine-disrupting chemicals (EDCs) with childhood obesity. There is limited information, however, on exposure to EDCs and childhood obesity in India. In this study, urinary levels of 26 EDCs were determined in 49 obese and 27 non-obese Indian children. Eleven EDCs, including 2,2-bis(4-hydroxyphenyl)propane (BPA), 4,4′-sulfonyldiphenol (BPS), methyl paraben (MeP), ethyl paraben (EtP), propyl paraben (PrP), 4-hydroxybenzoic acid (4-HB), 3,4-dihydroxybenzoic acid (3,4-DHB), triclosan (TCS), benzophenone-3 (BP3), bisphenol A diglycidyl ether (BADGE), and bisphenol A bis (2,3-dihydroxypropyl) glycidyl ether (BADGE·$2H_2O$) were found in >70% of urine samples. No significant associations were found between childhood obesity and most target chemicals studied, except for 3,4-DHB, which showed a significant positive association. Urinary concentrations of 3,4-DHB were higher in obese children than in non-obese children, independent of age, sex, family income, parent education, physical activity, and urinary creatinine. Urinary concentrations of several EDCs were higher in Indian children than the concentrations reported for children in the USA and China. To our knowledge, this is the first study to report urinary concentrations of several EDCs in Indian children.

1. Introduction

Obesity (defined in this study as the body mass index greater than 95th percentile for age), is a significant global public health problem that has reached epidemic proportions worldwide (Newbold et al., 2007). Globally, over one billion adults are overweight, and over 300 million are obese (Wasan and Looije, 2005). Obesity is a risk factor for a variety of diseases, such as type 2 diabetes and those of cardiovascular function (Grundy, 2004). Whereas obesity is a health risk for adults, it is an even more serious concern for children. Childhood obesity has been identified as one of the serious public health challenges in the 21st century, as it is difficult to treat and increases the risk for developing other non-communicable diseases (Newbold et al., 2007). The prevalence of childhood obesity has increased at an alarming rate globally, with over 42 million children below the age of 5 years who were overweight in 2010, of whom 35 million live in developing countries (WHO, 2014). However, very few epidemiological studies have been conducted on childhood obesity. Further, India, one of the world's most rapidly developing countries, has witnessed a dramatic increase in obese and overweight children in the past decades (Ramachandran et al., 2002).

The etiology of obesity has been studied extensively, but it is still not well understood. Obesity has been considered an effect of the high-caloric modern diet and sedentary lifestyle (Newbold et al., 2007). Although genetic factors play a role in the etiology of obesity, this alone cannot account for the large increase in obesity in recent years (Grun and Blumberg, 2006a; Herrera and Lindgren, 2010). Although high calorie intake and decreased physical activity play a role in obesity, mounting evidence has suggested the role of environmental factors (Baillie-Hamilton, 2002; Grun and

* Corresponding author at: Wadsworth Center, Empire State Plaza, P.O. Box 509, Albany, NY 12201-0509, USA.
E-mail address: Kurunthachalam.Kannan@health.ny.gov (K. Kannan).

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Blumberg, 2006a; Hatch et al., 2010). Human exposure to industrial chemicals has been reported to occur in parallel with the rising obesity epidemic (Baillie-Hamilton, 2002). Exposure to endocrine-disrupting chemicals (EDCs) during critical windows of perinatal development can modify normal cellular and tissue development and alter developmental programming, which can lead to increased susceptibility to metabolic and hormonal disorders later in life (Schug et al., 2011). In addition, childhood and adolescence stages are marked by continued maturation of key endocrine systems, which are susceptible to chemical exposures (Schug et al., 2011). Therefore, it is possible that childhood obesity, in recent years, is associated with increasing exposure to a variety of EDCs.

EDCs that can contribute to obesity are referred to as "obesogens" (Grun and Blumberg, 2006a; Newbold et al., 2007). Bisphenol A (BPA), a widely used compound in polycarbonate plastic, is considered an obesogen (Huang et al., 2012; vom Saal et al., 2012). Several *in vitro* and *in vivo* studies have shown that BPA exposure at low doses during the perinatal period can result in an increase in adiposity (Masuno et al., 2002; Sakurai et al., 2004; Somm et al., 2009). Diethylhexylphthalate (DEHP), extensively used as a softener and plasticizer in polyvinyl chloride (PVC) products, and tributyltin (TBT), primarily used in antifouling paints on ship and boat hulls, are known to induce adipogenesis through PPARγ activation (Desvergne et al., 2009; Grun et al., 2006b). A large number of *in vitro* and experimental animal studies have examined the toxic mechanisms of obesogens. However, epidemiological studies that examine the association between EDC exposure and obesity are limited, especially in developing countries.

Alkyl esters of *p*-hydroxybenzoic acid (parabens), bisphenol A diglycidyl ether (BADGE), bisphenol F diglycidyl ether (BFDGE), bisphenols (BPs), triclosan (TCS), and benzophenone-3 (BP3) are used in a broad range of consumer and industrial applications. Parabens are a group of chemicals that include methyl- (MeP), ethyl- (EtP), propyl- (PrP), butyl- (BuP), benzyl- (BzP), and heptylparabens (HepP) and are used as antimicrobial preservatives in cosmetics, pharmaceuticals, and foodstuffs (Andersen, 2008; Soni et al., 2005). 4-Hydroxybenzoic acid (4-HB) and 3,4-dihydroxybenzoic acid (3,4-DHB) are common metabolites of parabens in the human body (Abbas et al., 2010). BADGE- and BFDGE-based phenolic resins are used predominantly in the interior coating of food cans (May, 1988). The epoxide groups of BADGE and BFDGE are highly reactive and result in the formation of a variety of reaction products with HCl and/or H_2O. BPs are a group of chemicals with two para-hydroxyphenyl functionalities; BPA is the prototype chemical in this class. BPs can be used as additives or raw materials in plastic linings for food containers and thermo-sensitive coatings for papers (Delfosse et al., 2012). TCS is an antimicrobial agent and is well known for its widespread use in personal care products (PCPs), such as soaps and toothpastes. BP3 is the most commonly used ultra-violet (UV) light filter in cosmetic formulations (Vidal et al., 2007). All aforementioned chemical classes are considered endocrine disruptors and have displayed estrogenic and/or anti-estrogenic activities *in vitro* and/or in animal studies (Gomez et al., 2005; Grignard et al., 2012; Henry and Fair, 2013; Laws et al., 2000; Nakazawa et al., 2002). Widespread exposure of humans, including children, to these chemicals has been reported (Calafat et al., 2008a, 2008b; Frederiksen et al., 2011; Liao et al., 2012; Vidal et al., 2007; Wang et al., 2012; Wang et al., 2013; Wang and Kannan, 2013a, 2013c; Zhang et al., 2011).

In this study, urinary concentrations of 26 EDCs were measured in 76 Indian children aged 2–14 years (49 obese and 27 non-obese). The objectives of this study were to determine the magnitude of exposure to EDCs by Indian children and to examine the association between urinary EDC concentrations and childhood obesity. This is the first study to report these select EDCs exposures in Indian children.

2. Materials and methods

An observational prospective case-control pilot study was conducted in the Department of Endocrinology, Amrita Institute of Medical Sciences, Kochi, India, that comprises consecutive visits of obese/overweight children (aged 2–14 years) to the Endocrinology Outpatient Department during period from July 2012 to September 2013. Seventy five obese/overweight children and their parents/guardians were explained about this study, of which 60 agreed to participate. Children with a body mass index (BMI, weight/height) $>$95th percentile for age were considered obese and children with BMI $>$85th percentile for age were considered overweight. Patients with endocrine obesity, including hypothyroidism, Cushing's syndrome, hypothalamic syndromes, and genetic obesity were excluded. Children in the control group were selected based on the clinical evidence of no major diseases, after undergoing a routine physical examination (BMI at 50th percentile). An informed consent was obtained from parents/guardians of 34 children with normal weight/BMI. Socioeconomic and demographic factors of children were collected. Of the 94 children recruited, demographic data for 11 children in the case group and 7 children in the control group were incomplete. Thus, a total of 49 obese and 27 non-obese children were included in this study. The study participants underwent a detailed history evaluation, anthropometry, and clinical examination. Spot urine samples were obtained from children at the time of visit. Institutional review board (IRB) approvals were obtained from Amrita Institute of Medical Sciences and Research Center, Kochi, for the collection and analysis of urine samples from children.

3. Analysis of EDCs

Analytical standards of BADGE ($\geq$97%), bisphenol A (2, 3-dihydroxypropyl) glycidyl ether (BADGE·H_2O, $\geq$97%), bisphenol A (3-chloro-2-hydroxypropyl) glycidyl ether (BADGE·HCl, ~95%), bisphenol A bis(2,3-dihydroxypropyl) glycidyl ether (BADGE·$2H_2O$, $\geq$97%), bisphenol A bis(3-chloro-2-hydroxypropyl) glycidyl ether (BADGE·2HCl, $\geq$99%), bisphenol A (3-chloro-2-hydroxypropyl)(2,3-dihydroxypropyl) glycidyl ether (BADGE·H_2O·HCl, $\geq$98%), BFDGE (~97%), bisphenol F bis(3-chloro-2-hydroxypropyl) glycidyl ether (BFDGE·2HCl, ~95%), bisphenol F bis(2,3-dihydroxypropyl) glycidyl ether (BFDGE·$2H_2O$, $\geq$97%), BP3 (~98%), 3,4-DHB ($\geq$97%), 2,2-bis(4-hydroxyphenyl) propane (BPA, $\geq$97%), 4,4′-(hexafluoroisopropylidene)-diphenol (BPAF, ~97%), 4,4′-(1-phenylethylidene)bisphenol (BPAP, ~99%), 4,4′-(1,4-phenylenediisopropylidene)bisphenol (BPP, ~99%), 4,4′-sulfonyldiphenol (BPS, ~98%), 4,4′-cyclo-hexylidenebisphenol (BPZ, ~98%), and TCS (~97%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Analytical standards of MeP, EtP, PrP, BuP, BzP, HepP, and 4-HB were purchased from AccuStandard, Inc. (New Haven, CT, USA). 2,2-bis(4-hydroxyphenyl) butane (BPB, ~98%) was purchased from TCI America (Portland, OR, USA). D_6-BADGE was obtained from Toronto Research Chemicals Inc. (Toronto, Ontario, Canada). ^{13}C-Isotopically labeled 2-OH-4-MeO-BP ($^{13}C_{12}$-BP3), $^{13}C_{12}$-BPA, $^{13}C_6$-MeP, $^{13}C_6$-BuP, and $^{13}C_6$-4-HB were purchased from Cambridge Isotope Laboratories ($\geq$99%; Andover, MA, USA). $^{13}C_{12}$-TCS was obtained from Wellington Laboratories Inc (Guelph, Ontario, Canada). The molecular structures of these compounds are shown in the Supplementary information (Table S1). β-Glucuronidase from *Helix pomatia* (145,700 units/mL β-glucuronidase and 887 units/mL sulfatase) was purchased from Sigma-Aldrich (St. Louis, MO, USA). The stock solutions of target