Toxicology 320 (2014) 6–14

Contents lists available at ScienceDirect

Toxicology

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

Exposure to p,p′-dichlorodiphenyldichloroethylene (DDE) induces fasting hyperglycemia without insulin resistance in male C57BL/6H mice

George E. Howell III[a,*], Edward Meek[a], Jessica Kilic[b], Mariel Mohns[b], Charlee Mulligan[a,b], Janice E. Chambers[a]

[a] Center for Environmental Health Sciences, Department of Basic Sciences, Mississippi State University College of Veterinary Medicine, Mississippi State, MS 39762, United States
[b] Department of Biological Sciences, Mississippi College, Clinton, MS 39058, United States

ARTICLE INFO

Article history:
Received 19 November 2013
Received in revised form 30 January 2014
Accepted 10 February 2014
Available online 26 February 2014

Keywords:
Persistent organic pollutants
Organochlorine compounds
DDE
Glucose
Diabetes
C57BL/6 mice

ABSTRACT

Approximately 8.3% of the United States (U.S.) population have either diagnosed or undiagnosed diabetes mellitus. Out of all the cases of diabetes mellitus, approximately 90–95% of these cases are type 2 diabetes mellitus (T2D). Although the exact cause of T2D remains elusive, predisposing factors include age, weight, poor diet, and a sedentary lifestyle. Until recently the association between exposure to environmental contaminants and the occurrence of diabetes had been unexplored. However, recent epidemiological studies have revealed that elevated serum concentrations of certain persistent organic pollutants (POPs), especially organochlorine pesticides, are positively associated with increased prevalence of T2D and insulin resistance. The current study seeks to investigate if this association is causative or coincidental. Male C57BL/6H mice were exposed to DDE (2.0 mg/kg or 0.4 mg/kg) or vehicle (corn oil; 1 mL/kg) for 5 days via oral gavage; fasting blood glucose, glucose tolerance, and insulin challenge tests were performed following a 7 day resting period. Exposure to DDE caused significant hyperglycemia compared to vehicle and this hyperglycemic effect persisted for up to 21 days following cessation of DDE administration. Intraperitoneal glucose tolerance tests and phosphorylation of Akt in the liver, skeletal muscle, and adipose tissue following insulin challenge were comparable between vehicle and DDE treated animals. To determine the direct effect of exposure to DDE on glucose uptake, in vitro glucose uptake assays following DDE exposure were performed in L6 myotubules and 3T3-L1 adipocytes. In summary, subacute exposure to DDE does produce fasting hyperglycemia, but this fasting hyperglycemia does not appear to be mediated by insulin resistance. Thus, the current study reveals that subacute exposure to DDE does alter systemic glucose homeostasis and may be a contributing factor to the development of hyperglycemia associated with diabetes.

1. Introduction

The prevalence of obesity (body mass index $\geq 30\,kg/m^2$) has increased at a staggering rate in the U.S. and is approaching epidemic proportions. Analysis of data from the National Health and Nutrition Examination Survey (NHANES) conducted during 2009–2010 revealed that 35.7% of U.S. adults over the age of 20 years were classified as being obese and 16.9% of U.S. children and adolescents were obese (Flegal et al., 2012; Ogden et al., 2012). These statistics are particularly alarming given that childhood obesity greatly increases the likelihood of being obese as an adult (Biro and Wien, 2010; Whitaker et al., 1997). These data are of vital consequence to the healthcare system in the U.S. given that being obese or even overweight is intimately associated with other disease

Abbreviations: DDE, p,p′-dichlorodiphenyldichloroethylene; DDT, p,p′-dichlorodiphenyltrichloroethane; T2D, type 2 diabetes mellitus; POPs, persistent organic pollutants; OC, organochlorine compound; NHANES, National Health and Nutrition Examination Survey; AAALAC, Association for the Assessment and Accreditation of Laboratory Animal Care; DMSO, dimethylsulfoxide; ACE, accelerated solvent extractor; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; HOMAIR, homeostasis model assessment value for insulin resistance; TNFα, tumor necrosis factor alpha; IL-6, interleukin 6; MCP-1, monocyte/macrophage chemotractant protein 1; i.p., intraperitoneal; IPGTT, intraperitoneal glucose tolerance test; GC/MS, gas chromatography/mass spectroscopy.

* Corresponding author. Tel.: +1 6014204707.
E-mail address: thowell@cvm.msstate.edu (G.E. Howell III).

http://dx.doi.org/10.1016/j.tox.2014.02.004
0300-483X/

processes including dyslipidemias, hypertension, and T2D. Indeed, in 2011, 25.8 million people in the U.S. or 8.3% of the population were reported to have either diagnosed or undiagnosed diabetes (CDC, 2011). Out of these cases of diabetes, it is estimated that 90–95% will be T2D (CDC, 2011). The primary pathophysiological alteration in T2D is the development of insulin resistance in the liver, skeletal muscle and adipose tissue which leads to hyperinsulinemia and eventual hyperglycemia (DeFronzo, 1997, 2004).

While the exact etiology of insulin resistance and the resulting T2D remains an enigma, it is most likely the result of a multifactorial process including several well established components such as genetic predisposition, diet, and sedentary lifestyle. However, these processes are most likely not sufficient to explain the ever increasing prevalence of T2D. Until recently, an environmental exposures component had not been proposed as a risk factor for the development of T2D. A major classification of chemicals that has recently been implicated as a possible causative factor in the development of T2D is the persistent organic pollutants (POPs). Common properties of these chemicals include long half-lives, bioaccumulation, and biomagnification up the food chain. One of the first POPs to be implicated through epidemiological associations with T2D was 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Exposure to TCDD as a contaminant of Agent Orange has been positively associated with the development of T2D in veterans of the Vietnam Conflict (Kern et al., 2004; Michalek and Pavuk, 2008). Empirical studies have shown that exposure to TCDD causes insulin resistance and subsequent T2D in experimental animals (Liu and Matsumura, 1995). While TCDD is a POP, it is not as prevalent as some of the organochlorine insecticides or their bioaccumulative metabolites based on prevalence of detection in the general U.S. population. TCDD was detected in the serum of 7% of subjects in the 1999–2002 NHANES study whereas organochlorine pesticide compounds (OCs) such as DDE, trans-nonachlor, and oxychlordane were detected in the serum of 99.7%, 92.6%, and 82.9% of subjects in the 2003–2004 NHANES study, respectively (Lee et al., 2006; Patterson et al., 2009).

Like TCDD, these OCs have been positively associated with the occurrence of insulin resistance and diabetes through epidemiological studies. Recent studies have demonstrated through retrospective analysis of the 1999 to 2002 NHANES data that increasing serum concentrations of trans-nonachlor and oxychlordane are positively associated with insulin resistance (Lee et al., 2007). In addition, increasing serum concentrations of these OCs, including DDE, are positively associated with increased prevalence of diabetes and/or the metabolic syndrome (Lee et al., 2006). Interestingly, in this study, there was no association between obesity and diabetes in subjects with non-detectable levels of POPs (when all six categories of POPs were taken collectively). This observation suggests that the elevated serum concentration of POPs and not obesity promotes diabetes in these subjects. The possibility that background exposure to DDE may promote diabetes has been further substantiated by studies in non-diabetics where increased serum DDE concentrations predicted elevated homeostasis model assessment value for insulin resistance (HOMA-IR) values as well as dyslipidemias (Lee et al., 2011). More recent studies by Kim et al. (2014) have revealed that elevated adipose concentrations of DDE are positively associated with diabetes and systemic insulin resistance indicated by increased HOMA-IR values. In studies examining the prevalence of diabetes in Swedish men and women, there was a significant correlation between serum DDE and prevalence of diabetes (Rignell-Hydbom et al., 2007, 2009; Rylander et al., 2005). In addition to these studies of the Swedish population, Turyk et al. (2009a) determined in a cohort of Great Lakes sport fish consumers who were followed from a healthy, non-diabetic state to development of diabetes that DDE exposure was significantly associated with the incidence of diabetes. Taken together, these epidemiological studies provide a strong line of evidence suggesting elevated serum concentrations of POPs, especially the organochlorine pesticides or their metabolites, may play a role in the pathogenesis of T2D and metabolic dysfunction.

While the currently growing body of epidemiological evidence suggests a role of environmental exposures, especially OC exposures, in the pathogenesis of T2D, there is a lack of empirical evidence showing causality. Ruzzin et al. (2010) recently demonstrated that consumption of a diet contaminated with a mixture of POPs, including OCs, in conjunction with high fat feeding promoted increased weight gain, insulin resistance, and hepatic steatosis in a rodent model. In addition to these studies, Ibrahim et al. (2011) also demonstrated that consumption of diets contaminated with POPs promoted insulin resistance and other pathophysiological alterations associated with the metabolic syndrome. A common feature of these two studies is that they both utilized a mixture of POPs and did not seek to delineate the role of individual OC compounds in these phenomena. Given the lack of empirical data to determine if exposure to POPs, especially the OCs alone and in combination, results in insulin resistance and altered glucose homeostasis, the current study was designed to determine if exposure to a highly prevalent organochlorine compound, DDE, alone can cause hyperglycemia and insulin resistance in a murine model and in vitro models of insulin sensitive tissues.

2. Materials and methods

2.1. Chemicals

DDE (98% purity; Chem Service) was dissolved in corn oil (Sigma Aldrich) at concentrations of 0.4 mg/mL or 2.0 mg/mL for in vivo administration. For in vitro glucose uptake assays, DDE was dissolved in dimethylsulfoxide (DMSO; Sigma Aldrich). D-Glucose and solvents used for DDE extraction were obtained from Sigma Aldrich. Human insulin (25 U/mL) used for intraperitoneal insulin challenge and glucose uptake assays was obtained from Gibco. [H^3] 2-deoxy-D-glucose used for glucose uptake assays was obtained from Perkin Elmer.

2.2. Animal care

Male C57BL/6H mice were obtained from Harlan Laboratories at six weeks of age. Animals were housed individually in polycarbonate cages in an AAALAC-approved animal facility under a 12 h light-dark cycle with food and water ad libitum unless they were subjected to fasting prior to glucose measurements or insulin challenge. All animal use protocols were approved by the Mississippi State University Animal Care and Use Committee. Animals were allowed a four to 5 day acclimation period prior to administration of experimental compounds.

2.3. Experimental design

To determine the effect of exposure to DDE on fasting blood glucose concentrations, male C57BL/6H mice ($n = 10$/group) were administered vehicle (corn oil; 1 mL/kg) or DDE (0.4 or 2.0 mg/kg) via oral gavage daily for five consecutive days which represent a subacute exposure. The 2.0 mg/kg dose was chosen as the high dose for the repeated administration paradigm currently utilized because previous studies revealed behavioral alterations (slight lethargy vs. 2.0 mg/kg) with a 10.0 mg/kg dose for 5 days. Administration of DDE over the span of 5 days was utilized to provide a low amount of DDE over a period of time rather than a single, large bolus of DDE to elevate systemic DDE concentrations. Following cessation of DDE or vehicle administration, animals were allowed to rest for 7 days prior to fasting blood glucose measurements. Seven days following the last administration of DDE or vehicle, animals were fasted for 6 h and blood glucose concentrations were measured with a handheld glucometer (AlphaTrak; Bayer Animal Health) via a tail nick (Ayala et al., 2010). Following blood glucose measurement, blood samples were obtained via cardiac puncture and tissues (liver, epididymal fat pads, and gastrocnemius muscle) were harvested and immediately snap frozen in liquid nitrogen and stored at −80 °C. Whole blood samples were allowed to clot for 30 min on ice and then centrifuged at 10,000 × *g* for 10 min at 4 °C to separate the serum and cellular components. Serum samples were stored at −80 °C until further analysis.

2.4. Time course of DDE-induced hyperglycemia

Analysis of fasting blood glucose levels 7 days following the cessation of DDE administration revealed significant hyperglycemia compared to vehicle. To determine the duration of this hyperglycemic effect of DDE, male C57BL/6H mice ($n = 12$–13/group) were administered either vehicle (corn oil; 1 mL/kg) or DDE (2.0 mg/kg) via oral gavage for five consecutive days as described above. Following