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Permethrin alters glucose metabolism in conjunction with high fat diet by potentiating insulin resistance and decreases voluntary activities in female C57BL/6J mice

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ABSTRACT

Permethrin, a type 1 pyrethroid insecticide, was previously reported to promote adipogenesis in 3T3-L1 adipocytes and insulin resistance in C2C12 muscle cells; however, the effects of permethrin exposure on glucose and lipid metabolisms *in vivo* remain unknown. The purpose of this study was to investigate the effects of permethrin exposure on glucose and lipid homeostasis as well as voluntary movement in female mice in response to dietary fat. We tested three doses of permethrin (50, 500, & 5000 μg/kg body weight/day) in low fat diet-fed (4% w/w of diet) and high fat diet-fed (20% w/w of diet) female C57BL/6 J mice for twelve weeks. Our results demonstrated that permethrin treatment potentiated high fat diet-induced insulin resistance as indicated by insulin tolerance tests, glucose tolerance tests, and homeostasis model assessment - insulin resistance (HOMA-IR) without altering weight or fat mass. Permethrin treatment significantly decreased voluntary movement and elevated blood glucose and insulin levels. Western blot results further showed that permethrin impaired insulin signaling via the Akt signaling pathway in the gastrocnemius muscle. Taken together, these results suggest that oral administration of permethrin potentiated high fat diet-induced insulin resistance, possibly increasing the risk of type 2 diabetes without altering weight gain in female C57BL/6 J mice.

1. Introduction

Permethrin [(±)-3-phenoxybenzyl 3-(2,2-dichlorovinyl)-2,2 dimethylcyclopropanecarboxylate] is a synthetic insecticide that belongs to the pyrethroid family, which possesses a structural resemblance to natural pyrethrins. Synthesized in 1973, permethrin was first registered for use by the U.S. Environmental Protection Agency in 1979 (Elliott et al., 1973; Toynton et al., 2009). Permethrin demonstrates significantly improved photostability compared with the natural pyrethrins without sacrificing their potent insecticidal activities and low acute mammalian toxicity (Soderlund et al., 2002). Permethrin is one of the most widely-used synthetic pyrethroid insecticides in agricultural, veterinary, medical, and household settings (Tornero-Velez et al., 2012). Other applications of permethrin include public health mosquito control programs, hair treatment for head lice infestations, and clothing impregnation as an ectoparasite repellant. Thus, human exposure to permethrin is quite likely. The use of pyrethroids has dropped slightly since 1997 (Casida and Durkin, 2013), but they are still the second largest insecticide class currently on the market, accounting for 16% of the global insecticides sales in 2013 (Sparks and Nauen, 2015).

Permethrin was previously reported to promote adipogenesis and induce insulin resistance in cell culture models, similar to other types of membrane-depolarizing insecticides (Howell and Mangum, 2011; J. Kim et al., 2013a, 2014; Moreno-Aliaga and Matsumura, 2002; Park et al., 2013; Shen et al., 2017; Sun et al., 2016a). There is a lack of *in vivo* studies, however, determining the effect of low doses of permethrin on glucose and lipid metabolism.

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Thus, the purpose of this study was to investigate the effect of permethrin exposure on the development of dietary fat-induced obesity and type 2 diabetes using a mouse model.

2. Material and methods

2.1. Materials

Permethrin (98%, mixture of 38.7% *cis* and 59.4% *trans* isomers) and high-density lipoprotein (HDL)/low-density lipoprotein (LDL) cholesterol quantitation kits were purchased from Sigma Aldrich Co. (St. Louis, MO). Insulin (human recombinant) was acquired from Novo Nordisk Inc. (Princeton, NJ). D-Glucose solution (50%) was obtained from Hospira Inc. (Lake Forest, IL). Glucose, cholesterol and triglyceride kits were from Thermo Fisher Scientific (Rockford, IL). Insulin ELISA kit was purchased from Mercodia (Winston Salem, NC). Leptin ELISA kit was from R&D systems (Minneapolis, MN). Free fatty acid assay kit was purchased from Cell Biolabs Inc. (San Diego, CA). Pierce BCA protein assay kits (Thermo Fisher Scientific, Rockford, IL) was used for protein quantification. Rabbit anti-mouse antibodies of phosphorylated phosphoinositide-dependent kinase (pPDK), phosphorylated protein kinase B at threonine 308 (pAkt Thr308) and serine 473 (pAkt Ser473), Akt and glucose transporter 4 (GLUT4) were purchased from Cell Signaling Technology (Danvers, MA). Rabbit anti-mouse antibody of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Horseradish peroxidase-conjugated goat anti-rabbit secondary antibody was obtained from Cell Signaling Technology (Danvers, MA). All other chemicals were either purchased from Sigma Aldrich Co. (St. Louis, MO) or Fisher Scientific (Waltham, MA).

2.2. Animals and diet

All animal care and experimental procedures were approved by the Institutional Animal Care and Use Committee of the University of Massachusetts Amherst (protocol number 2013–0014). Female C57BL/6 J mice were purchased from the Jackson Laboratory (Bar Harbor, ME) at three weeks of age and were housed at two mice per cage with a 12 h light-dark cycle in a temperature and humidity controlled room. Semi-purified AIN-93-based diets from Harlan Laboratories (TD94048 for low fat and TD07518 for high fat diets, Madison, WI) in powdered form were used. The diet composition is shown in Table 1. Permethrin was dissolved in soybean oil and mixed with other ingredients in the diet. Diet and water were given to mice *ad libitum* throughout the experiment period except when fasting was conducted prior to glucose measurement. After a week of adaptation with the low fat diet (4 w/w % fat), all mice were given a baseline test for insulin tolerance in the second week of adaptation and a glucose tolerance test in the 3rd week of adaptation. Then, animals were randomly divided into two dietary groups: a low fat diet (4 w/w % fat) and a high fat diet group (20 w/w % fat). Within each dietary group, a control diet (no permethrin) and three different doses of permethrin-containing diet were given to mice for twelve weeks. Permethrin doses used in the current study were chosen based on the acceptable daily intake of permethrin, which is 50 μg/kg body weight (BW)/day, and the chronic no observed adverse effect level (NOAEL) of permethrin, which is 5000 μg/kg BW/day (CEPADP, 1987; WHO, 1990). Permethrin concentrations were 0.26, 2.6, and 26 μg/g in the low fat diet, and 0.36, 3.6, and 36 μg/g in the high fat diet to deliver 50, 500, and 5000 μg/kg BW/day. Since calorie densities are different between low and high fat diets, doses of permethrin were adjusted accordingly to achieve comparable permethrin doses delivered (Supplementary Fig. 1). Body weight and food intake were measured weekly. Food intake was measured as the total food intake per cage. Estimated permethrin intake in low fat diet fed animals were 33 ± 1, 334 ± 2, and 3387 ± 93 μg/kg BW/day for 50, 500, and 5000 μg/kg BW/day, respectively. Estimated permethrin intake in high fat diet fed animals were 31 ± 2, 374 ± 11 and 3491 ± 100 μg/kg BW/day for 50, 500 and 5000 μg/kg BW/day, respectively. There were no significant differences in three permethrin doses delivered between low vs. high fat diets. At the end of the study, mice were fasted for 4 h and sacrificed by CO_2 asphyxiation. Blood was immediately collected by cardiac puncture and then sera were collected by centrifugation at 3000 g for 20 min at 4 °C. Internal organs (livers, heart, pancreas, kidneys, spleen, and white adipose tissues, including omental, retroperitoneal, and mesenteric) were weighed at sacrifice.

Table 1
Composition of experimental diets.

Ingredient	Low fat diet	High fat diet
	g/kg	
Corn starch	465.7	288.5
Maltodextrin	155	132
Casein	140	169.1
Sucrose	100	100
Cellulose	50	50
Soybean oil	40	200
Mineral Mix, AIN-93M-MX (TD 94049)	35	42.8
Vitamin Mix, AIN-93-VX (TD 94047)	10	12.4
Choline bitartrate	2.5	3
L-Cystine	1.8	2.2
tert-Butylhydroquinone (TBHQ)	0.008	0.04
Total	1000	1000

Permethrin concentrations in low fat diet were 0.26, 2.6, & 26 μg per gram of diet to deliver 50, 500, & 5000 μg/kg BW/day. These concentrations were calculated based on estimated food intake for low fat diet is 4 g/mouse/day. Permethrin concentrations in high fat diet were 0.36, 3.6, & 36 μg per gram of diet to deliver 50, 500, & 5000 μg/kg BW/day based on estimated food intake 3 g/mouse/day.

2.3. Determination of glucose homeostasis

Insulin tolerance test (ITT) was conducted three times during the experiment (adaptation period, weeks 4 and 10). Animals were fasted for 4 h before the test and tail-vein blood samples were obtained at 0, 15, 30, 60, and 120 min after intraperitoneal injection of insulin (0.75U/kg BW). Intraperitoneal glucose tolerance tests (GTT) were conducted in the adaptation period, at weeks 5 and 11. Mice were fasted for 6 h prior to the test. A bolus of glucose solution (2 g/kg BW) was injected into the intraperitoneal cavity, and blood was obtained from the tail end to measure glucose level at 0, 15, 30, 60, and 120 min. All blood glucose levels were tested using a glucometer with test strips (Advocate, Pharma Supply Inc, Wellington, FL). The areas under the curve (AUC) were calculated using SigmaPlot 11.0 (Systat Software, Inc., San Jose, CA). Blood samples at 0, 30, 60, and 120 min were also used to determine insulin level based on a method described previously (Christensen et al., 2009). HOMA-IR was calculated using the HOMA2 calculator (Wallace et al., 2004).

2.4. Voluntary movement measurement (non-exercise physical activity test)

Voluntary movement (non-exercise physical activity) was measured in weeks 1 and 8 by using a method described previously (J. H. Kim et al., 2013b; Y. Kim et al., 2015). Briefly, an individual mouse was put into a clear cage during the dark cycle (6:00 p.m. to 6:00 a.m.). Diet and water (HydroGel, Portland, ME, USA) were provided *ad libitum* to mice during the measurements. Total travel