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Deltamethrin increases the fat accumulation in 3T3-L1 adipocytes and *Caenorhabditis elegans*

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ABSTRACT

Research has shown that permethrin, a Type-I pyrethroid, increases triglyceride (fat) accumulation in adipocytes. Little is known, however, about any similar effect of deltamethrin, a Type-II pyrethroid, which produces a distinct syndrome of poisoning in mammals compared with permethrin. This study was therefore aimed to explore the role of deltamethrin on fat accumulation in 3T3-L1 adipocytes and *Caenorhabditis elegans*. Deltamethrin (10 μM) significantly increased the fat accumulation in 3T3-L1 adipocytes and wild type *C. elegans* compared to respective controls. Deltamethrin decreased the ratio of phosphorylated AMP-activated kinase (pAMPKα) over AMPKα and phosphorylated acetyl-CoA carboxylase (ACC) over ACC, while it increased expression of CCAAT/enhancer-binding protein (C/EBPα) and peroxisome proliferator-activated receptor-γ (PPARγ) in 3T3-L1 adipocytes. Similarly, deltamethrin potentiated fat accumulation in *C. elegans* without affecting growth or pharyngeal pumping rate. Moreover, deltamethrin significantly reduced the total progeny number and locomotive activities in *C. elegans* in a dose-dependent manner. Deltamethrin increased fat accumulation via *aak-2* (an ortholog of AMPKα) and *nhr-49* (a homolog of peroxisome proliferator-activated receptor-α and also downstream target of *aak-2*) mediated mechanisms. The current work is the first report of the effects of deltamethrin on increased fat storage by 3T3- L1 adipocytes and *C. elegans* via *aak-2* (AMPKα ortholog)-mediated mechanism.

1. Introduction

Pyrethroid insecticides, synthetic derivatives of naturally occurring pyrethrins, are widely used in agriculture and households for pest control. Pyrethroids are often regarded as “safer” alternatives to organophosphates and carbamates owing to their relatively low mammalian toxicity (Armstrong et al., 2013; Yoon et al., 2008) and thus, have been used intensively in the United States and Europe since early 1980s (Kim et al., 2010). In recent years, however, significant levels of pyrethroid metabolites have been found in the urine of humans, including pregnant women and children (Berkowitz et al., 2002; Morgan et al., 2007; Naeher et al., 2010). Pyrethroids act primarily by binding to voltage-sensitive sodium channels in neurons and prolonging their opening (Choi and Soderlund, 2006), which cause convulsion, paralysis and even death of the organism. Based on their structure and toxic effects, pyrethroids have been traditionally divided into two classes (Gammon et al., 1981). Type I compounds, which do not contain a cyano group, can cause tremors and skin parathesia. Type II compounds possess a cyano group, can cause salivation, hyperexcitability, and choreoathetosis (Kim et al., 2008). The acute neurotoxic potency, signs of poisoning, and mechanisms of action can vary from one pyrethroid to another (Soderlund et al., 2002).

Deltamethrin is a potent Type-II pyrethroid and it is also the primary metabolite of another pyrethroid, tralomethrin (“Deltamethrin Risk Characterization June 13, 2000,”). Deltamethrin is used widely for agricultural (treatment of cotton), residential, industrial, and institutional (flowers and ornamentals) purposes (“Deltamethrin Risk Characterization June 13, 2000,”). The LD_{50} values of deltamethrin for rats range from 30 mg/kg body weight (with an oily vehicle) to >5000 mg/kg body weight (in an aqueous vehicle) (“Pesticides residues in food, 2000, Deltamethrin, 2001”).

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It has been reported that the elimination half-life of deltamethrin in rats was 38.5 h when orally-administered and 33.0 h when intravenously administered (Anadon et al., 1996). Deltamethrin has been studied for its toxicokinetics and tissue distribution in both immature and adult rats and the accumulation of deltamethrin was found in various tissues, including plasma, brain, fat, skin and muscle (Berkowitz et al., 2002; Kim et al., 2008, 2010; Morgan et al., 2007; Naeher et al., 2010; Soderlund et al., 2002). There is emerging evidence suggesting the relationship between exposure to insecticides and the development of obesity. Previously it was reported that permethrin, a Type-I pyrethroid, increased the triglyceride accumulation as well as the expression of key markers of adipogenesis and lipogenesis in adipocytes (Kim et al., 2014). In contrast, little is known about the effect of deltamethrin on fat accumulation.

Caenorhabditis elegans (*C. elegans*), a free-living nematode, has been extensively utilized because of its relatively short lifespan, quick turnover, easy maintenance, large brood size and well-known genetic pathways with a large number of mutant strains available (Stiernagle, 2006). More than 65% of the genes related to human diseases are conserved in *C. elegans* and it has been employed as a model in obesity studies (Lemieux et al., 2011; Sun et al., 2016b; Zheng and Greenway, 2012). Thus, the purpose of the current study was to determine effects of deltamethrin on adipogenesis using 3T3-L1 adipocytes. In addition, we also determined effects of deltamethrin on fat accumulation using *C. elegans* as an *in vivo* model.

2. Materials and methods

2.1. Materials

All the chemicals used were from Thermo Fisher Scientific Inc. (Pittsburgh, PA) unless stated otherwise. Household bleach (The Clorox Company, Oakland, CA, USA) was used for bleaching the worms. Fluorodeoxyuridine (FUdR) and carbenicillin were purchased from Sigma-Aldrich Co. (St. Louis, MO). All the strains were obtained from the Caenorhabditis Genetics Center, University of Minnesota, USA. 3T3-L1 preadipocytes were obtained from American Type Culture Collection (Manassas, VA). Fetal bovine serum (FBS), Dulbecco's modified Eagle's medium (DMEM), methylisobutylxanthin, insulin, dexamethasone and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich Co. (St. Louis, MO). Deltamethrin (Lot No. 344–15A, 99% pure) was purchased from Chem Service (West Chester, PA). Rabbit antibodies for PPARγ (1:400) and horseradish peroxidase conjugated goat anti-rabbit IgG (1:5000) were purchased from Abcam Inc. (Cambridge, MA). Rabbit antibodies for ACC (1:1000), phosphorylated ACC (pACC, 1:1000), AMP-activated protein kinase α (AMPKα, 1:1000), phosphorylated AMPKα (pAMPKα, 1:1000), C/EBPα (1:1000) and β-actin (internal control, 1:1000) were purchased from Cell Signaling Technologies (Danvers, MA).

2.2. 3T3-L1 culture

3T3-L1 preadipocytes were cultured as previously described (Park et al., 2013). Briefly, 3T3-L1 preadipocytes were maintained in DMEM with 10% FBS to confluence at 37 °C. 2 days after confluence (designated day 0), adipocyte differentiation was induced with a mixture of methylisobutylxanthin (0.5 mM), dexamethasone (1 μM), and insulin (1 μg/ml) in DMEM containing 10% FBS. On day 2, the medium was changed to DMEM containing 10% FBS and insulin only. From day 4, cells were maintained in DMEM containing 10% FBS, and the medium was changed every 2 days. Cells were treated with deltamethrin (0.1, 1, and 10 μM) or 0.02% DMSO as a control from day 0. These concentrations of deltamethrin had no influence on cell viability measured by a 3-(4,5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT) based assay (data not shown) (Gerlier and Thomasset, 1986).

2.3. C. elegans culture

M9 buffer, S-complete and nematode growth medium (NGM) used for *C. elegans* culture were prepared as previously described (Solis and Petrascheck, 2011; Stiernagle, 2006). Strains used in this study were: N2, Bristol (wild type), *sbp-1(ep79) III*, *nhr-49(ok2165) I*, *tub-1(ok1972) II* and *aak-2(ok524) X*. Synchronized populations were obtained according to the previous protocols (Kenyon et al., 1993). Treatment (either deltamethrin at 0.1, 1, and 10 μM or 0.1% DMSO as a control) started with synchronized L1 worms in 12-well plates at 20 °C. After 7 days of treatment, worms were collected for triglyceride and protein quantification.

2.4. Growth rate, progeny production, pumping rate and locomotive activity

The number of worms at each developmental stage after 48 h was counted to calculate growth rate (Lemieux et al., 2011). Worms were shifted to fresh NGM plates every day during the reproduction period, and the eggs left were allowed to hatch and grow to L4 stage before counting the number of progeny of each worm (Chen et al., 2014). For pumping rate, the number of pharyngeal contractions of 12 randomly selected nematodes was counted under microscope for 1 min (Chen et al., 2014).

Locomotion behavior was analyzed by WormLab tracking system (MBF Bio-science, Williston, VT). Low-peptone NGM plates were covered with a thin layer of *E. coli* OP50 spread at 5 min before tracking (Ward et al., 2009). Worms were transferred to the plates and allowed to acclimate for 30 min before the tracking started. A 2-min recording (7.99 frames/second) was captured. WormLab software was then used to track and analyze the moving behavior of 45–55 animals per treatment.

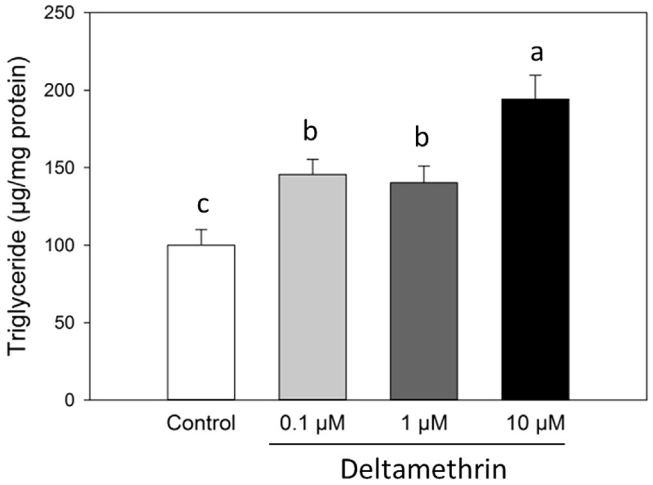

Fig. 1. Effect of deltamethrin on fat accumulation in 3T3-L1 adipocytes. Cells were treated with deltamethrin (0, 0.1, 1 and 10 μM) for 8 days. Triglycerides and protein were determined with commercial kits. Triglyceride content was normalized with protein concentration. Values represent different means ± S.E. (n = 12). [a-c]Means with different letters are significantly different ($p < 0.05$).