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Adulthood dietary exposure to a common pesticide leads to an obese-like phenotype and a diabetic profile in apoE3 mice

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ABSTRACT

Increasing evidence links the widespread exposure to organophosphate (OP) pesticides to the global epidemics of type 2 diabetes and obesity. Our recent data highlighted gene × environment interactions: mice expressing the human apolipoprotein E3 (apoE3) isoform were more prone to develop obesity than those expressing apoE2 or apoE4 upon dietary challenge with chlorpyrifos (CPF), the most used OP worldwide. Thus, we aimed to further explore the contribution of the *APOE3* genotype on the emergence of obesity and related metabolic dysfunctions upon subchronic exposure to CPF. Seven-month-old targeted replacement apoE3 and C57BL/6N male mice were orally exposed to CPF at 0 or 2 mg/kg body weight/day for 8 consecutive weeks. We examined body weight status, food and water intake, lipid and glucose homeostasis, metabolic biomarkers concentrations, insulin levels and insulin resistance, and leptin and ghrelin profiles. CPF exposure generally increased food ingestion, glucose and total cholesterol concentrations, and tended to elevate acyl ghrelin levels. Nonetheless, excess weight gain and increased leptin levels were inherent to apoE3 mice. Moreover, the propensity towards a diabetic profile was markedly higher in these animals than in C57BL/6N, as they showed a higher homeostatic model assessment for insulin resistance index and higher insulin levels. Although both genotypes were metabolically affected by CPF, the results of the present investigation revealed that apoE3 mice were the most vulnerable to developing obesity and related disturbances following CPF administration through the diet. Since the *APOE3* genotype is the most prevalent worldwide, current findings have particular implications for human health.

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1. Introduction

Over the last two centuries, the human lifespan has increased markedly because the development of industrialized societies has led to an improved quality of life. In this context, individuals are constantly and unconsciously exposed to a wide range of xenobiotics, the long-term effects of which are often unknown. Despite its obvious neurotoxic effect (Eaton et al., 2008), chlorpyrifos (CPF) is still the most widely used organophosphate (OP) pesticide in Europe, for both agricultural and urban purposes. It has been classified as a potent inhibitor of both systemic and brain cholinesterases (ChE), leading to the onset of acute neurotoxic symptomatology. However, an increasing body of reports have suggested that CPF also disrupts the serotonergic neurotransmitter system (Slotkin et al., 2015), targets serine hydrolase enzymes (Quistad et al., 2006b) and interferes with the signaling of hormones, some of which – for example, insulin and leptin-are related to energy homeostasis (Lassiter and Brimijoin, 2008; Slotkin et al., 2005). In accordance, sundry investigations have shown that CPF exposure induce a broad spectrum of effects, including metabolic disturbances (Lasram et al., 2014; Peris-Sampedro et al., 2014).

Abbreviations: OP, organophosphate; apoE, apolipoprotein E; CPF, chlorpyrifos; DTNB, 5, 5'-dithiobis-(2-nitrobenzoic acid); HOMA-IR, homeostatic model assessment for insulin resistance; ChE, cholinesterase; WHO, World Health Organization; apoE TR mouse model, apoE targeted replacement mouse model; AEBSF, 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride; AST, aspartate transaminase; ALT, alanine transaminase; ACh, acetylcholine; BChE, butyrylcholinesterase

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Type 2 diabetes accounts for over 90% of all cases of diabetes. Sedentary lifestyle, obesity, careless dietary habits, low socio-economic status and genetic vulnerability are well-known risk factors that contribute to its emergence (Zimmet et al., 2001). Nowadays, the prevalence of obesity and type 2 diabetes worldwide is increasing at epidemic rates. According to the World Health Organization (WHO), 13% of the adult population was obese (body mass index ≥ 30 kg/m^2) in 2014, while the predictions of the incidence of type 2 diabetes are not very encouraging, pointing to 366 million type 2 diabetes patients in 2030 (Wild et al., 2004). In the light of this trend, the risk factors commonly studied fail to explain by themselves the global boom of both diseases. Hence, "non-traditional" risk factors have been reconsidered (Arrebola et al., 2015; Howell et al., 2015). Some epidemiological evidence links general pesticide exposure (Arrebola et al., 2013, 2015; Suarez-Lopez et al., 2015) and more specifically OP exposure (Montgomery et al., 2008; Saldana et al., 2007) to a higher incidence of type 2 diabetes and related metabolic dysfunctions. Nevertheless, experimental studies are scarce. Very little research has investigated the metabolic and endocrine effects that emerge following adulthood exposure to CPF in rodents, being most studies focused on early-life exposure (Lassiter and Brimijoin, 2008; Slotkin et al., 2005). Current knowledge of adulthood exposure to CPF is limited to four studies carried out in rats. From these, two revealed a weight gain in treated subjects (Ehrich et al., 2004; Meggs and Brewer, 2007) and the other two pointed to disturbances of both glucose and lipid metabolisms in exposed animals (Acker and Nogueira, 2012; Elsharkawy et al., 2013). In general, these protocols were based on high CPF doses.

Apolipoprotein E (apoE) is a glycoprotein mainly involved in the maintenance of plasma lipid homeostasis, and is basically synthesized in the liver, but also in the brain and adipose tissue (Frühbeck, 2004; Gee and Keller, 2005). The human *APOE* gene is polymorphic and presents three major allelic variants (*ε2*, *ε3*, *ε4*), coding for three main isoforms associated with a low-to-high prevalence following the apoE2 < apoE4 < apoE3 rank order (Corbo and Scacchi, 1999). While apoE3 is accepted as the healthy phenotype, recent experimental data have shown that it tends to be more prone to developing diet-induced obesity (Arbones-Mainar et al., 2008; Huebbe et al., 2015; Karagiannides et al., 2008), and more vulnerable to decabromodiphenyl ether (Reverte et al., 2013). In a recent study, we found that apoE3 mice were more vulnerable to gain excess weight upon CPF exposure than apoE2 and apoE4 mice (Peris-Sampedro et al., 2015).

The apoE targeted replacement (TR) mouse model was originally created by Sullivan et al. (1997). These animals have a C57BL/6N background but their murine *apoE* gene has been replaced by one of the three most prevalent human *APOE* alleles. Thus, apoE TR mice differ from C57BL/6N in that they carry and express functional human apoE isoforms at physiological levels. It has been established that this expression does not alter any known endogenous regulatory sequence (Sullivan et al., 1997), being the subsequent phenotype in mice similar to that found in humans (Hauser et al., 2011).

Based on our previous results and from evidence gathered in the literature, the main objectives of the current investigation were: (a) to provide greater insight into the metabolic disturbances, ranging from hormonal imbalance to disturbed eating behavior, as a result of CPF exposure, and (b) to investigate how the human *ε3* allele might favor their emergence. For these purposes, the metabolic profile of both apoE3 and C57BL/6N male mice were assessed and compared after an 8-week period of oral exposure to CPF.

2. Material and methods

2.1. Chemicals

CPF (*O*,*O*-diethyl *O*-3,5,6-trichloropyridin-2-yl phosphorothioate, purity 99.5%) was supplied by Sigma-Aldrich (Seelze, Germany). Standard rodent chow (Panlab, Barcelona, Spain) was supplemented with CPF at a concentration intended to deliver a dose of 2 mg/kg body weight/day, based on the results of our recent study (Peris-Sampedro et al., 2015). The protease inhibitor 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride (AEBSF) was also purchased from Sigma-Aldrich.

2.2. Animal care

Seven-month-old apoE TR male mice and C57BL/6N male mice were used. Mice homozygous for the human *ε3* allele were obtained from Taconic (Taconic Europe, Lille Skensved, Denmark), and C57BL/6N mice were purchased from Charles River (Charles River France, L'Arbresle, France). After a quarantine period, the animals were properly housed in plastic cages containing 2–3 individuals in an environmentally controlled room equipped with a 12-h light–dark automatic light cycle (light: 08:00–20:00 h), a temperature of 22 ± 2 °C, and a relative humidity of 50 ± 10%. Mice were allowed access to food and fresh water *ad libitum* and given a standard chow diet (Panlab, Barcelona, Spain) before the experiment started. The use of animals and the experimental protocol design were supervised and approved by the Animal Care and Use Committee of the Rovira i Virgili University (Tarragona, Spain). Likewise, efforts were made to alleviate animal suffering as established by the Spanish Royal Decree 53/2013 and and the European Communities Council Directive (86/609/EEC).

2.3. Treatment protocol

The animals were weighed and then distributed into four experimental groups ($n=10$/group): control apoE3, control C57BL/6N, CPF-exposed apoE3, and CPF-exposed C57BL/6N. Mice were fed either a standard or a CPF-supplemented rodent chow (2 mg/kg body weight/day) for 8 consecutive weeks, and were checked for cholinergic signs twice a week. After the treatment period, animals were subjected to a 3-h fast before being anesthetized with carbon dioxide and euthanized by cardiac puncture. Blood was immediately collected into 500 μL tubes containing EDTA (BD Microtainer®, Plymouth, United Kingdom), and centrifuged at 3000 rpm for 20 min at 4 °C to obtain plasma, which was aliquoted and stored at −80 °C.

2.4. Plasma cholinesterase activity

Plasma ChE activity was evaluated as an indicator of the systemic CPF effect (Eaton et al., 2008). It was determined spectrophotometrically using a commercial available kit, as recommended by the supplier. Briefly, the cholinesterase enzyme hydrolyzes butyrylthiocholine to give thiocholine and butyrate. The reaction between thiocholine and 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) produces 2-nitro-5-mercaptobenzoate, a yellow compound which can be measured at 405 nm. The enzymatic activity of exposed animals was calculated on the basis of the activity value of the control mice, and represented as a percentage.

2.5. Body weight status and food and water consumption

The body weight status of the mice was recorded weekly over the treatment period. Food intake was estimated on a daily basis for a 7-day period by subtracting the uneaten pellets at the end of

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