Toxicology Letters 215 (2012) 8–15

Contents lists available at SciVerse ScienceDirect

Toxicology Letters

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

# Metabolic impacts of high dietary exposure to persistent organic pollutants in mice☆

Mohammad Madani Ibrahim[a,b], Even Fjære[a,c], Erik-Jan Lock[a], Livar Frøyland[a], Niels Jessen[d], Sten Lund[e], Hubert Vidal[f], Jérôme Ruzzin[a,g,*]

[a] National Institute of Nutrition and Seafood Research, Postboks 2029, 5817 Bergen, Norway
[b] Institute of Biomedicine, University of Bergen, Postboks 7804, 5020 Bergen, Norway
[c] Department of Biology, University of Copenhagen, Ole Maaløes Vej 5, 2200 Copenhagen, Denmark
[d] Aarhus University Hospital, Research Laboratory for Biochemical Pathology, DK-8000 Aarhus, Denmark
[e] Aarhus University Hospital, Department of Internal Medicine and Diabetes and Institute of Experimental Clinical Research, DK-8000 Aarhus, Denmark
[f] INSERM U-1060, INRA U-1235, Lyon 1 University and CarMeN Laboratory, F-69921 Oullins, France
[g] Department of Biology, University of Bergen, Postboks 7803, 5020 Bergen, Norway

## HIGHLIGHTS

- Persistent organic pollutants are potent endocrine disruptors.
- Elevated dietary exposure to POPs reduced body weight gain in mice.
- Despite elevated POP accumulation, mice remained sensitive to insulin.
- Nutrition may modulate the toxicity of environmental pollutants.

## ARTICLE INFO

*Article history:*
Received 6 June 2012
Received in revised form
24 September 2012
Accepted 26 September 2012
Available online 4 October 2012

*Keywords:*
Endocrine disruptors
Metabolic syndrome
Type 2 diabetes
Pollution
Xenobiotics
Marine food

## ABSTRACT

Persistent organic pollutants (POPs) have been linked to metabolic diseases. Yet, the effects of high exposure to dietary POPs remain unclear. We therefore investigated whether elevated exposure to POPs provided by whale meat supplementation could contribute to insulin resistance.

C57BL/6J mice were fed control (C) or very high-fat diet (VHF) containing low or high levels of POPs ($VHF_{+POPs}$) for eight weeks. To elevate the dietary concentrations of POPs, casein was replaced by whale meat containing high levels of pollutants.

Feeding $VHF_{+POPs}$ induced high POP accumulation in the adipose tissue of mice. However, compared with VHF-fed mice, animals fed $VHF_{+POPs}$ had improved insulin sensitivity and glucose tolerance, and reduced body weight. Levels of ectopic fat in skeletal muscles and liver were reduced in mice fed $VHF_{+POPs}$. These mice also gained less adipose tissue and had a tendency to reduced energy intake. In pair-feeding experiments, improved insulin action and reduced body weight gain were still observed in $VHF_{+POPs}$ compared to VHF pair-fed mice.

We concluded that mice fed VHF contaminated with POPs derived from whale meat remain sensitive to insulin and glucose tolerant despite significant body burden of POPs. This indicates complex interactions between organic pollutants and nutrition in the development of metabolic disorders.



*Abbreviations:* ANOVA, analysis of variance; C, control diet; DDTs, dichloro-diphenyl-trichloroethanes; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; VHF, high-fat diet; $VHF_{+POPs}$, high-fat diet containing high levels of POPs; HxCDD, hexachlorodibenzo-*p*-dioxin; ip, intraperitoneal; LSD, least-square difference; n-3 PUFAs, n-3 polyunsaturated fatty acids; PCBs, polychlorinated biphenyls; POPs, persistent organic pollutants; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TAG, triacylglycerols.

☆ *Funding sources*: JR and MMI were supported by the Research Council of Norway (#204473 and #185567, respectively). EF was supported by the Danish Council for Strategic Research (#2101-08-0053).

* Corresponding author at: Department of Biology, University of Bergen, Postboks 7803, 5020 Bergen, Norway. Tel.: +47 55584400; fax: +47 55584450.
*E-mail address:* jerome.ruzzin@bio.uib.no (J. Ruzzin).

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http://dx.doi.org/10.1016/j.toxlet.2012.09.022

## 1. Introduction

The origins of type 2 diabetes remain poorly known, and the incidence of this disease continues to increase at alarming rates worldwide (Danaei et al., 2011). Although physical inactivity and excess energy intake have been traditionally viewed as important risk factors, there is evidence that some environmental pollutants, like persistent organic pollutants (POPs), can act as endocrine disruptors and contribute to metabolic disorders and type 2 diabetes (Alonso-Magdalena et al., 2011; Ruzzin et al., 2012). POPs are hazardous chemicals, like dioxins, polychlorinated biphenyls (PCBs) and organochlorine pesticides, which are mostly produced through industrial activities and are omnipresent in the food chain. Because of their lipophilic properties and high resistance to degradation, POPs bio-accumulate in fatty rich tissues of organisms for many years. In the general population, POP exposure mainly occurs through intake of animal fat, like fatty fish, milk and meat products (Fromberg et al., 2011; Schecter et al., 2010), and this chronic background exposure to POP mixtures may represent a potential risk to human health (Ruzzin, 2012).

Initially recognized for their ability to promote cancer and affect reproductive functions (Skene et al., 1989), numerous studies reported a positive association between the exposure to low doses of POPs and diabetes, which has been especially linked to organochlorine pesticides and PCBs (Airaksinen et al., 2011; Lee et al., 2006, 2010, 2011; Rignell-Hydbom et al., 2009; Turyk et al., 2009; Vasiliu et al., 2006). There is also experimental evidence for a causal role of POPs in the development of metabolic disorders linked to insulin resistance. Rats fed crude salmon oil containing environmental levels of POPs developed insulin resistance, visceral obesity, non-alcoholic fatty liver, and chronic low-grade inflammation, whereas animals fed refined salmon oil with low POP concentrations did not (Ruzzin et al., 2010). Furthermore, we have demonstrated that, among different POP mixtures mimicking those present in crude salmon oil, mixtures of dichloro-diphenyl-trichloroethanes (DDTs) and PCBs had the strongest inhibitory effect on insulin action in 3T3L1 cells (Ruzzin et al., 2010). More recently, the presence of POPs in a high-fat diet (VHF) was found to negatively modulate the health outcomes associated with farmed salmon fillet consumption (Ibrahim et al., 2011).

The effects of high POP exposure on insulin resistance and type 2 diabetes in humans remain, however, unclear. Indeed, while prospective studies of people exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) or PCBs in accidental or occupational settings have reported increased risk of diabetes, impaired glucose metabolism or insulin resistance (Bertazzi et al., 1998; Longnecker and Michalek, 2000; Vena et al., 1998; Wang et al., 2008), other studies reported no clear positive association between high exposure to TCDD and diabetes (Steenland et al., 1999; Zober et al., 1994). Furthermore, in the Greenland Inuit population, who is highly exposed to POPs due to the consumption of large amount of marine mammals like whale or seal (Bjerregaard et al., 2001), no association between POPs and insulin resistance or glucose intolerance was found (Jorgensen et al., 2008). More recently, a role of nutrients in the modulation of toxicity of environmental pollutants was emphasized (Hennig et al., 2012).

To get insight into the relationships between organic pollutants and nutrition in metabolic disorders, the aim of the present study was to assess whether mice submitted to VHF and exposed to elevated dietary concentrations of POPs from marine mammal meat developed insulin resistance and glucose intolerance, two critical features of type 2 diabetes.

## 2. Methods

### 2.1. Animals

In the present study, we used male mice, and animal experiments were performed in accordance with the Norwegian State Board of Biological Experiments with Living Animal. Eight-week old male C57BL/6J mice were purchased from Taconic (Ry, Denmark). At arrival, mice were allowed to acclimatize for five days, and were housed on a 12-h-light/-dark cycle with free access to chow (C; 2018 Teklad Global, Harlan Laboratories, The Netherlands) and tap water. Then, animals were weight matched and assigned to C, VHF or VHF containing elevated amount of POPs through the incorporation of whale meat in the diet ($VHF_{+POPs}$). Since we previously used VHF to investigate the impacts of POPs present in fatty fish (Ibrahim et al., 2011), a similar dietary model was performed in the present investigation to compare our different studies. Additional animals were submitted to pair-feeding experiment. In this trial, food intake of *ad libitum* $VHF_{+POPs}$-fed mice was measured every day at 10.00 AM, and the following day, the individually housed VHF-fed mice were given the same amount of food. All animals were fed for eight weeks, and body weight and food intake were assessed weekly.

### 2.2. Experimental diets

VHF was prepared as described previously (Ibrahim et al., 2011), and $VHF_{+POPs}$ was made by using meat of whale, a high trophic marine mammal accumulating considerable amount of POPs (Table 1). Level of protein in the diets was adjusted at the expense of carbohydrate to obtain an isonitrogenous content whereas corn oil was adjusted according to the levels of lipid present in casein and whale meat. Accordingly, VHF and $VHF_{+POPs}$ contained (in percentage of calories), 16% protein, 12% carbohydrate (sucrose), 72% fat (50% lard and 50% corn oil for VHF and 50% lard, 33% corn oil and 17% whale oil from whale meat for $VHF_{+POPs}$), and were isocaloric.

### 2.3. Blood glucose and plasma insulin

Blood was collected from tail vein of animals one week before the end of the feeding trial. Blood glucose levels were measured with a glucometer (Ascensia Contour, Bayer Healthcare, Oslo, Norway) whereas plasma insulin concentrations were determined using an ELISA kit (Mouse Insulin Ultrasensitive ELISA, DRG, Marburg, Germany).

### 2.4. Insulin and glucose tolerance tests, and assessment of beta cell function

After seven weeks, an intraperitoneal (ip) injection of insulin (1.0 U/kg body weight), or glucose (1.0 mg/g body weight) was performed in fed and fasted (5 h) mice, respectively. Blood glucose levels were measured before the injection and after 15, 30, 60, and 90 min. Other fasted animals received an ip injection of glucose (2 mg/g body weight), and plasma insulin was measured at 0 and 15 min post-injection.

### 2.5. Ex vivo muscle glucose uptake

*Ex vivo* muscle glucose uptake was carried out as described previously (Ruzzin and Jensen, 2005). Briefly, slow-twitch soleus muscles were mounted on apparatuses at their approximate resting length. Soleus muscles were pre-incubated for at least 30 min at 30 °C in flask containing 6 mL of Krebs–Henseleit buffer with 5.5 mM glucose, 2 mM pyruvate, 5 mM HEPES, and 0.1% BSA, pH 7.4, under continuous flow of gas (95% $O_2$–5% $CO_2$). After pre-incubation, muscles were incubated in Krebs–Henseleit buffer containing 0.25 μCi/mL 2-[1,2$^3$H(N)]-deoxy-D-glucose (DuPont, New England Nuclear) and 0.1 μCi/mL [1-$^{14}$C]-D-mannitol (DuPont, New England Nuclear) without or with the addition of insulin (10,000 μU/mL), and glucose uptake assessed as described previously (Ruzzin et al., 2005).

### 2.6. Tissue sampling

Mice were fasted overnight and sacrificed by cardiac puncture under isoflurane anesthesia. Liver, gastrocnemius muscles, and white adipose tissue (epipidymal, retroperitoneal and inguinal fat pad) were quickly removed, weighed, snap-frozen in liquid nitrogen, and stored at −80 °C for further analysis.

### 2.7. Liver and muscle triacylglycerols (TAG)

Concentrations of TAG in liver and gastrocnemius muscles were determined in frozen samples by using high performance thin layer chromatography as described previously (Ruzzin et al., 2010).

### 2.8. Assessment of fat absorption

Total lipid excreted in feces was analyzed as described before (Ibrahim et al., 2011).