

Liver metabolic disruption induced after a single exposure to PCB126 in rats

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Abstract Polychlorinated biphenyls (PCBs) have been recognized as metabolic disruptors. The liver plays a pivotal role in detoxification of an organism. Fatty liver results from altered intra-, and extra-hepatic mediators and is associated with increased glucose-related protein 78 (GRP78), commonly used as a marker for endoplasmic reticulum (ER) stress signaling. This pilot study aimed to study the effects of a single exposure on fatty liver metabolic parameters. The objective of the study is to characterize the effects of 3,3',4,4',5-pentachlorobiphenyl (PCB126) on ER stress protein chaperon GRP78 and CCAAT-enhancer-binding protein homologous protein (CHOP) and intra-hepatic mediators such as microsomal triglyceride transfer protein (MTP), sterol regulatory element-binding protein 1c (SREBP1c), and peroxisome proliferator-activated receptor alpha (PPAR α), as well as extra-hepatic factors such as non-esterified fatty acid (NEFA) and tumor necrosis factor alpha (TNF α). Hepatic GRP78 mRNA and protein levels, indicating the presence of ER stress, were significantly increased following a single PCB126 exposure in rats. Intra-hepatic mechanisms such as lipoprotein secretion pathway (i.e., MTP), lipogenesis de novo (i.e., SREBP1c), and oxidation (i.e., PPAR α) were altered in PCB126-treated rats. In addition, a state of inflammation

measured by higher TNF α plasma levels was present in contaminated rats. These data indicate that a single injection of PCB126-modulated expression of GRP78 associated with hepatic ER stress and systemic inflammation in rats.

Keywords Hepatic steatosis · Rats · Inflammation · Unfolded protein response · PCB126

Introduction

Polychlorinated biphenyls (PCBs) were commercially produced as dielectric insulating fluids for transformers and capacitors. However, as a result of increasing environmental PCBs residues during the 1960s and 1970s, production and use of PCBs have been banned for over 30 years in most industrialized countries (Agency 2002). PCBs are widely distributed, mostly found in air, soil, and water. Most of the animal studies that have investigated the effects of PCBs were carried out with commercial mixtures. Working with mixtures makes it difficult to interpret the toxicity of single congeners. According to the toxic equivalency factor (TEF) who were developed for dioxin-like compounds and used for estimating the potential risk associated with exposure to these compounds, PCB126 is considered the most toxic PCB congener in the environment among a family of 209 congeners dioxin-like compounds (Van den Berg et al. 2006; Lee and Yang 2012; Liu et al. 2016; van Ede et al. 2016). The role of PCB126 in the development of metabolic diseases is not fully described. However, it has been shown that in vivo PCB126 exposure induces alterations in preadipocytes metabolism (Gadupudi et al. 2015) and impairs glucose homeostasis (Baker et al. 2013).

In addition to functions in intracellular calcium reserve and hepatic lipoprotein metabolism, the endoplasmic reticulum

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(ER) also plays a crucial role in protein metabolism. The ER ensures quality control in protein synthesis in the organism. Therefore, properly folded proteins are conducted in secretory pathway, while misfolded proteins are degraded by proteasomes via a process called ER-associated degradation (ERAD) (Ellgaard et al. 1999). High concentrations of chaperone-folding proteins, such as GRP78, are usually present in the ER lumen ensuring a balance between proteins influx and folding ability (Munro and Pelham 1986). Chaperone protein GRP78 is the most abundant member of the heat shock protein 70 kDa (Hsp70) family in the ER lumen, reaching a concentration in the millimolar range (Guth et al. 2004). Thus, GRP78 is a main regulator of the unfolded protein response (UPR) and is commonly used as a marker for ER stress signaling (Malhotra and Kaufman 2007). When these translational and transcriptional pathways are insufficient to restore homeostasis, components of apoptosis pathways are then engaged by CHOP (Oyadomari and Mori 2004).

Because PCBs have been related to metabolic disorder such as fatty liver, we hypothesized that PCB126 can alter lipid accumulation pathways accompanied with the presence of ER stress. Therefore, the main objective of this study is to assess the effects of a single congener, PCB126, exposure on non-alcoholic fatty liver disease (NAFLD) and ER stress-related molecular mechanisms and potential mediators.

Methods

Animal care Female Sprague-Dawley strain rats (Charles River, St-Constant, Quebec, Canada), weighing 200–250 g upon their arrival were housed in pair and had ad libitum access to food and tap water. Their environment was controlled in terms of light (12:12-h light-dark cycle starting at 6:00 a.m.), humidity, and room temperature (20–23 °C). Following 1 week of acclimatization, animals were given a single i.p. injection of vehicle (corn oil; 0.14 ml/kg body weight) or vehicle with 1.05 µmol/kg body weight (326 µg/kg body weight) of PCB126 (eight rats per group). Animals were sacrificed after a week from the PCB 126 challenge. This method of exposure was chosen based on two recent studies, in which a single administration of PCB 126 at 1 µmol/kg was shown to induce fatty liver (Lai et al. 2010; Boucher et al. 2015). All procedures were approved by the Animal Care Committee of the University of Ottawa and adhered to the guidelines established by the Canadian Council on Animal Care.

Tissue sampling Seven days after the injection and after complete anesthesia (sodium pentobarbital, 65 mg/kg, i.p.), the abdominal cavity was rapidly opened following the median line of the abdomen. Blood was rapidly (<45 s) drawn from

the abdominal vena cava (~4 ml) and transferred into tubes pretreated with 15 % ethylenediaminetetraacetic acid (EDTA). Blood was centrifuged (3000×g for 10 min, 4 °C), and the plasma was stored at –80 °C until analyses. The liver was excised, and the median lobe was immediately frozen and used for triacylglycerol determination and protein quantification.

Western immunoblotting Briefly, 200 mg of liver were homogenized in liver lysis buffer (SDS 2.3 %, EDTA 1 mM, ethyleneglycotetraacetic acid 1 mM, sodium fluoride 20 mM in phosphate-buffered saline (PBS) with sodium orthovanadate (Na₃VO₄) 1 mM and protease inhibitor cocktail using a polytron, sonicated and centrifuged at 13,000×g, 4 °C for 20 min. The infranatant was collected with a blunt-tipped Pasteur pipette and stored at –80 °C until protein determination. All samples (50 µg of proteins) were separated on a 10 % SDS-polyacrylamide gel and electrotransferred onto nitro-cellulose membranes. Membranes were blocked 1-h in PBS containing 0.05 % Tween 20 (PBST 0.05 %) and 5 % non-fat dry milk at 4 °C. The blot was then incubated with primary antibodies overnight. Protein quantification of GRP78 (cat no. NB300-520, Novus Biologicals, Littleton, CO) was used to assess the presence of ER stress; CHOP (cat no. L63F7, Cell Signaling, Danvers, MA) to determine cell apoptosis; and cytoplasmic protein FABP1 (cat no. AF1565, Novus Biologicals, Littleton, CO) to evaluate the binding and transport of intracellular non-esterified fatty acid (NEFA). After five washes in PBS-Tween 20 (0.05 %), the membrane was incubated for 45 min with secondary antibody at room temperature. Then, the membrane was washed five times for 15 min each time in PBST (0.05 %) before revealing with West Pico (Fisher Scientific Company). The resulting signal was acquired with ChemiDoc-It Imaging System (Fisher Scientific Company), and the bands were quantified with the use of VisionWorks@LS (UVP, LLC, Upland, CA) software and expressed as arbitrary units. Equal protein loading was determined using β-actin (Sigma, Saint Louis, MO, USA).

RNA extraction and cDNA synthesis Total RNA was extracted using TRIzol from Invitrogen Live Technologies (cat no. 15596-026) following the manufacturer's instructions, with the exception that samples were homogenized in 1 ml of TRIzol in a Retsch Mixer Mill Type MM301 for 3 min at 20 beats/s. The RNA was precipitated overnight at –20 °C. The RNA pellet was air dried and then re-suspended in 500 µl of UltraPure Distilled water (Invitrogen cat no. 10977-015) and stored at –80 °C. RNA concentration was determined using a NanoDrop 2000 from Thermo Scientific. A total of 500 ng of RNA was then reverse transcribed using iScript complementary DNA (cDNA) synthesis kit from BioRad (cat no. 170-

8890) in a total volume of 20 μ l. The manufacturer's instructions were followed exactly and cDNA was stored at -20°C .

Quantitative RT-PCR Real-time experiments were conducted on a BioRad CFX machine (BioRad cat no. 184-5096) using SsoFast Evagreen Supermix (cat no. 172-5201) following the manufacturer's instructions. The final primer concentration was 0.2 μM ; 3 μ l of template were used in all reactions. GAPDH was used as the housekeeping gene (see Table 1 for primer details). Samples were diluted as follows: 5 fold for primers Hspa5; 25 fold for primers Mtp and GAPDH; and 50-fold for Ppia. Five microliters of PCR product from a sample with high expression and the controls was analyzed on a 2.0 % agarose gel in 0.5 \times TBE at 90 V for 60 min with 0.25 μg quick-load 50 bp DNA ladder and all samples were confirmed to be the correct size.

Droplet digital PCR After running this series of tests, it was decided to try Srebfl in droplet digital PCR (ddPCR) because of the low expression of this sample. QX200 ddPCR EvaGreen Supermix (BioRad cat no. 186-4033) was used as per the manufacturer's instructions with a final primer concentration of 0.2 μM . Three microliters of cDNA was added to a total reaction volume of 23 μ l. Twenty microliters of the PCR reaction and 70 μ l of droplet generation oil (BioRad cat no. 186-4005) were used to generate droplets QX200 Droplet Generator (BioRad cat no. 186-4002). A total of 40 μ l of the droplets was transferred to a 96-well PCR plate (Fisher cat no. 951020303) and heat sealed (PX1 PCR plate sealer, BioRad cat no. 181-4000 and cat no. 181-4040) for 5 s at 180°C . The following cycling protocol was used: 95°C 5 m (95°C 30 s, T_{A} 1 m ramp rate of $2^{\circ}\text{C}/\text{s}$) \times 40, 4°C 5 m, 90°C 5 m, and hold at 10°C (C1000 Touch Thermocycler, BioRad cat no. 184-1196). Samples were then counted by QX200 Droplet Reader and QuantaSoft Software (BioRad cat no. 186-4003).

Analytical procedures Plasma TNF α was measured by enzyme-linked immunosorbent assay (ELISA) (EMD Millipore Corporation, cat no. EZRTNFA). Plasma NEFA was determined by a coupled enzyme assay and read by colorimetry at 570 nm with a commercial kit (Sigma-Aldrich, St. Louis, MO, USA, cat. no. MAK044).

Statistical analysis Values are expressed as mean \pm S.E. Statistical analyses were performed using a one-way analysis of variance (ANOVA) for non-repeated measures. Prior to analysis, data were assessed for homogeneity of variance by use of Levene's test and for normality by use of the Kolmogorov-Smirnov test, and data were log-transformed if necessary. Significance was accepted at $P < 0.05$. All analyses were performed using SPSS Statistics 20.0, SPSS Inc., IL, USA.

Results

No changes in body weight were noticed between PCB126 and corn oil groups (320 ± 4 vs 315 ± 6 g; $P = 0.48$) (data not shown).

PCB126 exposure increases GRP78 mRNA and protein expression but not apoptosis

There was a significant increase by 2.5-fold of GRP78 protein content (1.2 ± 0.1 vs 3 ± 0.6 AU; $P < 0.01$) (Fig. 1a) and gene expression by 1.5-fold (0.9 ± 0.1 vs 1.4 ± 0.2 AU; $P < 0.05$) (Fig. 1b) in rats treated with PCB126 compared to vehicle-treated rats. However, exposure to PCB126 did not change CHOP protein content (Fig. 1c).

PCB126 exposure increases circulating NEFAs and inflammation factor

A single treatment with PCB126 in rats resulted in a significant elevation of the predominant extra-hepatic fatty liver contributing factor, plasma NEFA, compared to corn oil-treated rats (0.25 ± 0.02 vs 0.37 ± 0.3 nmol; $P < 0.05$) (Fig. 2a). In addition, extra-hepatic potential ER stress contributing mediator and inflammation factor plasma TNF α levels were significantly higher (100 %) following PCB126 injection compared to placebo (7.3 ± 1.2 vs 14.8 ± 2.9 pg/ml; $P < 0.03$) (Fig. 2b).

Table 1 Gene accession

GOI	Accession no. NCBI	Sense primer	Antisense primer	Amplicon length (bp)
GAPDH	NM_013083	ccctctggaagctgtgg	agtggatgcagggatgatg	63
Hspa5	NM_031347	ccgtaacaatcaaggtctacga	aaggtgacttcaatctgggta	124
Srebfl	L16995	tacagcgtggctgggaac	ggctgagcgatacagttcaa	98
Mtp	NM_009127	gcgagtctaaaaccgagtg	cactgtgatgtcgtggttatt	62
Ppia	NM_001107727.1	ccaaacacaaatggtccagct	attcctggacccaaaacgct	135

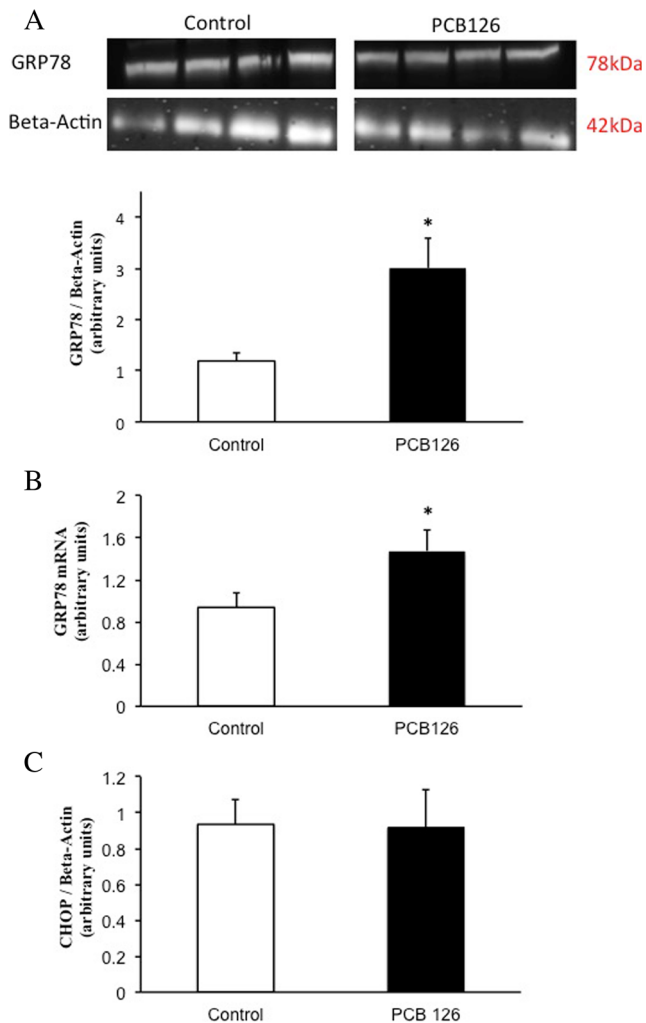


Fig. 1 One-week PCB126 post-injection is related to the presence of ER stress. Protein content of ER stress marker GRP78 assessed by western blot is increased (a). Similar high results are seen in GRP78 gene expression (mRNA) (b). Exposure to PCB126 for 1 week does not affect apoptosis measured by marker CHOP (c). Significant difference from control rats (* $P < 0.05$). $n = 7-8$. Control = white; PCB126 = black. Values are means, with their standard errors represented by vertical bars

PCB126 exposure alters protein expressions related to hepatic lipid accumulation

NAFLD in these rats was confirmed in a previously published study by our laboratory where rats with a single PCB126 injection depicted 64 % higher hepatic triglycerides TAG concentrations after 1 week compared to control group administered corn oil (21.3 ± 1.3 vs 35.2 ± 3.1 mg/g; $P < 0.001$) (Fig. 3a) (Boucher et al. 2015). FABP1 was higher after 1 week from an acute injection of PCB126 compared to the corn oil group (1.2 ± 0.1 vs 0.75 ± 0.1 AU; $P < 0.02$) (Fig. 3b). qRT-PCR demonstrated an alteration in liver lipoprotein production and exportation key molecule MTP expression that was significantly decreased by 32 % following injection of PCB126 compared to corn oil injected rats (1.1 ± 0.1 vs

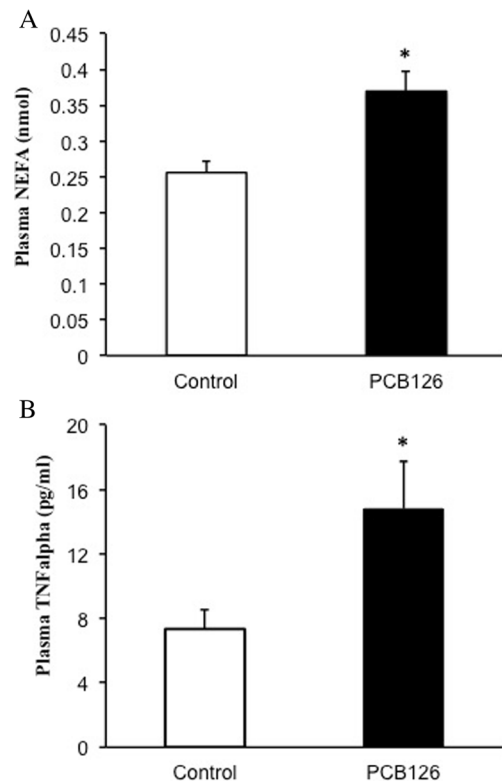


Fig. 2 PCB126 acute exposure has an effect on extra-hepatic parameters. Plasma NEFA concentrations (nmol) were elevated in PCB126-injected rats (a). Plasma inflammatory marker TNF α (pg/ml) levels are increased after a PCB126 challenge (b). Significant difference from control rats (* $P < 0.05$). $n = 6-8$. Control = white; PCB126 = black. Values are means, with their standard errors represented by vertical bars.

0.7 ± 0.07 AU; $P < 0.04$) (Fig. 3c). Lipogenic transcription factor SREBP1c messenger RNA (mRNA) levels were significantly increased by 52 % in contaminated animals versus control group (0.046 ± 0.009 vs 0.07 ± 0.01 AU; $P < 0.05$) (Fig. 4). PCB126 also significantly increased by 110 % genetic expression of lipid oxidation transcription factor PPAR α , compared to placebo rats (0.86 ± 0.12 vs 1.82 ± 0.4 AU; $P < 0.04$) (Fig. 5).

Discussion

The main finding of the present study is that a single injection of PCB126 was sufficient to induce ER stress development in liver measured by chaperone molecule GRP78. Moreover, intra-hepatic molecules and extra-hepatic factors contributing to hepatic lipid accumulation were also altered by an acute exposure to PCB126. These novel results enlighten the fact that a single exposure to PCB126 induces molecular consequences to the liver. Most importantly, these molecular effects were observed as early as 1 week after a single injection of PBC126.

Our results provide some evidences that PCB126 exposure is an important lipid metabolism disruptor and could, therefore, account for the presence of hepatic ER stress in rats.

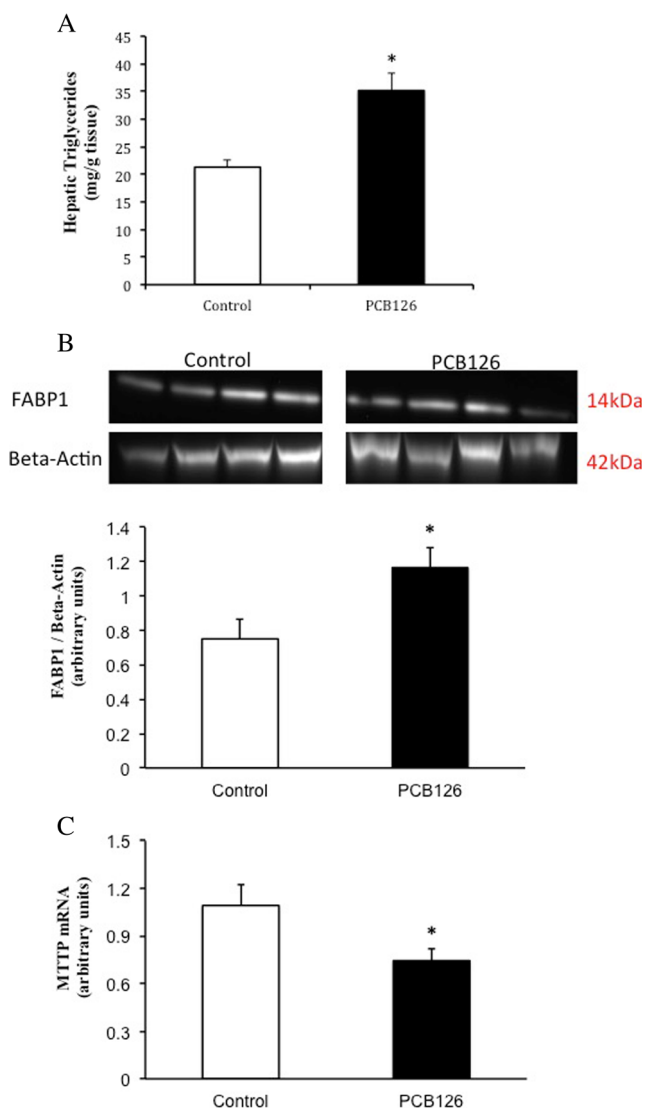


Fig. 3 The presence of fatty liver was confirmed in a previously published study by Boucher et al. (2015) reporting hepatic TAG content (mg/g tissue) in these same PCB126-injected rats. Significant difference from control rats ($*P < 0.05$). $n = 6-8$. Control = white; PCB126 = black (a). A single injection of PCB126 has an effect on intra-hepatic parameters. Western blot of intracellular plasma NEFAs transporter FABP1 protein content shows higher protein content in PCB126-contaminated rats compared to Control group (b). Decrease of very-low density lipoprotein production and exportation key-enzyme MTP gene expression (mRNA) in seen in PCB126-treated rat (c). Significant difference from control ($*P < 0.001$). $n = 9-11$. Control = white; PCB126 = black. Values are means, with their standard errors represented by vertical bars.

Western blotting and genetic expression assessments showed that compared to the control group, exposure to PCB126 in rats significantly increased hepatic GRP78 mRNA (1.5-fold) and protein content (2.5-fold) (Fig. 1). In line with our findings, an increase of 1.6-fold GRP78 protein expression has been found in islets of Langerhans following a 2-week PCB126 exposure in rats (Loiola et al. 2016).

As proposed by Oyadomari and Mori (2004) ER stress response seems to include two phases. The first early phase

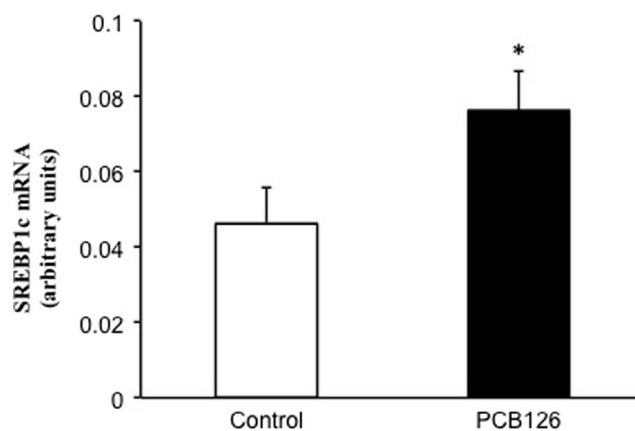


Fig. 4 PCB increases intra-hepatic molecular mechanism, lipogenesis de novo, assessed by measuring key-transcription factor SREBP1c gene expression (mRNA).

Significant difference from control rats ($P < 0.05$). $n = 7-8$. Control = white; PCB126 = black. Values are means, with their standard errors represented by vertical bars.

occurs to reduce mis- or un-folded protein accumulation in the ER through translational attenuation. If these mechanisms fail to reduce the mis- or un-folded protein load of the ER, the apoptosis pathway is then activated involving the CCAAT/enhancer-binding protein (C/EBP) homologous protein (CHOP) (Oyadomari and Mori 2004). Thus, our unchanged CHOP mRNA levels after 1 week exposure of PCB126 suggested that the liver of rats exposed during this study might have been in a first early phase of ER stress, as reported by Oyadomari and Mori (2004).

The development of hepatic ER stress is related to the presence of potential mediators such as TAGs. For example, incubation of McA-RH7777 hepatocytes with a TAG emulsion

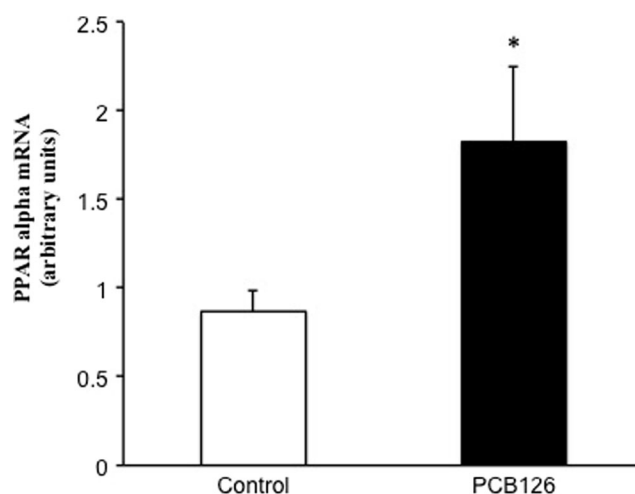


Fig. 5 Hepatic lipid oxidation measured by nuclear hormone receptor PPAR α mRNA levels in PCB126 injected rats is increased. Significant difference from control rats ($*P < 0.05$). $n = 8$. Control = white; PCB126 = black. Values are means, with their standard errors represented by vertical bars.

(500 mg/dl) for 6 or 16 h resulted in an increased GRP78 mRNA (2 to 4.5-folds) and GRP78 protein content after 16 h (2.7-fold) (Ota et al. 2008). Studies have demonstrated that the presence of environmental contaminants is thought to play a major role in hepatic TAG accumulation. We have recently reported that exposure to PCB126 for 1 week significantly increased hepatic TAG levels by 1.7-fold compared to corn oil injected rats (Fig. 3a) (Boucher et al. 2015). These studies strongly suggest a link between environmental contaminant exposure, accumulation of liver TAG, and increased GRP78 levels. However, the exact molecular mechanisms linking TAG accumulation to ER stress following environmental contaminants are still not fully elucidated.

Uptake Potential sources of TAG intrahepatic derive from circulating NEFAs (Gibbons and Burnham 1991). In our study, PCB126 exposure resulted in significant elevations of plasma NEFA levels compared to corn oil-injected group. In addition to stimulate TAG accumulation in the liver, it seems that NEFAs have also a direct role to induce hepatic ER stress. In C57BL/6J mice, infusion of oleic acid bound to albumin for 9 h resulted in increases of GRP78 mRNA and protein levels, ranging from 1.8-fold and 3-fold respectively, compared with saline infusions (Ota et al. 2008). Conversely, GRP78 overexpression attenuated the induction of ER stress by palmitate in HepG2 liver cells (Gu et al. 2010). Thus, the presence of high levels of plasma NEFAs could account in part for the presence of ER stress measured via increased hepatic GRP78 protein and gene levels seen in our PCB126-treated rats.

In our study, PCB126 exposure induced FABP1 protein expression by 55 % compared with our control rats. While FABP knockout mice fed a western diet accumulate significantly less hepatic TAG than western diet-fed controls (Newberry et al. 2006), a gain of function of FABP resulting from transfection with LFABP in rat hepatoma cell line McA-RH7777 resulted in an increase in FA uptake (Linden et al. 2002). Together, these observations are consistent with an indirect role for FABP1 in the development of ER stress in liver of rats following PCB126 exposure.

Export Microsomal triglyceride transfer protein (MTP) plays a central role in VLDL synthesis and secretion. It has been reported that deficiency of MTP in human and mice is associated with the development of hepatic steatosis (Raabe et al. 1999; Cuchel et al. 2007). Our results revealed that MTP mRNA levels were significantly decreased in rats after a week from a PCB 126 challenge. Accordingly, Sandberg and Glaumann (1980) reported that Aroclor 1254 administered daily (5 mg/100 g body weight), 6 to 8 times during 10 days gave rise to an accumulation of VLDL in the liver endoplasmic reticulum in rats. Thus, these observations suggest that exposure to PCB126 could contribute to hepatic lipid

accumulation by decreasing MTP protein content and, consequently, decreasing hepatic VLDL production and secretion.

Synthesis SREBP-1c is an ER localized transcription factor and is the major form expressed in the liver (Eberle et al. 2004). Transcription factor SREBP1c mRNA levels in our rats were higher following PCB126 exposure (Fig. 5). Accordingly, a study has shown a significant induction in the expression level of SREBP1c in rats fed farm salmon for 28 days containing various POPs including PCB126 (Ruzzin et al. 2010). Moreover, molecular links between ER stress and lipid biosynthesis have been demonstrated. Pharmacologic induced ER stress by tunicamycine, an agent known to promote unfolded or misfolded protein in ER (King and Tabiowo 1981), in C57Bl/6J mice leads to dramatic increases in cleaved (activated) SREBP1c protein levels in the liver (Lee et al. 2012). Accordingly, Werstuck et al. (2001) reported that homocysteine-induced ER stress, increased 6.7-fold GRP78 mRNA levels and activated SREBPs 1 and 2 gene expressions in HepG2 cells. These mice had higher hepatic lipid levels (Werstuck et al. 2001).

Connections between SREBP-1c modulation, ER stress, and inflammation marker TNF- α have been proposed (Carter-Kent et al. 2008). TNF- α is a pro-inflammatory cytokine having a central role in the development of hepatic steatosis (Abenavoli and Peta 2014). Endo et al. (2007) reported that intraperitoneal injection of mice with TNF- α resulted in fatty liver associated with the upregulation of SREBP-1c mRNA levels (Endo et al. 2007). Conversely, pretreatment with anti-TNF- α antibody decreased SREBP-1c expression in LPS-treated mice (Carter-Kent et al. 2008). Furthermore, TNF- α mRNA and protein levels were upregulated by GRP78 in bone marrow stem cells (Chen et al. 2013). Together, these observations support our present data where elevated TNF- α plasma and SREBP1c mRNA levels were found in our PCB126-induced ER stress rats.

Oxidation PPAR α is a transcriptional regulator abundantly expressed in liver involved in peroxisomal and mitochondrial β -oxidation (Xu et al. 2002). PPAR α activation improves non-alcoholic fatty liver disease (Staels et al. 2013) and reverses high-fat diet induced hepatocellular injury in *foz/foz* mouse (genetic model of Alström disease) (Arsov et al. 2006). Moreover, it has been recently reported that PPAR α activation can protect against injuries caused by oxidative and ER stress (Tang et al. 2014). In fact, PPAR α activation by WY14643, a selective PPAR α agonist, inhibited H₂O₂-induced GRP78 in HepG2 (Tang et al. 2014). However, despite higher levels of PPAR α reported in our current data, rats injected with PCB126 still depicted high GRP78 gene and protein expressions. The function of PPARs can be stimulated or inhibited by a number of

co-activators and co-repressors. Among endogenous ligands, NEFAs are natural agonist for PPAR α (Grygiel-Gorniak 2014; Nakamura et al. 2014). Based on these observations, we hypothesize that our higher PPAR α levels could be in response to elevated plasma NEFAs levels seen in our PCB126 exposed rats.

Conclusion

Taken together, these results provide evidence about PCB126 exposure and the development of hepatic ER stress and inflammation in rats. In fact, altered intra- and extra-hepatic mechanisms related to lipid accumulation seen in our contaminated rats could explain the relation between the presence of PCB126 and the increased GRP78 levels. As recent studies demonstrated that ER stress is critically involved in the initiation and progression of many diseases, such as metabolic disease, cardiovascular disease, neurodegenerative disease, and cancer, anti-toxic therapies reducing PCB126 levels in the organism could prevent hepatic ER stress development induced by environmental contamination.

PCBs are fat-soluble substances to which everyone is exposed through ingestion, inhalation, or dermal contact. A potential weakness of this study includes the i.p. route of administration of PCB126. We have opted for i.p. injection as route of exposure in order to assure that each rat received a similar dosage. Moreover, the fact that exposure to PCB126 was conducted for 1 week only could explain the moderate molecular response ranges obtained. Thus, future studies should emphasize on administrating PCBs or other environmental contaminants through ingestion and increase exposure period in order to be more relevant to health outcomes.

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Compliance with ethical standards All procedures were approved by the Animal Care Committee of the University of Ottawa and adhered to the guidelines established by the Canadian Council on Animal Care.

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Conflict of interest The authors declare that they have no conflict of interest.

Authors contribution MPB carried out the molecular studies on rats and helped drafted the manuscript. NAC conceived the study and its design, as well as coordinated and helped to draft the manuscript. All authors read and approved the final manuscript.

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