Toxicology Reports 1 (2014) 513–521

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Toxicology Reports

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Perfluorooctanoic acid exposure triggers oxidative stress in the mouse pancreas

Lisa M. Kamendulis[a], Qiangen Wu[a], George E. Sandusky[b], Barbara A. Hocevar[a,*]

[a] Department of Environmental Health, Indiana University School of Public Health, Bloomington, IN 47405, USA
[b] Department of Pathology, Indiana University School of Medicine, Indianapolis, IN 46202, USA

ARTICLE INFO

Article history:
Received 5 June 2014
Received in revised form 9 July 2014
Accepted 23 July 2014
Available online 2 August 2014

Keywords:
PFOA
Pancreas
Oxidative stress
Lipid peroxidation

ABSTRACT

Perfluorooctanoic acid (PFOA) is used in the manufacture of many industrial and commercial products. PFOA does not readily decompose in the environment, and is biologically persistent. Human epidemiologic and animal studies suggest that PFOA exposure elicits adverse effects on the pancreas. While multiple animal studies have examined PFOA-mediated toxicity in the liver, little is known about the potential adverse effects of PFOA on the pancreas. To address this, we treated C57Bl/6 mice with vehicle, or PFOA at doses of 0.5, 2.5 or 5.0 mg/kg BW/day for 7 days. Significant accumulation of PFOA was found in the serum, liver and pancreas of PFOA-treated animals. Histopathologic examination of the pancreas revealed focal ductal hyperplasia in mice treated with 2.5 and 5.0 mg/kg BW/day PFOA, while inflammation was observed only in the high dose group. Elevated serum levels of amylase and lipase were observed in the 2.5 mg/kg BW/day PFOA treatment group. In addition, PFOA exposure resulted in a dose-dependent increase in the level of the lipid peroxidation product 8-iso-$PGF_{2\alpha}$ and induction of the antioxidant response genes Sod1, Sod2, Gpx2 and Nqo1. Our findings provide additional evidence that the pancreas is a target organ for PFOA-mediated toxicity and suggest that oxidative stress may be a mechanism through which PFOA induces histopathological changes in the pancreas.
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1. Introduction

Perfluoroalkyl and polyfluoroalkyl substances (PFASs), such as perfluorooctanoic acid (PFOA), are widely used in consumer and industrial applications due to their unique hydrophobic properties. PFASs do not readily decompose in the environment and have been detected in air, soil, surface water, sediments, ice caps and wildlife worldwide [1]. Humans are exposed to PFOA by drinking water, dust in homes, food products or migration from food packaging and cookware [1,2]. Detectable levels of PFOA are found in 98% of the American population, with mean serum levels measured at 3.9 ng/ml [3]. PFOA is readily absorbed, but poorly eliminated with a predicted half-life of 3.8 years in humans.

Based on its biological persistence, it has been postulated that exposure to PFOA has the potential to contribute to development of chronic diseases in humans. Epidemiologic studies have shown an association between PFAS exposure and adverse health effects in humans [1,4]. Studies of occupationally-exposed workers, community residents exposed to contaminated drinking water, as well

* Corresponding author at: Department of Environmental Health, Indiana University, 1025 East 7th Street, Bloomington, IN 47405, USA.
Tel.: +1 812 856 2481.
E-mail address: bhocevar@indiana.edu (B.A. Hocevar).

http://dx.doi.org/10.1016/j.toxrep.2014.07.015
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as general population studies have identified a positive association between PFOA exposure and increased total serum cholesterol and non-HDL cholesterol [5–7]. A general population study revealed an increase in obesity, as well as serum insulin and leptin levels in 20-year-old female offspring exposed to PFOA *in utero* [8]. PFOA was also associated with increased mortality due to diabetes in an occupationally-exposed cohort [9]. In addition, a weak association was seen between PFOA levels and pancreatic cancer in a general population study [10].

In rodents, exposure to PFOA results in reduced body weight, liver enlargement, decreased triglycerides, and hepatic peroxisomal proliferation [1,11]. The hepatic effects of PFOA have been attributed in large part to activation of the nuclear receptor peroxisome proliferator-activated receptor alpha (PPARα), although PPARα-independent effects have also been reported [12]. More recent studies have shown that PFOA disrupts the endocrine and immune systems and exerts multiple developmental effects in mice [13–15]. Female mice exposed to low levels of PFOA *in utero* displayed elevated serum leptin and insulin levels and increased body weight [14]. Chronic exposure to PFOA has been shown to induce a "tumor triad" in Sprague-Dawley rats, consisting of liver, Leydig cell and pancreatic acinar cell tumors (PACTs) [16]. While liver tumor formation is proposed to be mediated predominantly through PPARα activation [17], the mechanism by which PFOA induces PACTs is not well understood [16]. Due to the fact that pancreatic ductal adenocarcinoma (PDAC) exhibits a ductal morphology, it was previously thought that PDAC arises from ductal epithelial cells [18]. However, more recent studies have shown that targeting expression of oncogenic KRas to adult mouse acinar cells leads to development of PDAC, firmly establishing the acinar cell as a cell of origin for pancreatic cancer [19–21].

Oxidative stress occurs when reactive oxygen species (ROS) production exceeds the capacity of the cells' detoxification mechanisms [22]. ROS can cause lipid, protein, and DNA damage and contribute to the pathology observed in several chronic diseases including cancer [22]. Previous experimental evidence exists demonstrating that PFOA induces oxidative stress. PFOA has been shown to stimulate ROS production in HepG2 cells [23–25] which led to oxidative DNA damage, assessed by the immunocytochemical detection of 8-hydroxydeoxyguanosine (8OHdG) [25], and activation of caspase-9 and apoptosis [24].

While the effects of PFOA in the liver have been extensively studied, few studies have evaluated the effects of PFOA on the pancreas. The goal of this study was to characterize the adverse effects of short-term exposure to PFOA in the pancreas.

2. Materials and methods

2.1. Chemicals

PFOA (96%), ammonium acetate, potassium hydroxide and ethyl acetate were purchased from Sigma–Aldrich (St. Louis, MO). Perchloric acid was purchased from ACROS (Fair Lawn, NJ). 8-iso-$PGF_{2\alpha}$ and 8-iso-$PGF_{2\alpha}$-d4 were purchased from Cayman Chemical (Ann Arbor, MI). n-Hexane was obtained from Baker Chemicals (Houston, TX). Water, acetone, methanol and acetonitrile were LC–MS grade and purchased from Fisher Scientific (Pittsburgh, PA).

2.2. Study design

Eight-week-old male C57Bl/6 mice were purchased from Harlan Laboratories (Indianapolis, IN), and were acclimated for 4 days prior to treatment. Mice were singly housed in polycarbonate cages with filter tops, and received LabDiet 5015 in pelletized form and de-ionized water *ad libitum*. The care and treatment of the mice were in accordance with the NIH Guide for the Care and Use of Laboratory Animals and was approved by the Indiana University Bloomington IACUC. Due to lack of gender-specific differences in elimination of PFOA [26], mice have been extensively used to evaluate the adverse effects of PFOA exposure [27]. Groups of 4 mice were treated with water (control) or PFOA (0.5, 2.5 or 5.0 mg/kg BW) *via* oral gavage (1×/day at a volume of 0.1 ml/10 g BW) for 7 days. These doses have previously been used in mouse studies evaluating the effects of PFOA [14]. An additional group of mice were treated with cerulein for 7 days (1×/day, 5 μg, i.p.) to stimulate pancreatitis. Mice were killed by CO_2 asphyxiation, and serum, pancreata and livers collected 24 h after the last treatment. Pancreata were divided in half along the longitudinal axis, from the head to tail of the pancreas. The top section was used for histology for all pancreata. Pancreas and liver sections were fixed in formalin for 48 h and then embedded in paraffin, sectioned and stained with H&E for histopathologic examination. Images were captured with an Aperio whole slide imaging system at 20× magnification. The remaining pancreata and liver were snap frozen in liquid nitrogen for further biochemical and gene expression analysis. For the quantitation of PFOA and 8-iso-$PGF_{2\alpha}$ in pancreas and liver, frozen tissues were homogenized in buffer containing 20 mM Tris buffer, 20 μM BHT at pH 7.4 on ice. Supernatants were collected following centrifugation at 16,000 × *g* for 5 min, and stored at −80 °C until use.

2.3. PFOA quantitation

One ml acetonitrile was added to serum or tissue homogenates, vortexed for 15 s and sonicated for 5 min. Samples were centrifuged at 16,000 × *g* for 3 min. An equal volume of supernatant was then mixed 1:1 with LC–MS grade water containing 3 mM ammonium acetate. PFOA was quantified using an Agilent 1260 UPLC system coupled with an Agilent 6430 triple quadrupole mass spectrometer. The samples were separated on an Agilent Zorbax Eclipse XBD-C18 column at 30 °C. Analytes were eluted in water containing 3 mM ammonium acetate and acetonitrile (50:50, v/v) at a flow-rate of 0.3 ml/min. The separated PFOA was detected by mass spectrometry with an electrospray ion source operating in the negative ion mode (ESI^-) using MRM. Precursor ion (*m*/*z* 413) and product ions (*m*/*z* 369 and *m*/*z* 169) were monitored at a fragmentation voltage of 66, cell acceleration voltage of 4, and collision energy of 1 and 13 for each product ion. PFOA levels were quantified from a standard curve prepared at final

concentrations between 0 and 50 pg/μl using Agilent Mass Hunter (v B.04.00) software.

2.4. Amylase and lipase measurements

Amylase and lipase activities in serum were measured spectrophotometrically, using kits from Pointe Scientific Inc. (Canton, MI) according to the manufacturers' instructions.

2.5. Measurement of 8-iso-$PGF_{2\alpha}$

Samples were prepared as described with modifications [28]. Briefly, standards or tissues were spiked with 1 ng of the internal standard, 8-iso-$PGF_{2\alpha}$-d4. 15% KOH (1:1, v/v) was added, vortexed, then hydrolyzed at 40 °C for 30 min. Formic acid (1.2 M final concentration) was added, then centrifuged at 16,000 × *g* for 3 min. Supernatants were transferred into glass tubes and extracted with ethyl acetate/hexane (3:1, 3× volume) twice at room temperature. The combined organic phases were evaporated under nitrogen and reconstituted in 80 μl mobile phase.

8-iso-$PGF_{2\alpha}$ and 8-iso-$PGF_{2\alpha}$-d4 were quantified using an Agilent 1260 UPLC system coupled to an Agilent 6430 mass spectrometer. The analytes were separated by gradient elution (flow fate of 0.35 ml/min) on an Agilent Poroshell 120 SB-C18 column (4.6 mm × 100 mm 2.7-μm), at 45 °C. Mobile phase A consisted of LC–MS grade water containing 0.01% of acetic acid; mobile phase B contained methanol and acetonitrile (50:50, with 0.01% of acetic acid). The column was first equilibrated to 65% B; following sample injection (20 μl) the solvent mix was increased to 77% B over 6 min, held at 77% B for 2 min, increased to 95% B over 0.5 min, then the column was reequilibrated to 65% B for 0.5 min. The UPLC system was coupled to an electrospray ion source operated in negative ion mode (ESI^-). Voltages for the fragmentor, collision energy, and cell accelerator were set at 135, 27, and 4, respectively. The ESI source temperature was set at 350 °C, with a gas flow of 10 L/min and a nebulizer pressure of 50 psi. 8-iso-$PGF_{2\alpha}$ and 8-iso-$PGF_{2\alpha}$-d4 were detected using multiple reaction monitoring for the following transitions: 8-iso-$PGF_{2\alpha}$, *m*/*z* 353.3 to 193.2, and *m*/*z* 353.3 to 247.3; 8-iso-$PGF_{2\alpha}$-d4, *m*/*z* 357.3 to 197.0, and *m*/*z* 357.3 to 251.3. An 8-iso-$PGF_{2\alpha}$ standard curve was constructed at concentrations between 0 and 200 pg/ml. 8-iso-$PGF_{2\alpha}$ was quantified from the response ratio of 8-iso-$PGF_{2\alpha}$:8-iso-$PGF_{2\alpha}$-d4 using Agilent Mass Hunter (v B.04.00) software.

2.6. Quantitative RT-PCR analysis

Total RNA was prepared from pancreas and liver using Trizol (Invitrogen). 5.0 μg of total RNA was reverse-transcribed (RT) with Superscript II reverse transcriptase (Invitrogen) using random hexamers (Roche) for priming. Real-time PCR was performed using FastStart Universal SYBR Green Master Mix (Roche) and gene-specific primers on an Illumina Eco Real-time PCR System. Primer pairs for specific genes were designed using the Primer Express program (Applied Biosystems), with β-actin amplification used as the endogenous control. Primer sequences are listed in Supplemental Data, Table 1. Samples were measured in triplicate and analyzed by the threshold cycle (Ct) comparative method. The $2^{-\Delta\Delta Ct}$ value was calculated, where $\Delta Ct = Ct_{target} - Ct_{\beta\text{-actin}}$ and $\Delta\Delta Ct = \Delta Ct_{sample} - \Delta Ct_{reference}$. Relative quantitation for each gene is shown, where control levels in the pancreas were set to 1.0.

Supplementary Table 1 related to this article can be found, in the online version, at doi:10.1016/j.toxrep.2014.07.015.

2.7. Statistical analysis

The data were analyzed by one-way ANOVA followed by a Dunnett's two-tailed test for comparison against controls when the overall model indicated a statistically significant effect. For all studies, treatment groups were considered significantly different from control values when $p < 0.05$.

3. Results

3.1. Effect of PFOA on organ weight, tissue histology and pancreatic enzymes

We hypothesized that treatment of C57Bl/6 mice with PFOA would lead to PFOA accumulation in the pancreas resulting in pancreatic damage. To address this, we conducted a 7 day exposure study in male C57Bl/6 mice. Water or PFOA dissolved in water was administered by gavage at doses of 0.5, 2.5, and 5.0 mg/kg BW/day for 7 days. Our design also included a cerulein treatment group (5 μg/day, i.p.) which elicits a mild form of chronic pancreatitis in rodents [29]. In this way, we could compare results obtained with an agent that induces oxidative stress selectively in the pancreas, *i.e.* cerulein, compared to PFOA, which produces effects in several tissues including the liver. Mice were sacrificed 24 h after the last treatment and absolute and relative pancreatic and liver weights were determined. Body weights were not different between controls and treatment groups (Table 1). However, PFOA caused a significant dose-related increase in absolute and relative liver weight, consistent with previous studies in rodents which demonstrate hepatomegaly following PFOA exposure [17]. PFOA significantly decreased the absolute pancreas weight in the 5.0 mg/kg BW/day treatment group; however, the relative pancreas weight was not significantly different from controls (Table 1).

Previously, levels of PFOA have been commonly measured in the liver, kidney and serum of rodents following PFOA treatment [11,27]; however quantitative measurements of PFOA in the pancreas have not been evaluated. In the present study, measurement of PFOA levels by LC–MS/MS demonstrates that the concentration of PFOA increases in a dose-dependent manner in the liver and serum following 7 days of treatment (Table 2). In addition, PFOA also exhibited a dose-dependent linear accumulation in the pancreas in all PFOA treatment groups (Table 2).

Histologic changes elicited by PFOA treatment were evaluated in H&E stained pancreatic sections (Fig. 1). As shown in Fig. 1B, PFOA exposure of 5.0 mg/kg BW/day led to the appearance of focal ductal hyperplasia (white arrows).

Table 1
Body, pancreas and liver weights of C57Bl/6 mice treated with PFOA for 7 days.[a]

	Final body weight (g)	Pancreas weight (g)	Relative pancreas weight (%)[b]	Liver weight (g)	Relative liver weight (%)[b]
Control	25.9 ± 0.6	0.122 ± 0.011	0.47 ± 0.05	1.30 ± 0.03	5.02 ± 0.05
PFOA 0.5 mg/kg	25.9 ± 1.6	0.126 ± 0.017	0.49 ± 0.04	1.45 ± 0.11*	5.62 ± 0.14*
PFOA 2.5 mg/kg	26.4 ± 0.7	0.138 ± 0.008	0.52 ± 0.02	2.08 ± 0.06*	7.87 ± 0.08*
PFOA 5.0 mg/kg	24.9 ± 0.1	0.104 ± 0.008*	0.42 ± 0.03	2.30 ± 0.02*	9.24 ± 0.06*
Cerulein	25.0 ± 1.2	0.116 ± 0.002	0.46 ± 0.10	1.31 ± 0.05	5.26 ± 0.11*

[a] Data represent the mean ± S.D. in = 4 mice/group.
[b] Relative organ weight = (organ weight/body weight) × 100.
* Significantly different from control ($p < 0.05$).

Table 2
PFOA levels in pancreas, liver and serum.[a]

	Pancreas (ng/mg tissue)	Liver (ng/mg tissue)	Serum (ng/ml)
Control	0.09 ± 0.02	0.10 ± 0.03	5.5 ± 1.9
PFOA 0.5 mg/kg	0.32 ± 0.07*	5.90 ± 0.52*	4162.6 ± 399.9*
PFOA 2.5 mg/kg	1.57 ± 0.13*	53.54 ± 5.27*	28,878.3 ± 2871.7*
PFOA 5.0 mg/kg	3.33 ± 0.87*	103.07 ± 2.10*	47,984.2 ± 4427.4*
Cerulein	0.17 ± 0.08	0.12 ± 0.04	9.5 ± 5.0

[a] Data represent the mean ± S.D. in = 4 mice/group.
* Significantly different from control ($p < 0.05$).

Focal ductal hyperplasia was observed in all mice in the high dose group (4/4) while 50% of mice (2/4) of the 2.5 mg/kg BW/day group displayed similar changes. The pancreata of mice in the control and 0.5 mg/kg BW/day treatment groups did not display these changes. Consistent with previous reports [30], we observed that cerulein treatment led to widespread areas of acinar-to-ductal metaplasia (ADM) (Fig. 1C). PFOA at a dose of 5.0 mg/kg BW/day, as well as cerulein treatment, increased the number of white blood cells in the vessel lumens, indicative of inflammation (Fig. 1E and F), as compared to the control pancreas (Fig. 1D). Serum amylase and lipase measurements were determined to assess the effect of 7 day exposure to PFOA on the pancreas. As shown in Fig. 2, serum amylase and lipase levels were significantly elevated in the 2.5 mg/kg PFOA exposure group (32% and 52% increases, respectively).

3.2. Effects of PFOA on oxidative lipid damage

We next determined whether PFOA exposure resulted in oxidative damage to the pancreas and liver, as assessed by quantifying the lipid peroxidation product 8-iso-$PGF_{2\alpha}$, also known as F2α-8-isoprostane. As shown in Fig. 3A, PFOA administration led to a dose-dependent increase in 8-iso-$PGF_{2\alpha}$ levels in the pancreas, with 2.3-fold higher levels observed in the high dose group, as compared to controls. Cerulein administration, which causes a mild form of pancreatitis, also led to a significant (2.6-fold) increase in 8-iso-$PGF_{2\alpha}$ levels in the pancreas. In contrast, PFOA at the highest dose produced only a 1.5-fold increase in 8-iso-$PGF_{2\alpha}$ levels in the liver (Fig. 3B). The level of 8-iso-$PGF_{2\alpha}$ was not significantly increased by cerulein in the liver. When comparing the levels of 8-iso-$PGF_{2\alpha}$ between the liver and the pancreas, the fold-changes were significantly

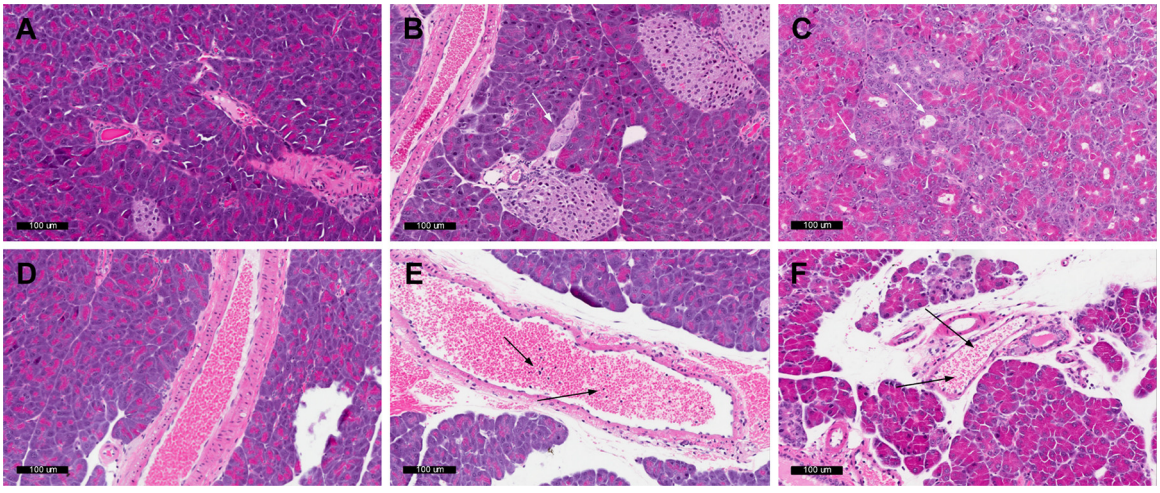

Fig. 1. PFOA treatment for 7 days leads to histopathologic changes in the pancreas. Representative H&E stained pancreas sections obtained from images captured with an Aperio whole slide imaging system (20× magnification) are shown from control (A, D), 5.0 mg/kg BW/day PFOA treatment (B, E) and cerulein (C, F) treatment groups. Depicted are examples of normal ductal morphology (A), focal ductal hyperplasia, white arrow (B) and acinar-to-ductal metaplasia, white arrows (C). The presence of inflammatory cells in the lumen of blood vessels is denoted by the black arrows (D–F). Scale bars: 100 μM.

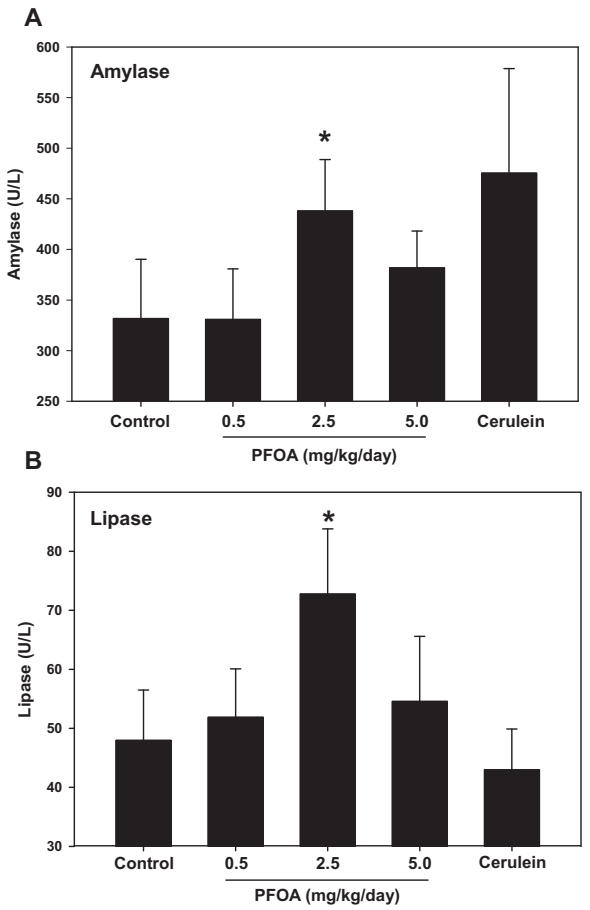

Fig. 2. PFOA treatment causes increased serum amylase and lipase levels. Amylase (A) and lipase (B) levels were measured in serum from mice ($n = 4$/group) treated with PFOA or cerulein for 7 days. Levels were determined 24 h after the last administration. Data are presented as mean ± S.D. *Significantly different from control ($p < 0.05$).

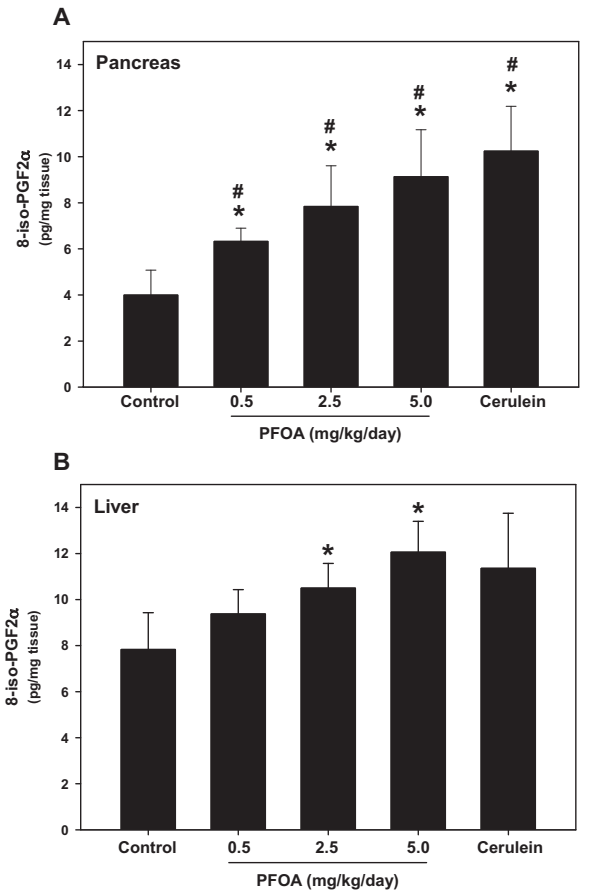

Fig. 3. PFOA treatment leads to increased lipid peroxidation in the pancreas of mice treated with PFOA for 7 days. 8-iso-$PGF_{2\alpha}$ was quantified as a measure of lipid peroxidation in the pancreas (A) and liver (B) of mice ($n = 4$/group) treated with PFOA or cerulein for 7 days using LC–MS/MS as detailed in Section 2. Data are expressed as mean ± S.D. *Significantly different from control ($p < 0.05$). #Significant difference between fold change in the pancreas as compared to the liver ($p < 0.05$).

higher in the pancreas when compared to the liver at all doses of PFOA (Fig. 3).

3.3. PFOA-mediated induction of antioxidant genes in the pancreas and liver

Induction of antioxidant genes commonly occurs as a response to oxidative stress. As PFOA has been shown to increase the activities of superoxide dismutase (Sod) and catalase (Cat) in HepG2 cells and cultured tilapia hepatocytes [31,32], we next determined whether gene induction occurs *in vivo* in the pancreas or the liver as a result of PFOA treatment. Although basal mRNA levels of the cytosolic form of Sod, Sod1, are 4-fold higher in the pancreas as compared to the liver, PFOA treatment led to a similar (2-fold) induction of mRNA levels in both the pancreas and the liver (Fig. 4A). PFOA treatment also increased mRNA levels of the mitochondrial form of Sod, Sod2, by approximately 3-fold in the liver and 2-fold in the pancreas (Fig. 4B). We observed that mRNA levels of glutathione peroxidase 1 (Gpx1) did not change with PFOA treatment in either the liver or pancreas (data not shown); however, Gpx2 levels increased 1.5-fold in the pancreas in the 2.5 and 5.0 mg/kg BW/day PFOA treatment groups, while these levels were not altered in the liver (Fig. 4C). In addition, basal levels of Gpx2 expression were found to be 152-fold higher in the pancreas relative to the liver. In contrast, basal levels of Cat mRNA were approximately 600-fold higher in the liver as compared to the pancreas. While Cat mRNA levels failed to be induced by PFOA treatment in the pancreas, PFOA exposure increased expression by 1.3-fold in the liver (Fig. 4D). Basal mRNA levels of NAD(P)H:quinone oxidoreductase 1 (Nqo1) were approximately 36-fold higher in the pancreas; however, comparable levels of mRNA induction (1.6–2-fold) were observed in both the pancreas and liver following 2.5 and 5.0 mg/kg BW/day PFOA treatment (Fig. 4E). Cerulein treatment led to significant induction of the antioxidant response genes Sod1, Sod2, Gpx2 and Nqo1 mRNA in the pancreas, but not the liver, further illustrating its pancreas-selective effects (Fig. 4).

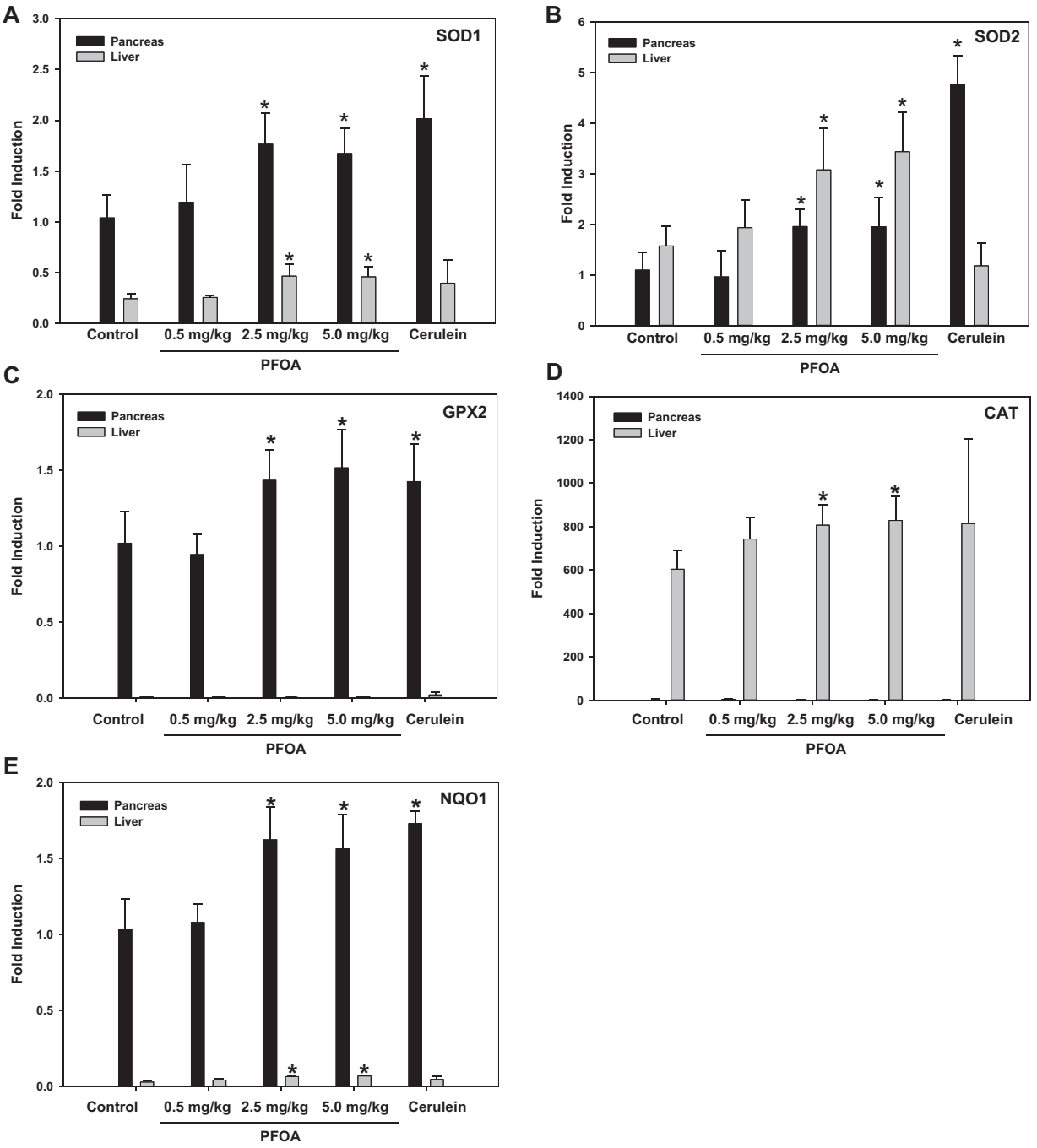

Fig. 4. Induction of antioxidant genes in the pancreas and liver of mice treated with PFOA or cerulein for 7 days. qRT-PCR was used to determine the relative mRNA expression of (A) SOD1; (B) SOD2; (C) GPX2; (D) CAT; and (E) NQO1 following normalization to β-actin as described in Section 2. The mRNA levels for each gene were determined in triplicate from individual mice and means were calculated from groups ($n = 4$ mice/group). Data are expressed as group mean ± S.D. Results are expressed relative to control levels in the pancreas, which were set to 1.0. *Significantly different from control ($p < 0.05$).

4. Discussion

PFOA has been detected in air and wildlife worldwide, with measurable PFOA levels found in more than 98% of the US general population [3]. Recent epidemiologic studies have shown that increased levels of PFOA are significantly correlated with development of diabetes in both occupationally exposed workers [9] and adult women that were exposed *in utero* to PFOA [8]. Further, a weak link was found between PFOA levels and development of pancreatic cancer [10]. While PFOA has been shown to accumulate in the serum, kidney and liver of treated rodents [1,11], levels of PFOA have not been previously measured in the pancreas. We show in this study that a 7 day exposure of mice

to PFOA leads to significant accumulation of PFOA in the serum, liver and also the pancreas (Table 2).

The function of the acinar cell, the major cell type of the exocrine pancreas, is to synthesize, store and secrete digestive enzymes, which are activated following their transport to the lumen of the small intestine. Pancreatitis results from the inappropriate intracellular activation of pancreatic enzymes, leading to autodigestion of the organ and an inflammatory response. A common experimental model of pancreatitis used in mice involves stimulation of acinar cells with the secretagogue cholecystokinin (CCK) or its synthetic analog cerulein [29,33]. Elevated serum amylase and lipase levels are used as a diagnostic indicator for acute pancreatitis in humans, and are observed after acute cerulein administration in rodents. While treatment with physiological concentrations of CCK stimulates pancreatic enzyme secretion, application of supraphysiological concentrations can cause inhibition of secretion [34]. With respect to the ability of PFOA to alter CCK, a study of PFOA production workers found no correlation between serum PFOA and CCK levels, suggesting that PFOA exposure does not modulate CCK levels [35].

In our study, serum amylase and lipase levels were significantly elevated only in the 2.5 mg/kg BW/day PFOA treatment group (Fig. 2). It has previously been demonstrated that serum levels of amylase return to normal within 24 h after cerulein treatment [36]. Since amylase and lipase levels were assessed 24 h after the last dose in our study, the levels may have been elevated at earlier time points and may provide an explanation for the lack of significant elevation of these levels in the cerulein treatment group and perhaps the high dose PFOA group.

Alternatively, ROS have been shown to both stimulate amylase release in mouse pancreatic acinar cells [37] as well as cause inhibition of CCK-stimulated amylase release *in vitro* [38,39]. At low doses, PFOA-mediated ROS production may thus act to stimulate enzyme secretion while at a higher dose (5.0 mg/kg BW/day) PFOA-stimulated ROS production may inhibit secretion. This may be due to the increased lipid peroxidation products observed in the high dose group, which have been shown to trigger loss of Ca^{2+} homeostasis, critical for stimulus-secretion coupling (reviewed in [40]). A definitive answer of whether PFOA stimulates acinar cell enzyme secretion and/or pancreatitis and the mechanism through which this occurs requires additional experimentation.

Chronic oxidative stress has long been associated with disease pathologies observed in atherosclerosis, aging and cancer [22]. Previous studies have shown that PFOA causes increased ROS generation and oxidative DNA damage in HepG2 cells [23,25] and increased malondialdehyde (MDA) levels in cultured tilapia hepatocytes [32]. Consistent with these results, we observed a dose-dependent increase in the lipid peroxidation product 8-iso-$PGF_{2\alpha}$ in the pancreas following exposure to PFOA for 7 days, similar to the induction seen with cerulein, an agent known to induce pancreatic inflammation and oxidative stress (Fig. 3A). Although PFOA levels in the liver were found to be 18–30-fold higher than in the pancreas (Table 2), the pancreas sustained significantly increased fold-changes in 8-iso-$PGF_{2\alpha}$ in all treatment groups when compared to the liver, suggesting that the pancreas may be more susceptible to oxidative damage at lower levels of PFOA exposure. ROS can be detoxified by cellular enzymes, such as Sod, Gpx, Cat, and Nqo1 which are commonly induced as a consequence of oxidative stress [22]. While PFOA stimulated the induction of Sod1, Sod2 and Nqo1 mRNA in both pancreas and liver, Gpx2 expression was induced only in the pancreas and Cat expression was induced only in the liver (Fig. 4). In addition, we observed differing levels of basal expression of antioxidant genes in the liver and pancreas. While the pancreas expresses greater levels of Gpx2 and Nqo1, the liver exhibited significantly higher levels (600-fold) of Cat mRNA. While mRNA levels do not always correlate with protein or activity levels, the lower Cat mRNA expression in the pancreas we observed is consistent with previous studies demonstrating that catalase activity in the pancreas is only 5% of the activity in the liver [41]. These differences in the expression of ROS detoxification enzymes, in particular Cat, may act to mitigate the potential oxidative stress elicited by higher PFOA concentrations in the liver relative to the pancreas.

5. Conclusion

We show here that exposure of mice to PFOA for 7 days triggers oxidative stress in the pancreas, which is associated with altered pancreatic enzyme secretion, focal ductal hyperplasia and inflammation. The conditions of pancreatitis, diabetes, and obesity, as well as other exposures which cause oxidative stress, such as smoking and alcohol consumption, are risk factors for the development of pancreatic cancer [42]. Long-term PFOA exposure has been shown to induce pancreatic acinar cell tumors in rats [1]. In addition, targeting expression of oncogenic $KRas^{G12V}$ to adult mouse acinar cells, in combination with mild pancreatitis, led to development of pancreatic ductal adenocarcinoma [19,20], firmly establishing the acinar cell as a cell of origin for PDAC [43,44]. As exposure to PFOA has been shown to increase ROS *in vitro* and we have shown here that PFOA causes oxidative stress in the pancreas, exposure to PFOA may stimulate an inflammatory response which may contribute to the development or progression of pancreatic cancer [45]. Given that measurable levels of PFOA are found in the general population, additional studies targeted at investigating the mechanism of PFOA-induced pancreatic toxicity will thus provide information that can be used to better characterize the potential human health risks associated with chronic exposure to PFOA.

Transparency document

The Transparency document associated with this article can be found in the online version.

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