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Sex Differences in Transcriptional Expression of FABPs in Zebrafish Liver after Chronic Perfluorononanoic Acid Exposure

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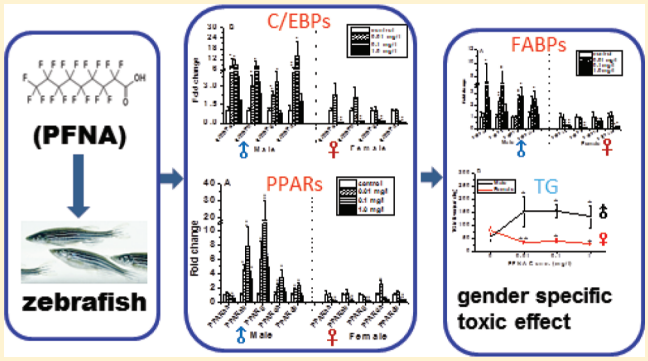
Supporting Information

ABSTRACT: Perfluorononanoic acid (PFNA), a nine carbon backbone of perfluorinated acids (PFAAs), has wide production applications and is found in environmental matrices as a contaminant. To understand the adverse effects of PFNA, adult male and female zebrafish were exposed to differing PFNA dosages (0, 0.01, 0.1, and 1.0 mg/L) for 180 days using a flow-through exposure system. Results showed body weight, body length, and hepatosomatic index (HSI) decreased in both sexes. The HPLC-MS/MS analysis found that PFNA concentrations were higher in male livers than in female livers with increasing significance in a dose-dependent manner. Total cholesterol levels increased in the livers of both sexes, whereas triglyceride (TG) levels increased in males and decreased in females. With the exception of FABP1b, the transcriptional expression levels of fatty acid binding proteins (FABPs) were up-regulated in males and down-regulated in females. A similar trend between sexes occurred for peroxisome proliferator-activated receptors (PPARs) and Ccaat-enhancer-binding proteins (C/EBPs), which may be the upstream regulatory elements of FABPs. The results indicated that PFNA exposure caused opposite adverse effects on liver TG levels between the sexes in zebrafish possibly due to the opposite expression of FABPs and its upstream genes.



INTRODUCTION

Perfluoroalkyl acids (PFAAs), a family of perfluorinated chemicals consisting of high-energy carbon−fluorine (C−F) bonds, have wide commercial and consumer applications due to their unique physicochemical characteristics.[1] This class of compounds is highly persistent and bioaccumulative, resulting in their broad distribution in the environment and in organisms.[2] Since 2000, the manufacturing practices for PFAAs have changed considerably. Consequently, emissions of PFAAs such as perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) have decreased.[3] However, hundreds of related chemicals, such as homologues with shorter or longer alkyl chains, remain unregulated [4] and continue to be released into the environment, especially in rapidly growing industrial and economic regions of the world.[5] The concentration of perfluorononanoic acid (PFNA), a nine carbon backbone of PFAAs, in aquatic environments and organisms is higher than PFOA and PFOS.[6] Detailed surveys have shown that PFNA concentrations are greater than PFOA in a variety of fish species.[7−11] As a result, PFNA concentrations in fish-eating marine mammals have increased rapidly.[12−15] Further, PFNA has been detected in human blood and tissue throughout the world with mean concentrations increasing every year, which likely relate to seafood being a major source of perfluorinated compounds in humans due to high dietary consumption.[16]

Subsequent research has found significant sex differences of PFAAs concentrations in human serum. For example, adult men have higher serum levels of PFOA and PFOS than women in the United States,[17] Japan,[18] Germany,[19] China,[20] and Australia.[21] One study found that serum PFNA content was higher in Australian women than men;[21] however, little information on sex differences in PFAAs accumulation is available for teleost fish. Recent laboratory study on tilapia (*Oreochromis niloticus*) found that the serum concentrations of both PFOS and PFOA were approximately five to six times higher in males than females, which may be attributable to the shorter half-life and higher apparent clearance of female fish compared to male.[22] A significantly higher PFNA concentration in male livers than female livers has also been detected in rodents, which may originate from the dramatically different elimination rates of PFNA between females and males.[23] In addition, toxicokinetic studies in rodents further indicated that females have a much shorter blood or plasma elimination half-life of PFNA than that of males.[24]

Bioconcentration of perfluorocarboxylic acids (PFCAs) occurs notably in the liver, with protein binding as a possible mode of bioconcentration, and it is consistent with the

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structural similarities between PFCA and hydrocarbon fatty acids.[25] Low-molecular-weight fatty acid binding proteins (FABPs) are members of the intracellular lipid-binding protein superfamily and are primarily involved in intracellular lipid trafficking and the regulation of gene expression.[26] So far, a total of 11 different FABP isotypes have been identified in zebrafish following the nomenclature proposed by Hertzel and Bernlohr.[27] Furthermore, several studies have demonstrated that PFAAs, as fatty acid analogues, affect fatty acid and cholesterol metabolism [28] via activation of the nuclear peroxisome proliferator-activated receptor (PPAR) isoforms and their target genes.[29,30] Perfluorononanoic acid is an agonist of the nuclear receptors PPARα and PPARγ, which have been reported in rodents.[31] However, the toxic effects mechanism of PFNA in teleostean livers remains unclear. Although FABPs have been extensively studied in mammals, including gene expression at the tissue and cellular level,[32] few studies have investigated FABPs in nonmammalian species, especially the large and evolutionary diverse teleost fish.

Those PFAAs with more than seven carbons in their backbone cannot be easily metabolized and excreted from the body; thus, longer carbon chain PFAAs such as PFNA have higher accumulation propensity and are more physiologically persistent than shorter chain PFAAs.[24,33,34] The objective of our study was to assess the accumulative levels and adverse effects of PFNA in zebrafish at 0.01, 0.1, and 1.0 mg/L doses after 180 days of flow-through exposure. In this article, we report on the classic end points and the PFNA concentrations in liver tissue between sexes. The lipid contents (triglycerides and total cholesterol) in the liver were measured. Finally, we compared the transcriptional profiles of FABPs genes and their upstream regulatory elements peroxisome proliferator-activated receptors (PPARs) and Ccaat-enhancer-binding proteins (C/EBPs) to investigate gene expression trends between sexes exposed to PFNA. This study will help evaluate the potential long-term ecological risks of PFNA on aquatic organisms.

MATERIALS AND METHODS

Materials. The PFNA was obtained from Sigma Aldrich (CAS number 375−95−1, 97% purity). Solvent-free stock solutions of PFNA were prepared by dissolving crystals in water with stirring. Three stock solutions of 30, 300, and 3000 mg/L were used to span the desired range of target solutions in exposure water.

Animals and Treatment. The 5-month-old zebrafish (n = 480) (wild-type, *Tuebingen* strain) were separated by sex and randomly assigned to nominal concentrations of 0 (control), 0.01, 0.1, and 1.0 mg/L of PFNA for 180 days using a flow-through exposure system (ISO7346−3) based on flow velocity of 30 mL/min, and were fed twice a day with live brine shrimp. The nominal low concentration of PFNA (0.01 mg/L) was based on our determination of PFOA (less one carbon atom in the backbone than PFNA) in environmental water sample (9.250 μg/L) from the fluorine chemical industrial zone in Jiangsu Province of China (unpublished). During 180 days of exposure, all fish were held under the same photoperiodic conditions of 16-h light:8-h dark, and a water temperature of 24−26 °C (pH 8.1−8.3). After exposure, all fish were ice-bath anesthetized for sampling. The body weight and length of fish were measured. Their livers were surgically removed after blood was taken from the tail fin using a glass capillary; one part was accurately weighed to analyze PFNA accumulation in the liver and the remainder was immediately frozen in liquid nitrogen and stored at −80 °C for RNA extraction. Hepatosomatic index (HSI) was calculated according to the formula (organosomatic index = organ weight ×100/body weight).

PFNA Accumulation in Liver Analysis. Concentrations of PFNA in liver samples from males (n = 8) and females (n = 6) of each group were quantified using high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). Briefly, livers were extracted with 5 mL of acetonitrile (ACN) in a 15 mL polypropylene (PP) tube, and all tubes were placed on a mechanical shaker for 20 min followed by centrifugation at 3000g for 10 min. The top layers, which contained PFNA (analytes and internal standards), were transferred into new PP tubes. The extraction procedure was repeated and a final solution of 10 mL of acetonitrile was combined and concentrated to 0.5 mL under nitrogen gas at 40 °C. After the addition of 0.5 mL MeOH, the final solution was diluted into 10 mL Milli-Q water for SPE cleanup. All samples were then extracted using an Oasis WAX cartridge (Oasis1 HLB; 150 mg, 6 cc; Waters). The cartridge was pre-equilibrated by the addition of a sequence of 4 mL of 0.1% NH_4OH in MeOH, 4 mL MeOH, and 4 mL water at a rate of 1 drop per second. Samples (11 mL) were then passed through these cartridges at a rate of 1 drop per second. After loading all samples, cartridges were rinsed with 5 mL Milli-Q water and then washed with 4 mL of 25 mM acetate buffer solution (pH 4). Any water remaining in the cartridges was removed by centrifugation at 3000 rpm for 2 min, and PFNA were eluted by 4 mL of 0.1% NH_4OH in MeOH and then concentrated to 1 mL under a stream of nitrogen.

The instrumental chromatographic setup consisted of a P680 binary gradient pump, an UltiMate 3000 autosampler, and a Chromeleon 6.70 chromatography workstation (Dionex, USA). Mass spectra were collected using an API 3200 triple quadrupole tandem mass spectrometer, fitted with an electrospray ionization source and operated in negative ionization mode. Quantification using these transitions was performed using *Analyst 1.4.1* software. Chromatographic separations were carried out on an Acclaim 120 C18 column (4.6 × 150 mm, 3 μm) (Dionex, USA), with a binary gradient. Methanol (A) and 50 mM ammonium acetate (NH_4Ac) (B) were employed as mobile phases. The flow rate was 1 mL/min and the injection volume was 10 μL. The elution gradient was: 0 to 4 min, from 28 to 5% B linearly; 4 to 7 min, 5% B; 7 to 10 min, 28% B.

A calibration curve was prepared from a series of concentrations (0, 10, 50, 100, 500, 1000, 5000, 20 000, and 50 000 pg/mL), and standard deviations were less than 20%. Blanks and recoveries were assessed following the same procedure as described above with each group of extractions. The blanks were all below the limit of quantifications (LOQs). Quality control is given in the Supporting Information. The recovery of PFNA was 94%. All native standards were spiked into samples and analyzed. The concentrations of PFNA in the experimental samples were not corrected for their corresponding recoveries.

Triglycerides (TG) and Total Cholesterol (TCHO) Contents in Liver Analysis. For determination of TG and TCHO content in the liver, 50 mg sections of liver from males (n = 8) and females (n = 6) of each group were powdered under liquid nitrogen and extracted for 16 h at 4 °C in 1 mL *N*-heptane/dimethylcarbinol (2:3.5). After the solution was centrifuged at 2000g for 10 min, the supernatant was obtained and the contents determined using enzymatic kits according to the manufacturer's directions (Biosino, Beijing, China).

Quantitative Real-Time PCR Assays. Total liver RNA was extracted from frozen liver tissues using TRIZOL reagent (Invitrogen Corp., Carlsbad, CA) according to manufacturer's instructions. The concentration was measured by absorbance at 260 nm using a UV1240 spectrophotometer (Shimadzu, Japan). The purity was assessed by determining the A_{260}/A_{280} ratio. The cDNA was then synthesized via reverse transcription (RT) using an oligo-$(dT)_{15}$ primer and the M-MuLV reverse transcriptase (Promega, Madison, USA) in accordance with manufacturer's recommendations. Real-time PCR reactions were performed with the Stratagene Mx3000P q-PCR system (Stratagene, USA). The SYBR Green PCR Master Mix reagent kits (Tiangen, Beijing, China) were used for quantification of gene expression according to manufacturer's instructions. Zebrafish-specific primers were designed for the genes of interest using *Primer Premier 5.0* software (Supporting Information). The housekeeping gene hypoxanthine guanine phosphoribosyltransferase (HPRT) was used as an internal control. The differences in efficiencies of amplification between the target genes and HPRT were all less than 5%. The PCR amplification procedure was as follows: 95 °C for 2 min followed by 40 cycles of 94 °C for 10 s, 58 °C for 15 s, and 68 °C for 15 s. Quantification of the transcripts was performed using the $2^{-\Delta\Delta Ct}$ method.[35]

Statistical Analysis. Raw data were analyzed using SPSS for Windows 13.0 Software (SPSS, Inc., Chicago, IL) and presented as means with standard errors (mean ± SE). Differences between the control and the treatment groups were determined using a one-way analysis of variance (ANOVA) followed by the Duncan's multiple range test. A p-value of <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

PFNA Concentration in Liver. To evaluate PFNA concentration level in fish, its content in tissues were quantified by HPLC-MS/MS. As the collected serum amount was insufficient (2−5 μL serum/per zebrafish) to analyze individual distribution of PFNA in serum, we only detected PFNA content in liver and eggs (collected in fresh water on the 180th day). The results showed that PFNA concentration in males, including the control group, tended to be considerably higher than that in females, and elicited a significant increase with a dose-dependent response (Figure 1). The differences between sexes were statistically significant in the 0.01 and 1.0 mg/L exposure groups ($p < 0.05$ and 0.01, respectively). For eggs, the content of PFNA was about one thousandth of that in liver and increased in a dose-dependent manner (insert in Figure 1).

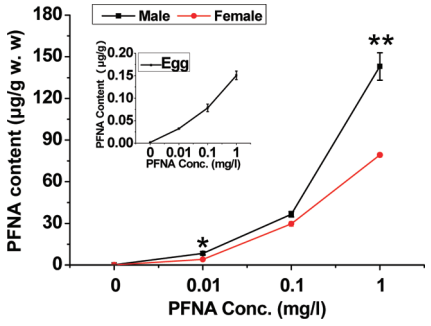

Figure 1. PFNA content in liver and egg of zebrafish. *$p < 0.05$ and **$p < 0.01$ indicate significant differences between the sexes.

This result is consistent with PFOA and PFOS accumulation studies in environmental samples collected from New York State, in which PFOS levels were higher in the liver of male smallmouth bass (*Micropterus dolomieu*) than in females.[36] It has been suggested that different PFAA concentrations between the sexes may originate from sex differences in the elimination of PFCs, although this is yet to be elucidated. In adult rats, the elimination of PFOA is down-regulated by testosterone in both female and castrated male rats [37] and up-regulated by estradiol in male rats.[38] Similar PFNA results in mice have also been reported by Kudo,[24] in which they considered that these differences may be due to organic anion transporter action in the kidney as several transporter proteins are expressed differentially in rats of both sexes. Some of these differences develop during sexual maturation.[39] In addition, PFOS transfer from mother to egg has been suggested for seabirds, such as the Common Guillemot (*Uria aalge*) from the Baltic Sea, whereby PFOS is accumulated in the liver and is associated with very-low-density lipoprotein during formation of the egg yolk protein, and subsequently is transferred to the eggs as a protein-PFOS complex.[40] In accordance with this, a recent study of PFOS in zebrafish showed that sex specific accumulation of PFOS may result from higher excretion rates in females through spawning rather than higher accumulation rates in males.[41] A similar study in humans suggested menstrual bleeding, pregnancy, and lactation in women are the routes for PFOS excretion, which contribute to their lower PFOS body burden than men.[42] Taken together with our study, it can be concluded that male zebrafish had a higher concentration of PFNA in the liver than did females, mainly relating to the higher elimination rates of PFNA in females than in males. Maternal transfer may be one possible route to eliminate PFNA in females by laying large numbers of eggs during the reproductive cycles (7 day intervals) from a single mating. However, PFNA concentration in zebrafish liver did not reflect real bioaccumulation because the body weight of fish was lower at all exposure groups compared to the control group.

PFNA Effects on Morphology. To understand the adverse effects of PFNA on zebrafish, classic end points were investigated, including body weight, body length, and hepatosomatic index (HSI) (n = 30 males and females, respectively) (Figure 2). Compared to their respective control groups, the body weight of both sexes significantly decreased in a dose-dependent manner ($p < 0.01$) (part A of Figure 2). However, a significant reduction in body length was only observed in the high exposure group (1.0 mg/L PFNA) of both males and females ($p < 0.05$ and 0.01, respectively), although it too displayed a dose-dependent manner (part B of Figure 2). Remarkably, HSI was reduced in the 0.01 mg/L PFNA exposure group in males ($p < 0.05$), as well as in the 0.01 and 0.1 mg/L PFNA exposure groups in females ($p < 0.01$ and 0.05, respectively) compared to their respective control groups. However, no significant changes were observed in the high exposure groups (1.0 mg/L PFNA) of either sex (part C of Figure 2). Similar results for body weight and lengths have been observed in previous reports on PFOS exposure in zebrafish, carp (*Cyprinus carpio*), and swordtail fish (*Xiphophorus helleri*).[43−45] In our study, the decrease in HSI was observed in low and intermediate PFNA concentration exposure groups, which was consistent with the study of common carp treated with PFOS for 14 days[44] but contradicted the results on swordtail fish exposed to PFOS for 21 days.[45] These differences in liver change following exposure to PFCs may originate from

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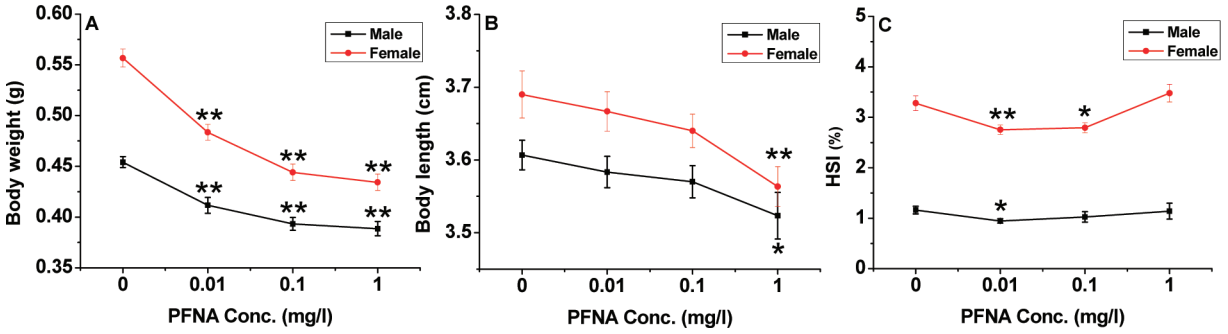

Figure 2. Body weight (A), body length (B), and HSI (C) in male and female zebrafish exposed to 0, 0.01, and 0.1 mg/L PFNA for 180 days. Results show the means of the 30 individual fish, and the error bars indicate standard errors; *p < 0.05 and **p < 0.01 indicate significant differences between the corresponding controls and exposure groups.

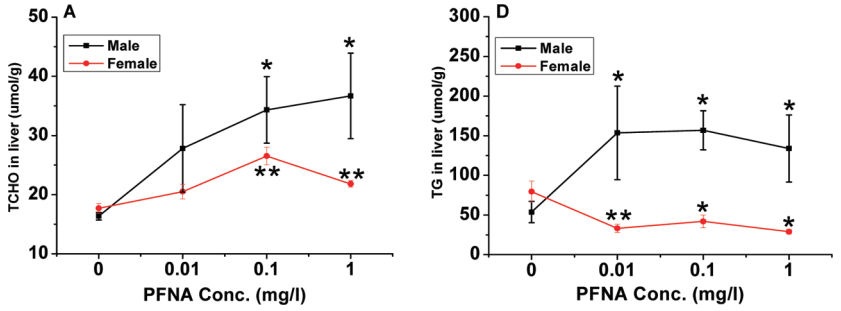

Figure 3. TCHO (A) and TG (B) levels in liver of zebrafish. *p < 0.05 and **p < 0.01 indicate significant differences between the corresponding controls and exposure groups.

differences in species or exposure schemes. Based on these data, it can be concluded that exposure to PFNA at the three test concentrations had significant inhibitory effects on the body growth of zebrafish, and the response to PFNA in body weight was the more sensitive morphological end point.

TCHO and TG Levels in Liver. To investigate the adverse effect of PFNA on liver lipid metabolism in zebrafish, total cholesterol (TCHO) and triglycerides (TG) were measured (Figure 3). Compared to the respective control groups, the liver TCHO levels of both sexes increased significantly in the 0.1 and 1.0 mg/L exposure groups (part A of Figure 3). However, the liver TG levels presented a wholly opposite trend in males and females. Compared to the respective control groups, the TG levels increased significantly in males but decreased in females at all exposure doses (part B of Figure 3). The increase in male liver TG following exposure to PFNA has also been found in PFNA studies in rodents [46] and PFOA has been reported to induce TG formation in the liver resulting in decreased secretion of TG into circulation.[47] For female TG levels, however, a significant reduction was observed in the liver compared to the control, which may indicate that PFNA produced a decline in triglyceride synthesis or accelerated fatty acid oxidation and inhibited uptake affinity for free fatty acids from circulation to liver tissue. In addition, our study showed that exposure to PFNA raised liver cholesterol levels. This finding is in agreement with several occupational studies in humans which found a positive association between PFOS and PFOA and cholesterol.[48] Our results suggest that zebrafish may be a potentially suitable model for studying the effects of PFAAs on cholesterol. Taken together, our research indicates that the sex specific toxic effect of PFNA involves a disorder metabolism of cholesterol and triglycerides in the zebrafish.

Transcriptional Expression of FABP Family in Zebrafish Liver. To further investigate the effect of PFNA on the transcriptional expression level of FABPs, the mRNA levels of 11 FABP isoforms (1a, 1b, 2, 3, 6, 7a, 7b, 10a, 10b, 11a, and 11b) were measured by quantitative real-time PCR. The transcriptional expressions of these FABPs were strikingly different between the sexes. Overall, the pattern was increased in males and decreased in females compared to their respective control groups (Figure 4), except for FABP1b which showed a significant decrease in the 0.01 (p < 0.05) and 1.0 mg/L (p < 0.01) exposure groups of males and significant increases in all exposure groups of females (p < 0.05). For male livers, FABP7b and 10b increased markedly in all exposure groups, FABP3 and 10a increased significantly in the 0.01 and 0.1 mg/L exposure groups, FABP7a increased in the 0.1 and 1 mg/L exposure groups, and the remaining isoforms (FABP1a, 2, 6, 11a, and 11b) increased in the 0.1 mg/L exposure group only. By contrast, no marked differences were exhibited in females in the low dose exposure group (0.01 mg/L). The FABP1a, 3, 10a, 10b, and 11a isoforms were significantly down-regulated in the 0.1 and 1.0 mg/L groups, FABP7a and b genes were only down-regulated in the 0.1 mg/L group, and FABP6 and 11b genes were only down-regulated in the 1.0 mg/L group. The FABP2 levels were also reduced, but the differences were not statistically significant.

To explore the different transcriptional patterns for FABPs subtype, amino acid sequences of all FABPs were aligned using CLUSTALW (Figure S1 of the Supporting Information), and a bootstrap neighbor-joining phylogenetic tree was constructed (Figure S2 of the Supporting Information). From the results, FABP1b showed high sequence identity and similarity with FABP1a, but the remarkably similar genes showed two diametrically opposed expressions. This can be explained by

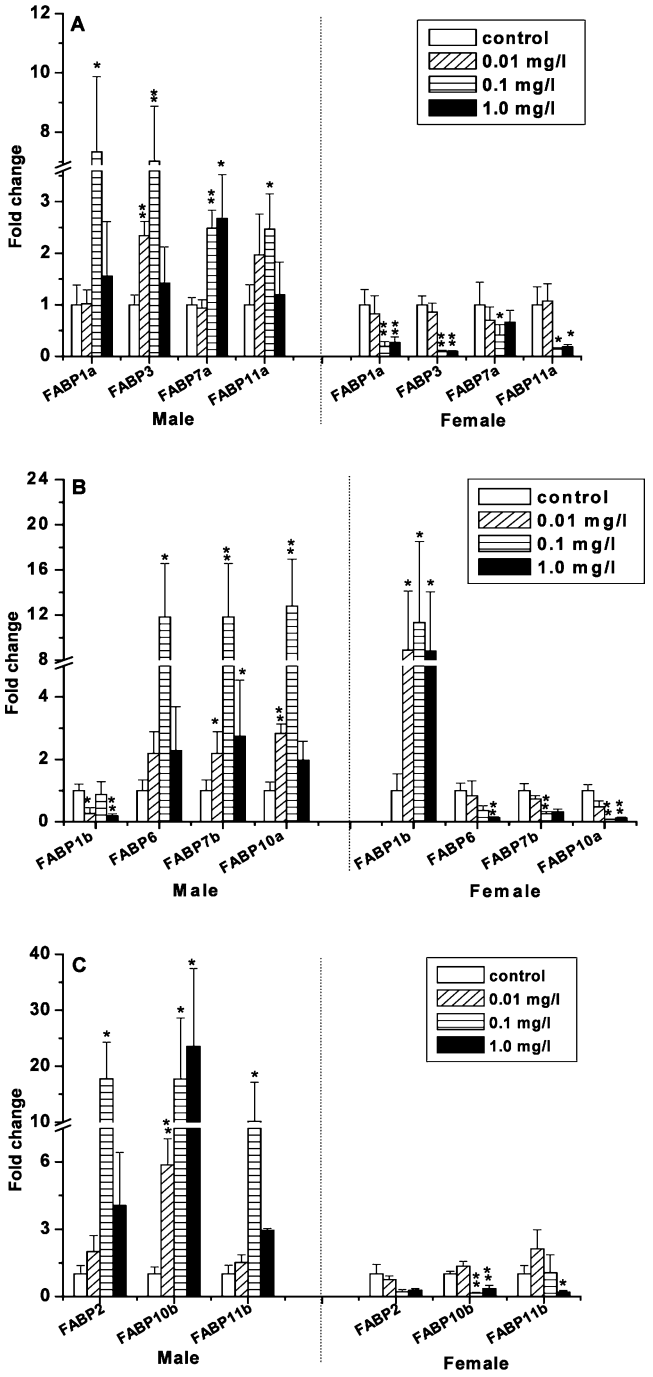

Figure 4. Differential transcriptional expression of FABP family in liver. *$p < 0.05$ and **$p < 0.01$ indicate significant differences between the corresponding controls and exposure groups.

phylogenetic analysis, in which FABP1a and FABP1b genes in zebrafish are orthologs of mammalian FABP1 and most likely arose by a whole-genome duplication event in the ray-finned fish lineage. The distinct expression pattern observed in our study suggests a division of function of these paralogous genes in zebrafish liver.[49]

Transcription Levels of PPARs and C/EBPs. We next explored the transcriptional levels of upstream genes of FABPs, and the mRNA levels of peroxisome proliferator-activated receptors (PPARs) and Ccaat-enhancer-binding proteins (C/EBPs) were analyzed. The three PPAR subtypes, PPARα, PPARg, and PPARd, have been identified in a wide range of vertebrates including zebrafish.[50] Interestingly, with the exception of the PPARαa gene, an opposite expression trend between the sexes was observed (part A of Figure 5). There was significant inhibition of the PPARαa gene in the 1.0 mg/L group for both males and females ($p < 0.01$). For the other PPARs subtypes in males, both PPARαb and g increased markedly in all PFNA exposure groups, while PPARda and db increased markedly in the 0.01 and 0.1 mg/L exposure groups, respectively. For females, however, PPARαb, g, and db were all significantly down-regulated in the 0.1 and 1.0 mg/L exposure groups, while PPARda increased significantly in the 0.01 mg/L exposure group but decreased in the 1.0 mg/L exposure group ($p < 0.05$). The mRNA levels of four C/EBPs (a, b, g, and d) are shown in part B of Figure 5. Compared to the respective control groups, significant increases were detected in the 0.01 and 0.1 mg/L exposure groups in males, but decreases were observed in the 0.1 and 1.0 mg/L exposure groups in females.

Previous evidence shows that FABPs play an important role in uptake, sequestering, and transport of fatty acids, and interact with other transport and enzyme systems.[32] Fluorochemicals may, with a similar structure to fatty acids, successfully compete with these natural ligands for FABP binding.[25] The molecular mechanisms for the induction of FABP genes by PFAAs in mammals suppose that FABPs transport PFAAs to the nucleus from the cytoplasm. Once inside the nucleus, FABPs interact with and transfer PFAAs to nuclear receptors, such as PPARα and PPARγ.[28−30] The PFAAs are known to activate these nuclear receptors, which, once activated, form heterodimers with retinoic acid receptors (RAR) or retinoid X receptors (RXR). These, in turn, bind to response elements such as FABP genes and stimulate their transcription. Our results showed an increase in the transcription of FABP genes in males, which is consistent with the above hypothesis. In females, however, the FABP genes were down-regulated in the liver, which accords with previous research on PFDoA in female zebrafish.[51] This previous research showed that decreased FABP expression in liver was associated with rapid increase in ovarian lipid deposition, suggesting liver-mediated lipid transport may occur during ovarian development.[52] Female ovaries require substantial lipid accumulation, especially fatty acids, during their propagative stages.[53] Transcriptional levels of FABPs, PPARs, and C/EBPs as well as TG content in female liver were reduced coincidentally in our study, which suggests that a liver-ovary feedback loop may play an important role in PFNA toxicity; however, the molecular mechanism requires further study.

Although a similar transcriptional pattern occurred between FABPs and PPARs and C/EBPs, which agrees with previous reports that FABPs are regulated by a combination of PPARs and C/EBPs,[32] other transcriptional regulation mechanisms of FABP genes was not excluded. For example, Berger et al. [54] suggested that the observed down-regulation of FABP5 mRNA levels in the liver of mice was mediated via transforming growth factor, beta 1.

Our results demonstrate that chronic PFNA exposure led to a decrease in body weight, body length, and liver weight in both sexes, although PFNA was less bioaccumulative in female livers than in male livers. The liver TG levels increased in males but decreased in females. Transcriptional levels of FABPs and their upstream regulators of PPARs and C/EBPs were significantly increased in males after chronic exposed to PFNA, whereas opposite trends were observed in females. Because FABPs play an important role in uptake, sequestering, and transport of fatty

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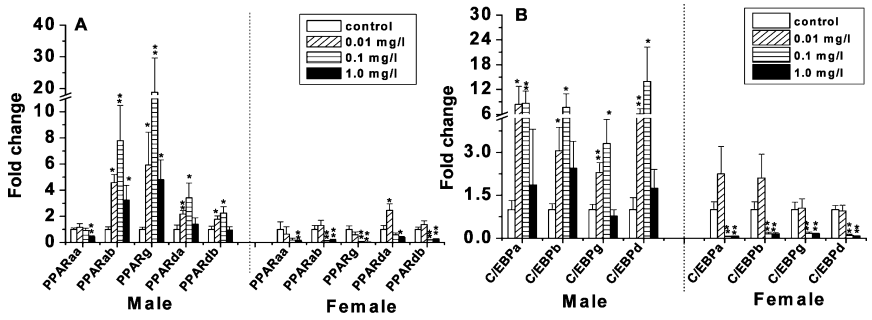

Figure 5. Transcription levels of possible regulation of FABP family in liver (A) PPARs, (B) C/EBPs. *p < 0.05 and **p < 0.01 indicate significant differences between the corresponding controls and exposure groups.

acids, our results imply that the disruption of hepatic TG metabolism by PFNA in different sexes of zebrafish was possibly due to the opposite expression of FABPs. The FABPs at least partially contributed the TG accumulation in zebrafish liver exposed to PFNA. However, the exact molecular mechanism requires further elucidation. The potential relationships of PFNA accumulation and FABPs transcriptional expression in zebrafish liver remain inconclusive because transcriptional level pattern of L-FABPs were the opposite between male and female zebrafish and the PFNA concentrations in the liver were elevated in both sexes although higher in males than female.

■ ASSOCIATED CONTENT

Supporting Information

Sequences of primers used for real-time RT-PCR amplification; amino acid alignments of FABPs in zebrafish; phylogenetic tree of FABPs in zebrafish; additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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