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The identification of apolipoprotein genes in rare minnow (*Gobiocypris rarus*) and their expression following perfluorooctanoic acid exposure

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ABSTRACT

Apolipoproteins play important roles in lipid transport and uptake in vertebrates, and they are associated with pathogenesis of many cardiovascular diseases. However, the diverse apolipoproteins in individual fish species have not been extensively characterized. Partial cDNA sequences encoding *ApoA-IV*, *ApoE*, *ApoM*, *ApoL*, and *ApoO*, and full-length cDNA sequences encoding *ApoA-I* were cloned from rare minnow (*Gobiocypris rarus*). Sequence analysis showed that these genes, as well as fragments of other known apolipoprotein genes (*ApoC-I*, *ApoC-II*, *ApoB*) of rare minnow had a high similarity (91–96%) to their orthologues in the spotted barbel *Hemibarbus mylodon* (Teleostei:Cypriniformes). The expression of these nine genes and their possible upstream genes, *PPARα*, *PPARγ*, and *HNF4α*, were investigated in rare minnow after subacute exposure to perfluorooctanoic acid (PFOA) for 14 days. Results showed that the expression of mRNA for *ApoA-I*, *ApoC-II*, and *ApoM* was significantly downregulated in all PFOA-treated animals. Only fish receiving the highest dose of PFOA showed downregulation of the expression of *ApoA-IV* and *ApoC-I*, while fish treated with 10 mg PFOA/L showed upregulation of expression of *ApoE*. Expression of *ApoB*, *ApoO*, and *ApoL* was unchanged between control and treated groups. In addition, the expression of *PPARα* was increased in all dosed fish, while the mRNAs for *PPARγ* and *HNF4α* were significantly altered with 30 and 3 mg PFOA/L doses, respectively. Therefore, subacute exposure to PFOA resulted in alteration of expression of apolipoproteins and related genes. These changes in gene expression may further influence lipid metabolism or other physiological functions in fish.

1. Introduction

Apolipoproteins, the protein component of various plasma lipoproteins, are synthesized in the liver, intestine, adipose tissues, and other tissues. The specific functions of apolipoproteins are to transport and redistribute lipids among various tissues, to act as cofactors for enzymes involved in lipid metabolism, and to maintain lipoprotein structure (Mahley et al., 1984). Aberrant apolipoprotein levels have been shown to lead to some diseases, such as atherosclerosis, stroke, and coronary heart disease (Kawakami and Yoshida, 2009). Thus, apolipoproteins and their mimetic peptides have been used in therapy for atherosclerotic lesions (Van Craeyveld et al., 2009), Alzheimer's disease (Handattu et al., 2009), and diabetes (Patel et al., 2009).

Fish use lipids rather than carbohydrates as their main energy source, and therefore lipid metabolism and lipoprotein physiology may be more important for their homeostasis (Kondo et al., 2005). In addition to the well-known roles in lipid transport and uptake, several fish apolipoproteins are also required for normal embryonic and ontogenic development, tissue regeneration (Lange et al., 2005; Zhou et al., 2005; Harel et al., 1990), and innate immunomodulation (Concha et al., 2004). Although gene sequences for different members of the apolipoprotein family have been isolated from several fish species (Kondo et al., 2005), the diverse apolipoproteins in individual fish species have not been extensively characterized. In addition, little information about the involvement of apolipoproteins during environmental stress, such as exposure to various pollutants, has been obtained in this lower vertebrate.

Exposure to some persistent organic pollutants, such as polychlorinated biphenyl (PCB), organochlorine, and perfluoroalkyl acids (PFAAs) affects the level of apolipoproteins and disrupts the balance of lipid metabolism in many organisms (Boon et al., 1984; Sakr et al., 2007; Lee et al., 2008). Perfluorooctanoic acid (PFOA), which is used to synthesize fluoropolymers during the manufacture of a variety of products, is a prominent PFAA detected in abiotic and biotic matrices worldwide (Lau et al., 2007). In humans, a positive relationship between serum PFOA and total cholesterol, low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL) was seen in occupationally exposed workers (Sakr et al., 2007). In mice, exposure to PFOA reduces the total level of A-I apolipoprotein in whole serum

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and in the high-density lipoprotein (HDL) and LDL subfractions (Xie et al., 2003). PFOA dissociates apolipoprotein B48 from lipoprotein particles and decreases secretion of VLDL from cultured rat hepatocytes (Okochi et al., 1999). These physiological functions of PFOA may be related to the activation of peroxisome proliferator-activated receptor (PPAR) because PFOA is a PPARs agonist, and PPARs play important roles in regulating a number of genes that encode enzymes involved in lipid metabolism. In addition, in mammals, PPARα, PPARγ, and hepatocyte nuclear factor 4α (HNF4α) are known to regulate genes involved in lipid transport, especially those encoding apolipoproteins (Mandard et al., 2004; Gonzalez 2008; Xie et al., 2009; Yue and Mazzone 2009).

Administration of PFOA to fish has been shown to alter the expression of the function of proteins involved in intracellular fatty acid transport (Wei et al., 2008). However, the effect of subacute PFOA exposure on the expression of fish apolipoproteins and their upstream genes is still unknown. Rare minnow (*Gobiocypris rarus*), which is small in size, has a high fertilization rate, a short embryonic development period, and a sensitivity to aquatic pollutants, has been used as a good native Chinese test species in toxicological tests by the Environmental Protection Agency of China since 2000 (Zhou et al., 1995). This study focused on the cloning and sequencing of the gene products coding for rare minnow apolipoproteins, and investigated the effect of PFOA exposure on the expression of apolipoproteins. In addition, the expression of *PPARα*, *PPARγ*, and *HNF4α*, genes upstream of apolipoproteins in mammals, was also examined after PFOA exposure to determine whether these genes play the same role in the regulation of apolipoprotein expression in fish.

2. Materials and methods

2.1. Fish, exposure, and sampling procedure

Nine-month-old male rare minnows with an average body mass of 1.3 ± 0.3 g were obtained from a laboratory hatchery. Fish were kept in an indoor aquaria system with flowing dechlorinated water at 25 ± 2 °C and a photoperiod of 16 h:8 h (light:dark). After acclimation for one week to ensure the absence of disease, fish were randomly assigned to 20 L glass tanks (10 individuals per tank) and exposed under flow-through conditions to various concentrations (0, 3, 10, or 30 mg/L) of PFOA (98% purity, Acros Organics, Geel, Belgium) for 14 days. Each treatment was in duplicate tanks. The flow rate of the test solution (8 L/h), dissolved oxygen (>80%), water temperature (25 ± 2 °C) and the functioning of the delivery system were monitored throughout the study. Commercial granule food (Tetra, Germany) was supplied at a rate of 0.1% body weight per day during the experiment. Waste and uneaten food were removed daily. No decrease in food consumption or other adverse effects was observed during the experiment. At the end of the exposure, ten fish per group were anesthetized on ice. The livers were removed, and six livers from each group were immediately frozen in liquid nitrogen and stored at −80 °C until analysis. Hepatic tissues of the other four individuals per group were quickly dissected and fixed in 10% formalin for histological examination.

2.2. Total RNA isolation and reverse transcriptase reactions

Total RNAs were extracted from the individual liver samples using an RNeasy Mini kit (Qiagen, Hilden, Germany) and treated with RNase-free DNase I (Qiagen) to remove any remaining genomic DNA. Isolated RNA was quantified based on the A_{260} value. The purity of the RNA was determined from the 28S:18S rRNA ratio on a MOPS/formaldehyde gel. Approximately 1 μg of total RNA from each sample was reverse transcribed using an oligo-(dT) 15 primer (Promega, Madison, WI, USA) and M-MuLV reverse transcriptase, as described by the manufacturer (New England Biolabs, Ipswich, MA, USA).

2.3. Amplification of rare minnow apolipoprotein fragments

Because there was no sequence information about rare minnow *ApoA-IV*, *ApoE*, *ApoM*, *ApoL*, *ApoO*, or *HNF4α*, oligonucleotides for PCR were mainly designed using the previously known sequences of the apolipoprotein and *HNF4α* genes of zebrafish (*Danio rerio*) and spotted barbel (*Hemibarbus mylodon*; Teleostei:Cypriniformes). Conserved regions were identified, and primers for *ApoA-IV*, *ApoE*, *ApoM*, *ApoL*, *ApoO*, and *HNF4α* were subsequently designed (Table 1). The amplification products from the PCR reactions were cloned into a pGEM®-T Easy Vector (Promega) and sequenced.

2.4. Cloning of the full-length cDNA of ApoA-I

The 5′- and 3′-ends of the *ApoA-I* cDNA were amplified using 5′-RACE and 3′-RACE reactions with a BD SMART™ RACE cDNA Amplification kit (BD Biosciences Clontech, San Jose, CA, USA). The gene-specific primers (GSPs) were designed and synthesized according to the partial sequences of *ApoA-I* ESTs (GenBank accession numbers EE392715.1) from our rare minnow adult liver cDNA library. The GSPs for *ApoA-I* are as follows: GSP1, 5′-CATGGCTCCCTTCTC CATCTGTTCCTT-3′ and GSP2, 5′- GCCCATTAGAGAGAATGTGAGTCCTG-3′. For 5′-RACE, the first strand cDNA was synthesized from total RNA using the 5′-CDS Primer and SMARTIIA™ oligonucleotide and amplified by PCR using GSP1 and 10× universal primer A mix. For 3′-RACE, the first strand cDNA was synthesized from total RNA using the 3′-CDS Primer A. The cDNA was amplified by PCR using GSP2 and 10× universal primer A mix. Amplification of cDNA ends was performed for 35 cycles with the following conditions: 94 °C for 30 s, 65 °C for 30 s, and 72 °C for 3 min. The amplified products were cloned into a pGEM-T vector and sequenced.

2.5. Sequence alignment and analysis

The full-length cDNA sequence of rare minnow *ApoA-I* was obtained based on the assembled sequence information from the RACE reactions. The predicted amino acid sequence was determined using the open reading frame finder programs in NCBI (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). A database search using the BLASTN and BLASTP programs was carried out to test the degree of nucleotide and amino acid sequence homology with *ApoA-I* sequences from other vertebrates. The predicted molecular mass and theoretical p*I*

Table 1
Primers used for amplification of apolipoproteins and *HNF4α* fragments.

Gene	GenBank accession no.	Forward primer (from 5′ to 3′)	Reverse primer (from 5′ to 3′)	Amplicon size (bp)
ApoA-IV	NM_001079861	CTCAAGAAACAGGTGGAT	AGTGGCCTTCAGGGT	225
ApoE	NM_131098	ATCCGCAACACCGTAG	GCCTGGACCTCCAACT	191
ApoM	XR_045224.1	CGTGTCTTCCTCCAGC	CTTGCCGTCCCAGAT	299
ApoL	NM_001030138.1	GCCTCATCTTGGCTCCTT	GGCACCGTCCTCATAG	311
ApoO	NM_001005777	AGCAATGCCAAAGAA	AACTCAGATGGAGGGTC	287
HNF4α	NM_194368	GCAGCTTACCCTCCATA	AGGCGTATTCATTGTCATC	420

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value were estimated using ProtParam (http://www.expasy.org/tools/protparam.html). The cleavage site of the signal peptide was predicted by the SignalP 3.0 program (http://www.cbs.dtu. dk/services/SignalP). Amino acid sequence data of rare minnow *ApoA-I* was aligned with the known fish homologues using ClustalW multiple alignment software in EBI. The Box Shade software (http://www.ch.embnet.org/software/BOX_form.html) was used to display the alignment results. The secondary structure of the protein from the amino acid sequence was predicted by the APSSP2: Advanced Protein Secondary Structure Prediction Server (Raghava, 2002). Conserved domains of ApoA-I were predicted using A Conserved Domain Database and Search Service, v2.17 (http://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml).

2.6. Histopathological analysis

Liver tissues were fixed in 10% neutral buffered formalin and embedded in paraffin according to standard histological procedures. Paraffin-embedded tissue samples were sectioned (4–5 μm), deparaffinized, rehydrated, and subjected to hematoxylin–eosin staining. Histopathological changes were observed under a light microscope.

2.7. Quantitative real-time PCR

Quantitative real-time PCR (qRT-PCR) reactions were performed with the Stratagene Mx3000P qPCR system (Stratagene, La Jolla, CA, USA), and the SYBR Green PCR Master Mix reagent kit (Takala, Dalian, China) was used for quantification of gene expression. Gene names, accession numbers, and forward and reverse primer sequences are listed in Table 2. Primers for *PPARs* were designed according to Liu et al. (2009). Based on our previous microarray study, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was chosen as an internal control for normalization (Wei et al., 2008). The amplification protocol was as follows: 95 °C for 10 s, followed by 40 cycles of 94 °C for 5 s, 55 °C for 15 s, and 72 °C for 10 s. All the samples were analyzed in triplicate. After PCR, a melting curve analysis was performed to demonstrate the specificity of the PCR product, as displayed by a single peak (data not shown). The amplification efficiencies between target genes and GAPDH were verified to differ less than 5%. Quantification of the transcripts was performed using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

2.8. Statistical analyses

Data were expressed as the means ± SE and analyzed using one-way analysis of variance (ANOVA). A Duncan multiple range test was used to identify means that differed significantly ($p \leq 0.05$) from one another. All analyses were carried out using SPSS for Windows13.0 Software (SPSS, Inc., Chicago, IL, USA).

Table 3
Fragments of rare minnow apolipoproteins.

Gene	GenBank accession number	Sequence homology	
		Hemibarbus mylodon	*Danio rerio*
ApoA-IV	GQ303439	97% to ApoA-IV	90% to ApoA-IV
ApoE	GQ303440	96% to ApoE-2	88% to ApoEb
ApoM	GQ303441	90% to ApoM	78% to ApoM
ApoL	GQ303443	–	92% to ApoL-1
ApoO	GQ303442	–	85% to ApoOb
ApoC-I	EE392760	84% to ApoC-I	68% to ApoC-I like
ApoC-II	EE395805.1	92% to ApoC-II	75% to ApoC-II
ApoB	EE396877.1	–	80% to ApoB

3. Results

3.1. The cDNA sequences of apolipoproteins and HNF4α in rare minnow

Fragments of nucleotide sequences of *ApoA-IV*, *ApoE*, *ApoM*, *ApoL*, and *ApoO* were determined and deposited in GenBank. These five sequences, as well as the fragments for *ApoC-I*, *ApoC-II*, and *ApoB* from our rare minnow expressed sequence tag (EST) database (Wei et al., 2008) were analyzed and compared to their orthologs from two other cyprinids, *H. mylodon* and *D. rerio*. Results are shown in Table 3. In addition, the 378 bp fragment of *HNF4α* was acquired and deposited in GenBank (accession numbers: GQ331028). It has 91% similarity to that of zebrafish, and 80% identity to mouse *HNF4α*.

3.2. Sequence analyses

The full cDNA sequence of rare minnow *ApoA-I* was obtained by RACE-PCR and deposited in GenBank with accession no. EU327775. It contained 1144 bp including 45 bp of 5′-untranslated region, 768 bp of open reading frame, and 329 bp of 3′-untranslated region. The coding region of the sequence was translated into 256 amino acids, with a MW of 29.86 kDa and a p*I* of 5.77. A signal peptide was predicted to be cleaved between A17 and R18, followed by a six amino acid propeptide (Fig. 1). The alignment of rare minnow *ApoA-I* and the known full-length *ApoA-I* from other fish indicated that both the sequence of the propeptide and the propeptide of the rare minnow *ApoA-I* are completely identical to *ApoA-I-1* of *H. mylodon*, but somewhat different from other fish (Fig. 1). Rare minnow *ApoA-I* had 87.5% and 44% amino acid identity with *H. mylodon* (Teleostei: Cypriniformes) *ApoA-I-1* and *ApoA-I-2*, respectively. Additionally, rare minnow *ApoA-I* had 41–71% identity with *ApoA-I* in other fish, and 23–29% identity with that of birds and mammals.

The secondary structures of rare minnow ApoA-I were conserved with a predicted 94.1% α-helical content, forming several 22 and 11-residue amphipathic α-helical tandem repeat units punctuated by proline residues (Fig. 1). Conserved domain analysis showed that

Table 2
Primers used in quantitative real-time PCR.

Gene	GenBank accession no.	Forward primer (from 5′ to 3′)	Reverse primer (from 5′ to 3′)	Amplicon size (bp)
GAPDH	EE397198	CGTGCTGCTGTCCAGTCCAA	GCCGCCTTCTGCCTTAACCT	138
ApoA-I	EU237775	CTCCAGGTTTACGCAGAT	CCTTGGTCCACTTGTCG	208
ApoA-IV	NM_001079861	CTCAAGAAACAGGTGGAT	AGTGGCCTTCAGGGT	225
ApoB	EE396877.1	GACATCACCAGCCCAACATT	TGGAGCAGCATAGCGAAAGA	51
ApoC-I	EE392760	GGAGGGTCACATATCAGGAA	TTGCCACTAAAACCAAGAGC	147
ApoC-II	EE395805.1	CATCCCGCAAGAAGA	ATGTTAGAGGGCACCAG	232
ApoE	NM_131098	ATCCGCAACACCGTAG	GCCTGGACCTCCAACT	191
ApoM	XR_045224.1	CGTGTCTTCCTCCAGC	CTTGCCGTCCCAGAT	299
ApoL	NM_001030138.1	ACTGCGGTTGGTCTG	CCCACAATGCGTTCC	113
ApoO	NM_001005777	AGCAATGCCAAAGAA	AACTCAGATGGAGGGTC	287
PPARα	–	CTGTGTGGGAGAGAGAAGTGG	GCAGAGTCACCTGGTCGTTG	141
PPARγ	–	GCCGCAACAAGTGCCAAT	GGCTTTGGTCAGAGGGAAGT	221
HNF4α	NM_194368	CATTCTGCGACCTCCC	AGGCGTATTCATTGTCATC	240

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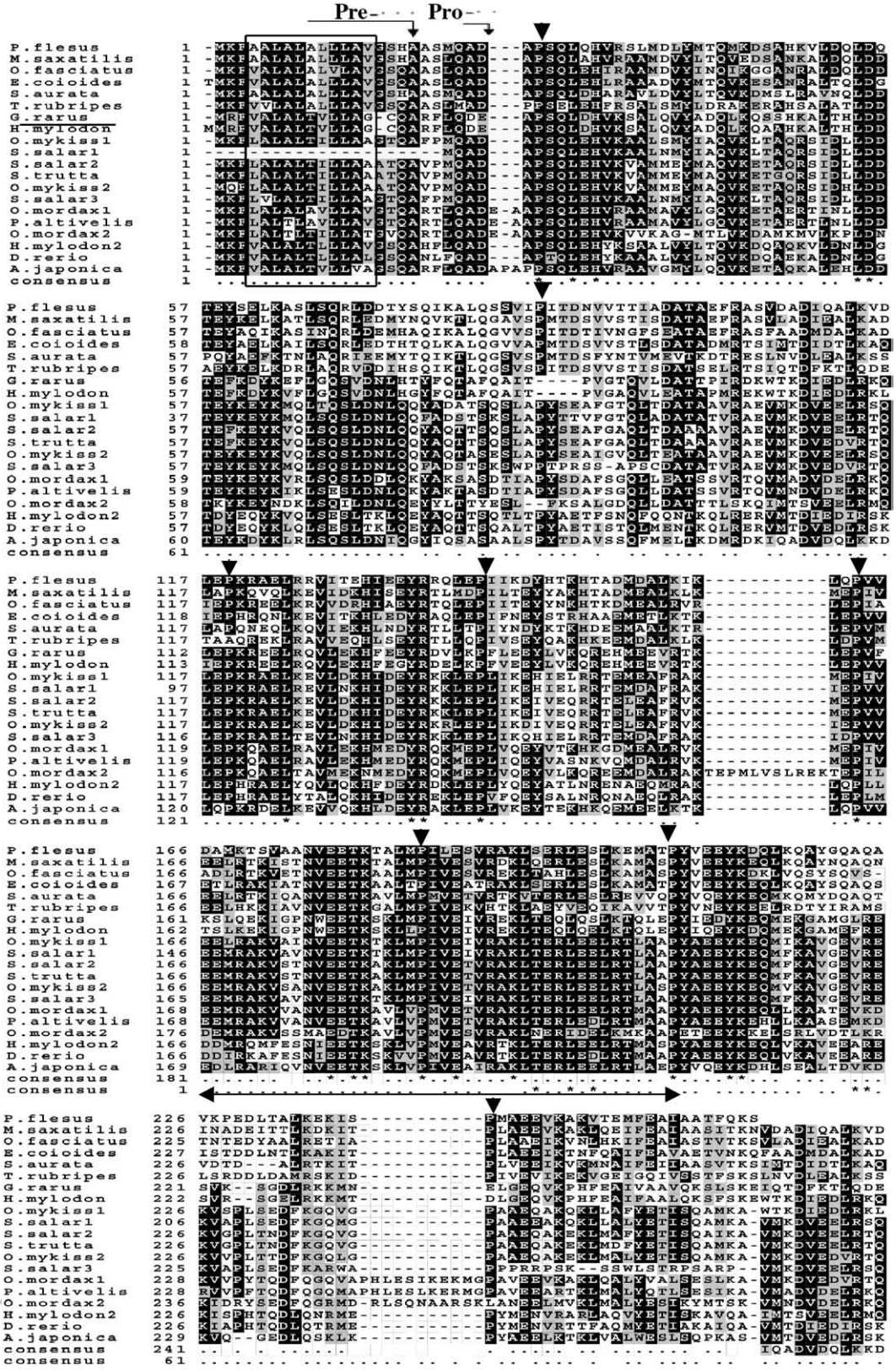

Fig. 1. Alignment of the deduced amino acid sequence of *ApoA-I* with other full-length fish *ApoA-I* sequences. Residues conserved in all sequences are enclosed in dark boxes. Identical amino acids are denoted with a star, and similar amino acids are indicated with a dot. Dashes represent gaps introduced to optimize alignment. The pre- and pro-segments are the areas of the sequence preceding the rectangled arrows. The area in the rectangle comprises the first conserved α helix with 11 aa. The eight bold arrows indicate highly conserved proline residues. The underlined region with an arrow at each end shows the area corresponding to mammalian *ApoA-1* LCAT binding regions (Frank and Marcel, 2000). The number behind the specific name indicates the different sequences from the same species deposited in Genbank.

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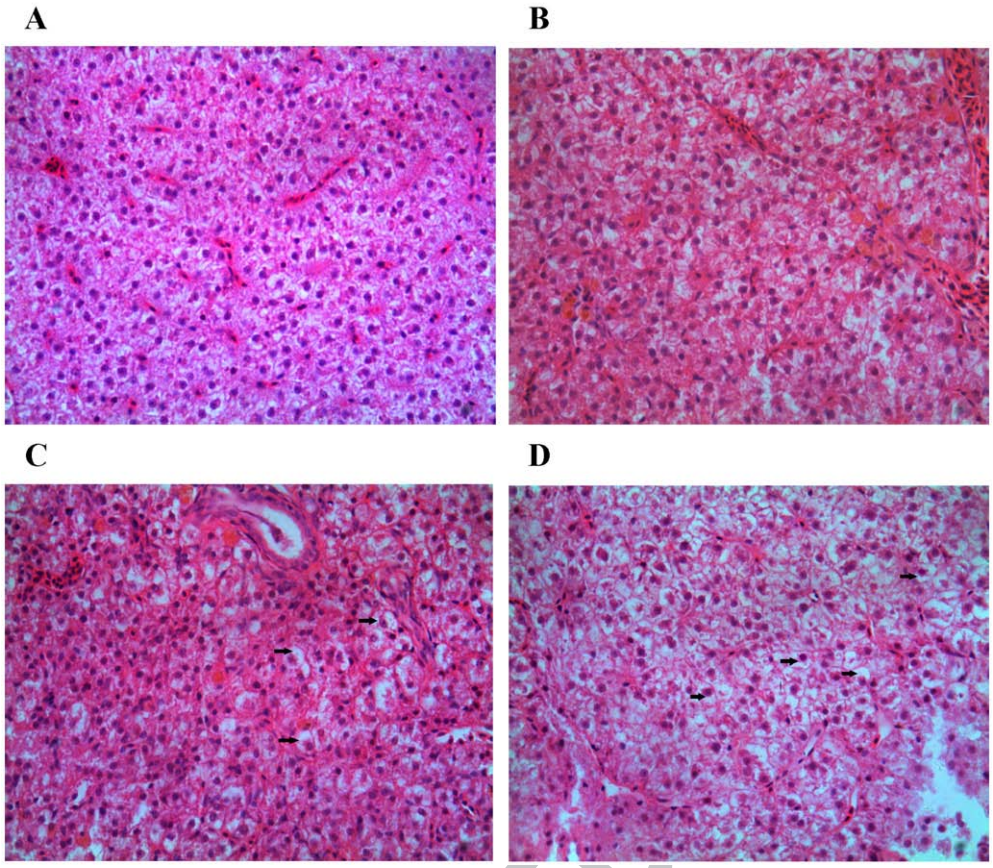

Fig. 2. Liver histopathology in male rare minnows following PFOA exposure. Normal tissue structure was observed in 0 and 3 mg/L PFOA-exposed fish livers (A and B). Several swollen hepatocytes (arrows) were found in 10 and 30 mg/L PFOA-exposed fish (C and D).

a conserved apolipoprotein A1/A4/E domain was located in the section of amino acids 63–247.

3.3. Hepatic histopathology

Histopathological examination of rare minnow liver revealed that there was no difference between control and 3 mg/L PFOA-treated fish (Fig. 2A, B). Exposure to 10 mg/L PFOA elicited obvious hepatic cell swelling and even hepatocellular vacuolar degeneration (Fig. 2C). This phenomenon became much more severe in fish exposed to 30 mg/L PFOA (Fig. 2D).

3.4. Expression of apolipoproteins and related genes following exposure to PFOA

Real-time quantitative PCR was performed on rare minnow hepatic samples. The results indicated that the mRNA expression of *ApoA-I*, *ApoC-II*, and *ApoM* was downregulated by about 2.5- to three-fold in all fish after PFOA exposure (Fig. 3A). Only fish in the highest dose group showed decreased expression of *ApoA-IV* (two-fold) and *ApoC-I* (three-fold) mRNA (Fig. 3B), while the fish exposed to 10 mg PFOA/L showed upregulation of expression of *ApoE* (Fig. 3C). *ApoB*, *ApoL*, and *ApoO* expression was unchanged between control

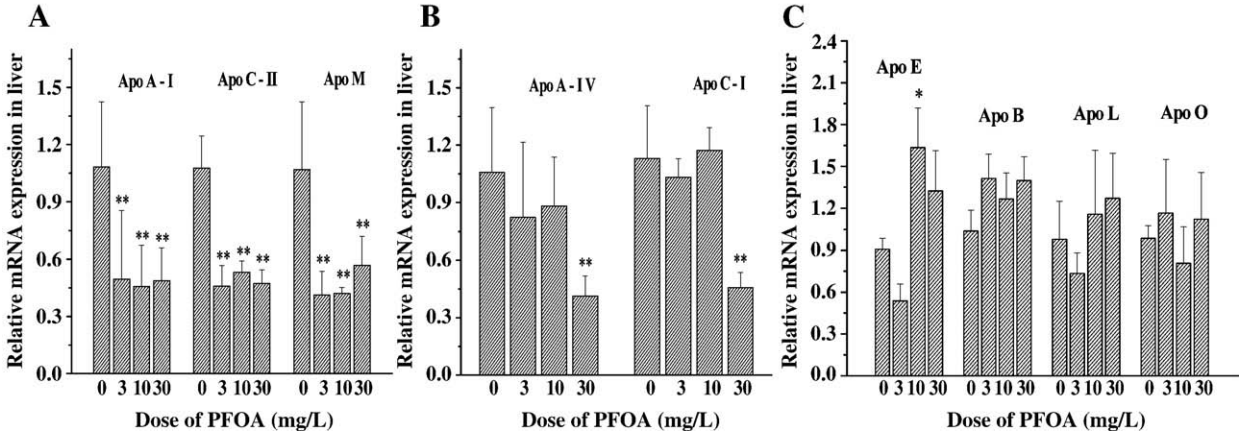

Fig. 3. Quantitative real-time PCR analysis of rare minnow liver mRNA expression levels of *ApoA-I*, *ApoC-II*, and *ApoM* (A); *ApoA-IV* and *ApoC-I* (B); *ApoE*, *ApoB*, *ApoL*, and *ApoO* (C). Gene expression levels are reflected by the relative mRNA expression compared to the control gene expression levels. Values indicate the mean ± SE for six fish per group. $^{*}p<0.05$; $^{**}p<0.01$.

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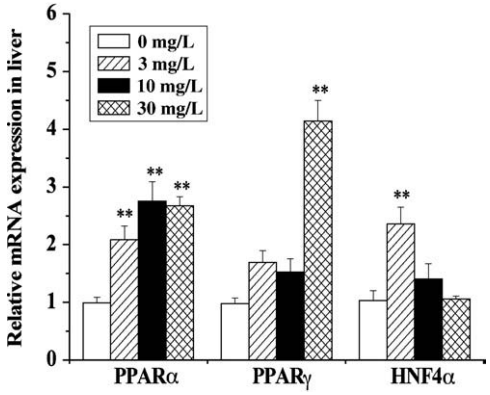

Fig. 4. Quantitative real-time PCR analysis of rare minnow liver mRNA expression levels of *PPARα*, *PPARγ*, and *HNF4α*. Gene expression levels are reflected by the relative mRNA expression compared to the control gene expression levels. Values indicate the mean ± SE for six fish per group. $^{*}p<0.05$; $^{**}p<0.01$.

and the dosed groups (Fig. 3C). Expression of *PPARα* mRNA was increased more than two-fold in all dosed fish, while *PPARγ* was upregulated four-fold in the highest dose group. *HNF4α* mRNA was only significantly increased following the 3 mg PFOA/L dose (Fig. 4).

4. Discussion

Gene sequences for different members of the apolipoprotein family have been isolated from several fish species, such as *D. rerio*, *Oncorhynchus mykiss*, and *H. mylodon* (Babin et al., 1997; Kim et al., 2009). Little information about the apolipoproteins in rare minnow, a sensitive test fish material, was available. In this study, partial cDNA sequences encoding *ApoA-IV*, *ApoE*, *ApoM*, *ApoL*, and *ApoO* were cloned from rare minnow. Sequence comparisons showed that these fragments, along with *ApoB*, *ApoC-I*, and *ApoC-II* were most closely related to their orthologs from *H. mylodon*. Because most fish have HDL in their plasma as the main component of lipoprotein, and because ApoA-I protein is one of the most abundant plasma proteins in fish (Chapman 1980; Amthauer et al., 1989), a full-length cDNA sequence encoding *ApoA-I* was isolated. Both the amino acid sequence and the secondary structure of rare minnow ApoA-I showed high similarity to the ApoA-I-1 of *H. mylodon*, a threatened fresh-water fish species found in the Korean Peninsula (Jang et al., 2003). Rare minnow and *H. mylodon* are fish species of the largest family Cyprinidae. The former are found mostly in upstream waters in the Yangtze River, Sichuan Province, China (Zhou et al., 1995). The latter is a vulnerable Korean endemic species that has been found only in the upper region of the Han River in recent years (Jang et al., 2003). It has been reported that the current fish fauna of the Korean Peninsula are biogeographically related to those in China and Japan, and were strongly influenced by the Palaeo-Hwang Ho River and Palaeo-Amur River two to five million years ago (Jang et al., 2003). Thus, the high identity in nucleotide sequences of all the known apolipoprotein genes indicates this close relationship between rare minnow and *H. mylodon*. Therefore, it is important to explore more conserved genes and to collect other information to investigate the phylogeny and evolution of these two species.

PFOA is a peroxisome proliferator that exerts a wide range of biological effects by activating PPARs in organisms (Permadi et al., 1992; Klaunig et al., 2003). In humans, activation of PPARα increases the expression of *ApoA-I*, *ApoA-II*, and *ApoA-IV* by binding to peroxisome proliferator response elements (PPREs) in their promoters (Vu-Dac et al., 1994,1995; Nagasawa et al., 2009). In contrast, previous studies using rodent livers demonstrated that a PPARα agonist tends to decrease *ApoA-I*, *ApoA-II*, and *ApoA-IV* mRNA expression (Staels et al., 1992; Anderson et al., 2004). Recently, PFOA has been reported to upregulate the expression of both *PPARα* and *PPARγ* in fish tissues (Liu et al., 2009). The expression of *ApoC-I* and *ApoB* was altered in different genders of fish administered PFOA for 28 days (Wei et al., 2008). In this study, subacute exposure of male rare minnows to PFOA produced a dramatic decrease in *ApoC-I*, *ApoC-II*, *ApoA-I*, and *ApoA-IV* expression, while *ApoB* mRNA was unchanged in liver. Interestingly, the expression of *PPARα* and *PPARγ*, genes that are upstream of apolipoproteins, showed opposite trends. This was similar to what was seen in a previous study showing that administration of several fibrate (clofibrate, fenofibrate, and gemfibrozil), agonists of PPARα, decreased the expression of *ApoA-I*, *ApoA-IV*, and to a lesser extent, *ApoA-II* in rat liver (Staels et al., 1992). In mammals, HNF4α regulates genes involved in glycolysis, xenobiotic and drug metabolism, as well as lipid transport, including *ApoA-I*, *ApoA-IV*, *ApoB*, *ApoC-II*, and others (Xie et al., 2009). In this study, *HNF4α* expression was upregulated at the lowest dose of PFOA in the livers of rare minnows, while *ApoA-I* and *ApoC-II* were downregulated with the same dose. Thus, the differential regulation of *PPARα*, *PPARγ*, *HNF4α*, and apolipoproteins suggests that transcriptional regulation of fish apolipoprotein is quite complex, and is more similar to rodent than to human. ApoA-I, the major component of HDL, promotes cholesterol efflux from cells, stimulates the enzyme lecithin-cholesterol acyltransferase (LCAT) to esterify cholesterol for reverse transport to the liver, and allows HDL uptake by hepatocytes (Temel et al., 1997; Xu et al., 1997). ApoC-I can modulate LCAT activity, and ApoC-II is an activator of lipoprotein lipase (LPL), a key enzyme in triglyceride hydrolysis (Mahley et al., 1984). ApoA-IV mediates both LCAT and LPL activity and plays a role in reverse cholesterol transport and in the catabolism of triglyceride-rich lipoproteins (Tso et al., 1995; Sato et al., 2002). Thus, decreased expression of these genes in liver may affect the essential enzymes of lipid metabolism and disrupt the balance of lipid transport and lipid accumulation in peripheral tissues.

Another water-soluble apolipoprotein, ApoE, is mainly produced and secreted by the liver (Mahley, 1988) and plays a central role in lipid metabolism due to its ability to interact with lipoprotein receptors (St Clair and Beisiegel, 1997; Schneider et al., 1997). PFOA (10 mg/L) significantly increased the *ApoE* mRNA level in rare minnow liver, and the expression of *PPARα* was upregulated with this dose as well. This is similar to effects of the PPARα agonist gemfibrozil, which has been demonstrated to increase hepatic ApoE synthesis and secretion in rat (Krause et al., 1984). Fish ApoE has been proposed to be important in lipid uptake and for redistribution in rapidly growing structures, such as during fin and scale development, and during fin regeneration (Monnot et al., 1999; Tingaud-Sequeira et al., 2006), as well as in supplying yolk nutrients to the developing embryo (Babin et al., 1997). Thus, exposure to the PFOA doses we used may cause little disruption of the physiological function of ApoE.

Apolipoprotein M (ApoM) is a recent addition to the long list of lipoprotein-associated proteins. Recent data suggest that ApoM affects HDL metabolism and that ApoM has anti-atherogenic functions by protecting LDL from oxidation and improving the efficiency of cholesterol efflux (Christoffersen et al., 2006). Thus, decreased ApoM may suppress the transport of lipid from liver to the peripheral tissues and may lead to accumulation of lipid in hepatic cells. Recently, our laboratory has found that perfluorododecanoic acid treatment of rats leads to hepatocellular hypertrophy, lipid droplet accumulation, and decreased expression of *ApoM* mRNA in liver (Ding et al., 2009). As in rat, downregulation of *ApoM* expression and hepatocellular hypertrophy simultaneously occurred in rare minnow liver after PFOA administration, further suggesting a similarity in the regulation of apolipoproteins in fish and rodents.

In addition, *ApoO* and *ApoL* expression were unchanged between control and dosed groups. Until now, no information of the specific function of fish ApoO and ApoL was available. In humans, ApoO promotes cholesterol efflux from macrophage cells (Lamant et al., 2006), and ApoL, which is upregulated by multiple pro-inflammatory

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signaling molecules (Monajemi et al., 2002; Sana et al., 2005), participates in the immune system, and is involved in functions such as killing parasites (Smith and Malik, 2009).

In addition to ApoL, other apolipoproteins such as ApoA-I, ApoA-IV, ApoC-I, and ApoE have been reported to participate in the regulation of innate immunity such as during infection and chronic inflammation, to display antimicrobial activity, and even to affect the complement system (Magnadottir, 2006; Schippers et al., 2008). Alteration of the expression of these apolipoproteins following PFOA exposure may potentially affect the fish innate immune system. Innate immunity is critical in fish, because their acquired immunity is less developed than in mammals (Plouffe et al., 2005). Moreover, many other functions of fish apolipoproteins have been proposed based on previous studies. For example, ApoE plays an important role in the development of the fish neural system (Babin et al., 1997). ApoA-I and ApoC-I are also indispensable to fish during early embryonic development (Babin et al., 1997; Wang et al., 2008). Thus, changes in apolipoprotein expression after PFOA exposure may affect the physiological function of multiple systems in fish.

In conclusion, the rare minnow apolipoproteins we examined show similarity to those of *H. mylodon.* PFOA exposure to rare minnows altered the expression of various apolipoprotein mRNAs and their possible upstream genes in liver. The trends of these alterations were more identical to rodents than to humans administrated PPAR agonists. Considering the multiple functions of these apolipoproteins in fish, PFOA exposure likely not only interfered with fish lipid metabolism, but also potentially affected other physiological processes. Much more work should be performed to further examine the potential risk of this chemical and its homologs on the various physiological functions of chemically sensitive fish, the indicators of aquatic ecosystem pollutants.

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