Supporting Information

Perfluorooctanesulfonate Induces Hepatomegaly and Lipoatrophy in Mice through Phosphoenolpyruvate Carboxykinase-mediated Glyceroneogenesis Inhibition

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Methods

Plasma lipids, glucose, and corticosterone levels analysis

Plasma total cholesterol (T-CHOL), triglyceride (TG), high-density lipoprotein cholesterol (HDL-ch), and low-density lipoprotein cholesterol (LDL-ch), as well as glucose levels, were measured by using commercial kits performed on Chemray-240 Automated Chemistry Analyzer (Rayto Life and Analytical Sciences Co., Ltd., China). Plasma corticosterone levels were determined by using corticosterone ELISA kit (Abcam, Cambridge, USA) according to the manufacturer's instructions.

Western-blotting analysis of PEPCK protein expression

30 mg frozen mouse liver tissues were homogenized in 600 μL cold radioimmuno-precipitation assay buffer (RIPA, with cocktail and PMSF) by using an electric homogenizer. Supernatant was separated by centrifugation at 14000 g for 5 min, 4℃. Concentration of total protein was measured by a BCA protein assay kit (Beyotime, Beijing, China). Quantitative samples were loaded and protein was separated by SDS-PAGE electrophoresis. The protein was then transferred from the gel to the PVDF membrane (Millipore, MA, USA) The membrane was blocked for 1 h at room temperature using 5% skimmed milk powder in 1X TBST buffer (TBS: TWEEN=1000:1). The membrane was incubated with appropriate dilutions of primary antibody (GAPDH and PEPCK) in blocking buffer. The dilution factor for GAPDH (Bioworld Technology, MN, USA) and PEPCK (Santa Cruz Biotechnology, TX, USA) was 1: 2000 and 1:200, respectively. The membrane was washed with 1X TBST buffer for three times and incubated for conjugated goat anti–rabbit IgG secondary antibody (1: 10000 in blocking buffer, Bioworld Technology, MN, USA) in blocking buffer at room temperature for 1 h. After washing for three time in 1X TBST buffer, the membrane was developed in a dark room for chemiluminescence. Obtained images were further analyzed in Image J software (National Institutes of Health, USA). Quantification data of PEPCK was normalized to house-keeping protein GAPDH, fold change to control group was calculated for PFOS-exposed groups.

In vitro exposure assay by using HepG2 cells and PEPCK gene expression analysis

HepG2 cells were cultured in DMEM/high glucose medium (Hyclone, UT, USA) with 10% fetal bovine serum and 1% penicillin/streptomycin (Gibco, Thermo-fisher Scientific, MA, USA). Cells were seeded in 6-well plate and treated by different concentrations of 15 different PFASs for 24 hours. PFASs and used concentration: 10 and 100 μM perfluorobutanoic acid (PFBA), 10 and 100 μM perfluorobutane sulfonate (PFBS), 10 and 100 μM perfluorohexanoic acid (PFHxA), 10 and 100 μM perfluorohexane sulfonate (PFHxS), 10 and 100 μM perfluoroheptanoic acid (PFHpA), 1 and 10 μM PFOS, 1 and 10 μM PFOA, 1 and 10 μM perfluorononanoic acid (PFNA), 1 and 10 μM perfluorodecanoic acid (PFDA), 1 and 10 μM perfluoroundecanoic acid (PFUnA), 1 and 10 μM perfluorododecanoic acid (PFDoA), 1 and 10 μM perfluorotridecanoic acid (PFTrDA), 1 and 10 μM perfluorotetradecanoic acid (PFTeDA), 1 and 10 μM perfluorooctadecanoic acid (PFOcDA), 1 and 10 μM

perfluorodecalin-2-sulfonate (PFDecS). All the PFASs except PFDecS were purchased from Sigma-Aldrich (Sigma-Aldrich, MO, USA). PFDecS was synthesized by a chemical plant in Hubei Province. All the PFASs have a good purity (≥ 97%). Post-exposure cells were washed by phosphate-buffered solution for three times. Total RNA was extracted from HepG2 cells and PEPCK gene expression was analyzed by real time PCR as described in the main text.

Molecular docking analysis for GR and PPARγ

The crystal structure of mouse GR in complex with cortisol (PDB ID: 3MNE) was used to predict the binding modes and scores of PFASs in this study. However, owing to the crystal structure of mouse PPARγ was not available to date, their 3D structures were constructed by the homology modeling method. The corresponding gene sequences of mouse PPARγ (NP_001295283.1) was obtained from NCBI. The LBD region (residues number: 208-476) of PPARγ from Homo sapiens (PDB ID: 3U9Q) was used as the template due to decanoic acid bound at the active pocket has a similar molecular structure to PFASs. The subsequent homology modeling was built by the Swiss-Model server and the Ramachandran plot analysis was done according to the method in homology modeling of mouse PEPCK. A model with 95.5% of the residues in the favored regions was used for the docking calculations. Molecular docking of PFOS to mouse GR and PPARγ was performed in LeDock program. During the docking calculations, the mouse GR and PPARγ structure were set to be rigid, while the structures of tested chemicals were flexible with 50 independent conformations samplings for each docking calculation.

Analysis of PFOS levels in liver, WAT and plasma

Plasma was separated, liver and WAT was collected following 7 and 14-day PFOS exposure. Quantified plasma, liver and WAT were prepared for chemical analysis of PFOS concentrations. Sample preparation and pretreatment procedures are performed according to the methods described in our previous study[1].

References

(1) Cao, H.; Zhou, Z.; Wang, L.; Liu, G.; Sun, Y.; Wang, Y.; Wang, T. and Liang, Y. Screening of potential PFOS alternatives to decrease liver bioaccumulation: Experimental and computational approaches. *Environ. Sci. Technol*. **2019**, *53*, 2811-2819.

Figures and Tables

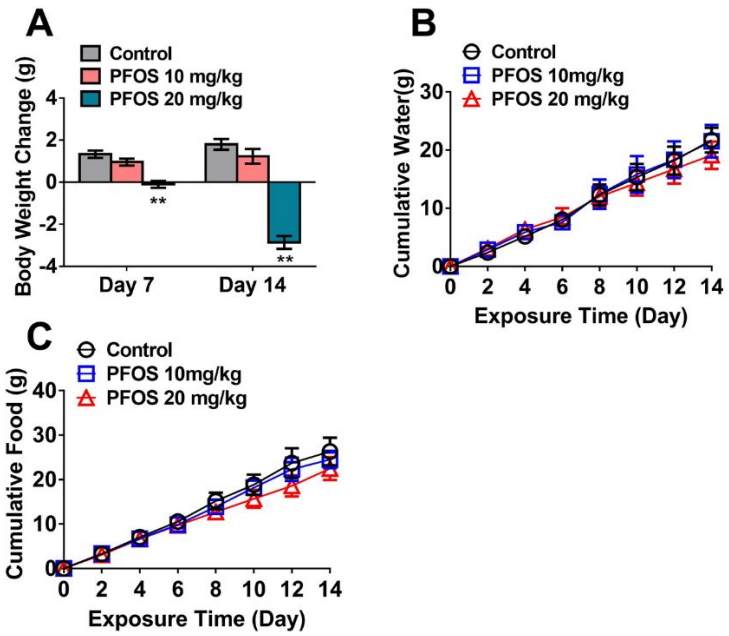

Figure S1 Body weight change (A), cumulative food intake (B), and water intake (C) of mice during PFOS exposure. Body weight change was calculated as body weight change of each mouse after 7 or 14-day exposure. Water and food intake were calculated as total water/food intake for each mouse through the exposure experiment. The data was expressed as the mean value ± SE of 11 individuals. * $p<0.05$, ** $p<0.01$ compared with the control group, using student's t-test.

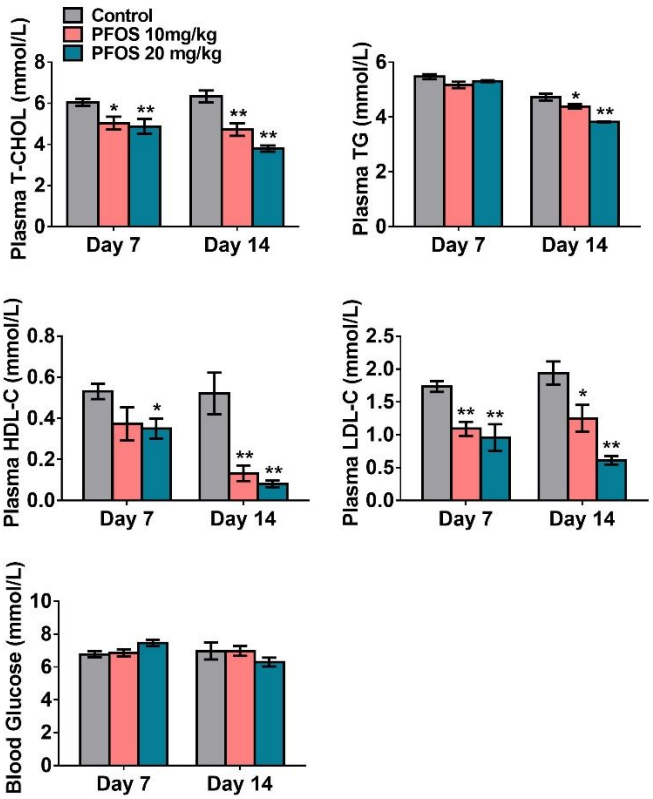

Figure S2 Plasma lipids and glucose levels of mice after 7 or 14-day PFOS exposure. Data were calculated as the mean value ± SE of 6 individuals. * p<0.05, ** p<0.01 compared with the control group, using student's t-test.

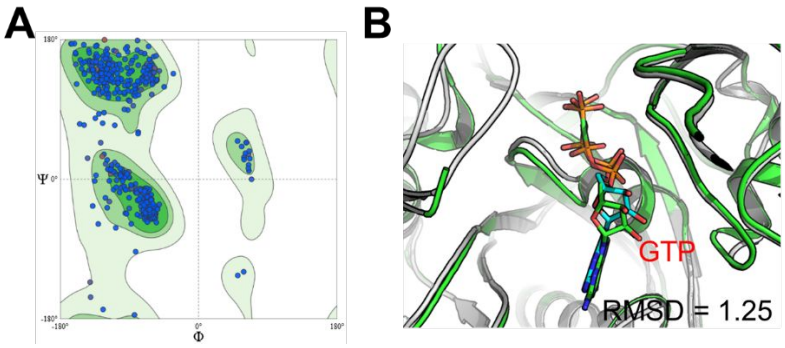

Figure S3 Ramachandran plot analysis for homology modeling structures of mouse PEPCK (A) and RMSD of heavy atoms between the best-scoring pose and native conformation of ligand (B).

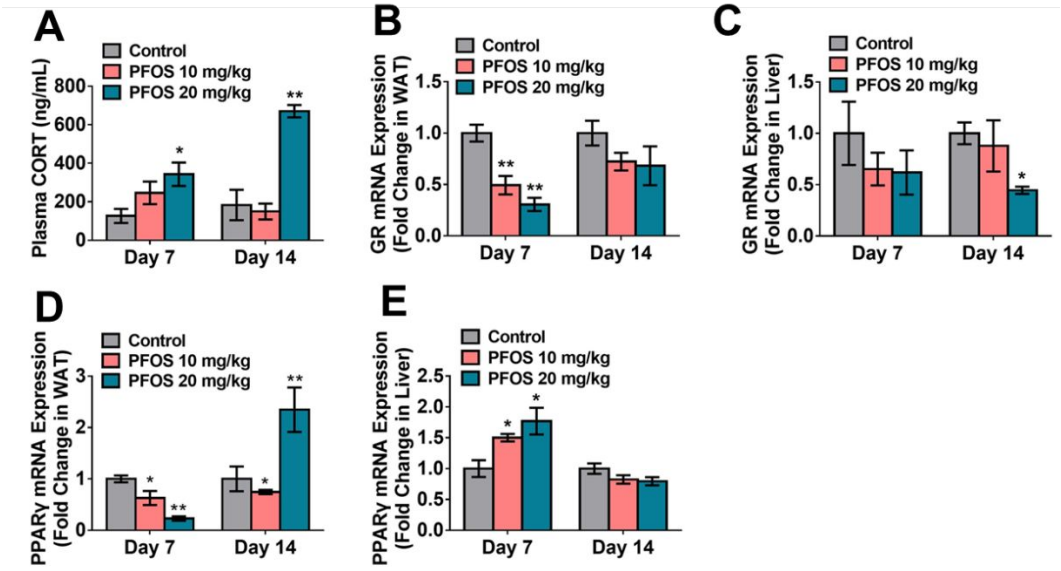

Figure S4 Alteration of key regulators involved in glyceroneogenesis process after PFOS exposure. A: Plasma corticosterone levels after 7 or 14-day PFOS exposure in mice; B and C: GR mRNA expression in livers and white adipose tissue after 7 or 14-day PFOS exposure in mice; D and E: PPARγ mRNA expression in livers and WAT after 7-day or 14-day PFOS exposure in mice. N ≥4 in each group. Data are expressed as mean value ± SE. * p<0.05, ** p<0.01 compared with the control group, using student's t-test.

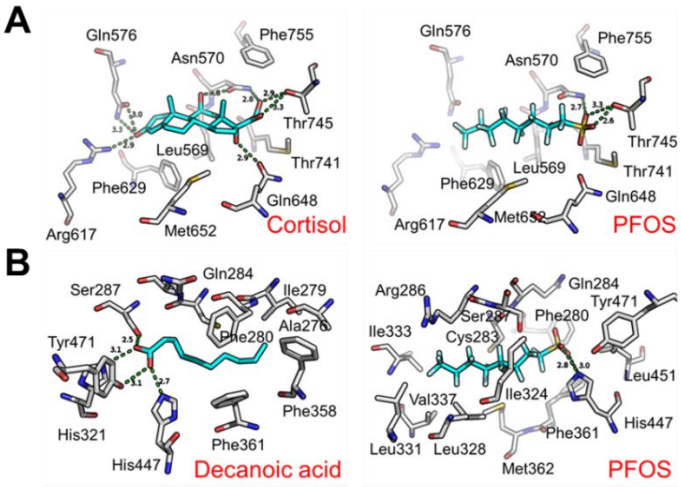

Figure S5 The binding mode of native ligand and PFOS with mouse GR (A) and PPARγ (B). Key amino residues interacting with the ligands are displayed and labeled.

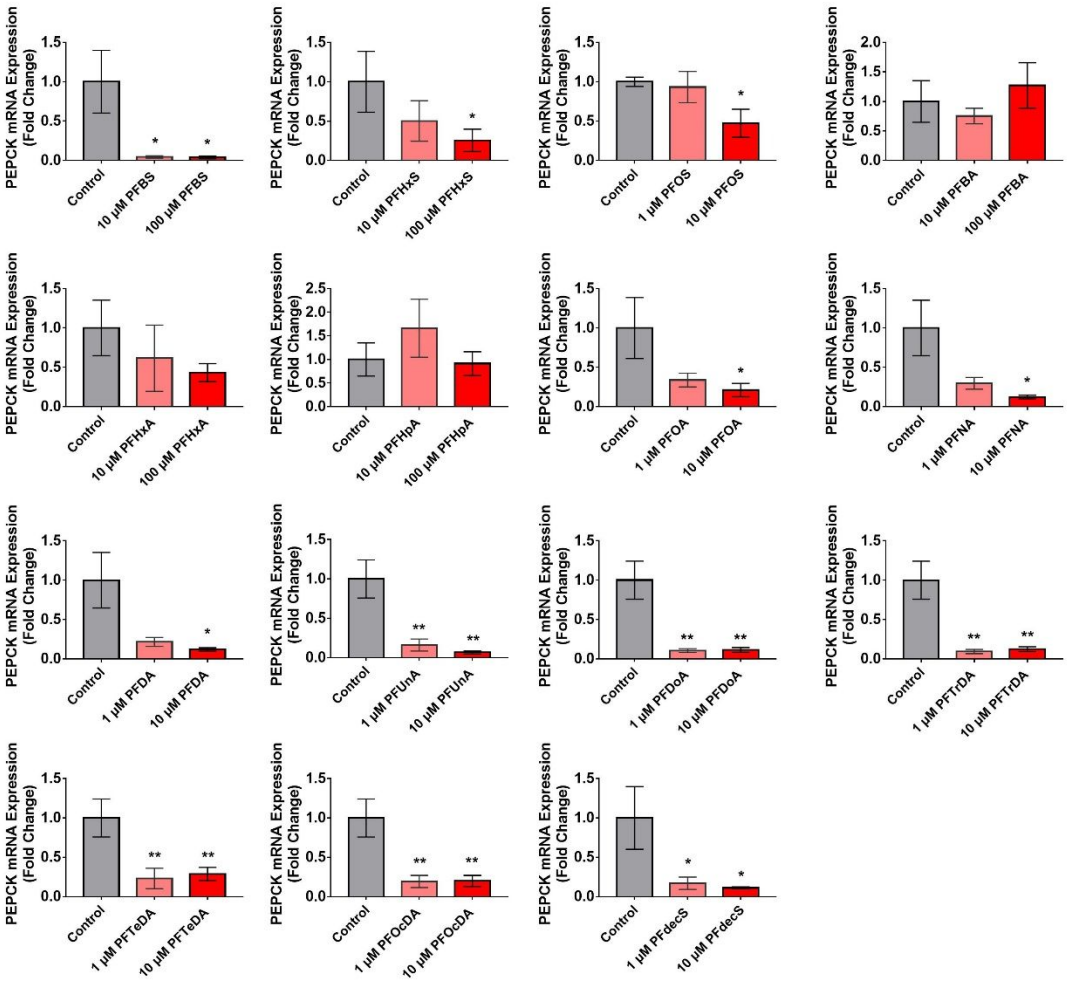

Figure S6 PEPCK gene expression in HepG2 cells after 24-h exposure to 15 various PFASs. The name and exposure concentrations of PFASs are indicated in the figure. Each group has at least 4 replicates, the data are expressed as mean value ± SE. * $p<0.05$, ** $p<0.01$ compared with the control group, using one-way ANOVA analysis.

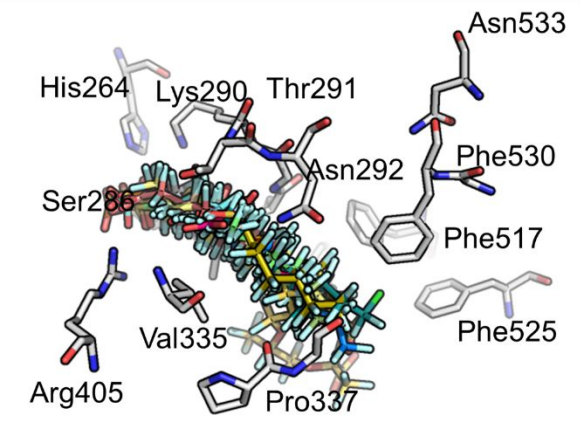

Figure S7 The binding mode of native ligand and multiple PFASs with mouse PEPCK. Key amino residues interacting with the ligands are displayed and labeled.

Table S1 Detailed information for primers used in this study

Gene Accession Number	Primer Name		Sequence (from 5‘ to 3’)	Amplification Efficiency (%)	PCR Product (bp)
NM_001289726.1	Mouse-GAPDH	F	AGGTCGGTGTGAACGGATTTG	92.3	123
		R	TGTAGACCATGTAGTTGAGGTC		
NM_001357943.2	Human-GAPDH	F	CCAGGGCTGCTTTTAACTC	92.3	238
		R	GCTCCCCCCTGCAAATGA		
NM_011044.3	Mouse-PEPCK	F	CAACTTCGGCAAATACCTG	94.0	183
		R	CTGTCTTCCCCTTCAATCC		
NM_008173.4	Mouse-GR	F	GCCGTCCAGAGAATCCCAAGA	92.1	712
		R	TCTCCAGACCCTTGGCACCT		
NM_001308354.1	Mouse-PPARγ	F	TCAAGGGTGCCAGTTTCG	92.7	150
		R	GAGGCCAGCATCGTGTAG		
NM_002591.4	Human-PEPCK	F	CTTTTTCGGTGTCGCTCCTG	95.9	151
		R	GACACCTGAAGCTAGCGGCT		

Table S2 PFOS levels in plasma (μg/mL), liver and WAT (μg/g wet weight) of mice after 7 or 14-day PFOS exposure

		Control	PFOS 10 mg/kg	PFOS 20 mg/kg
Day 7	Plasma	0.061 ± 0.010	265 ± 6.29	501 ± 77.0
	WAT	0.004 ± 0.002	14.2 ± 3.58	62.7 ± 32.4
	Liver	0.124 ± 0.061	430 ± 30.1	682 ± 38.9
Day 14	Plasma	0.028 ± 0.014	295 ± 28.1	725 ± 48.0
	WAT	0.020 ± 0.013	20.0 ± 2.02	301 ± 35.3
	Liver	0.114 ± 0.018	527 ± 18.5	1126 ± 72.4

Table S3 Docking scores of multiple PFASs with mouse PEPCK by using Ledock docking analysis

Compounds	Full Name	Score
PFBA	Perfluorobutanoic acid	4.42
PFBS	Perfluorobutane sulfonate	5.64
PFPA	Perfluoroheptanoic acid	5.32
PFHxA	Perfluorohexanoic acid	4.88
PFHxS	Perfluorohexane sulfonate	6.12
PFHpA	Perfluoroheptanoic acid	5.5
PFOA	Perfluorooctanoic acid	5.81
PFOS	Perfluorooctane sulfonate	6.21
PFNA	Perfluorononanoic acid	6.08
PFDA	Perfluorodecanoic acid	5.83
PFUnA	Perfluoroundecanoic acid	5.63
6:2 FTCA	6:2 Fluorotelomer carboxylic acid	5.98
6:2 FTSA	6:2 Fluorotelomer sulfonate	6.32
6:2 Cl-PFAES	6:2 Chlorinated polyfluorinated ether sulfonate	6.27
HFPO-DA	Hexafluoropropylene oxide dimer acid	5.14
HFPO-TA	Hexafluoropropylene oxide trimer acid	5.01
PFDecS	Perfluorodecalin-2-sulfonate	6.09
GTP	Guanosine triphosphate	5.39