Supplemental Information

Replacement per- and polyfluoroalkyl substances (PFAS) are potent modulators of lipogenic and drug metabolizing gene expression signatures in primary human hepatocytes

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List of the supporting information

Supplemental Table 1. Human and Environmental Exposure to per- and polyfluoroalkyl substances (PFAS)

PFAS	Human Half- Life	Detections in Human Blood/Serum	Detections in Human Liver	Detections in Environmental Exposed Animal Liver	Detections in Environmental (Soil/Water/Air)
PFBS (C4s)	25.8 days [1]	2–5	6	7,8	9–12
PFHxS (C6s)	8.5 years [13]	2–5,14	6,14,15	7,8,16,17	7,9–12,18
PFOS (C8s)	5.4 years [13]	2–5,14	6,14,15	7,8,16,17,19,20	7,9–12,18,21
PFBA (C4)	75 hours [22]	3	6	7,8	7,9,11,21
PFPeA (C5)	Unknown	3	6	7,8	7,9,11,12,21
PFHxA (C6)	32 days [23]	3	6	7,8	7,9,11,12,21
PFHpA (C7)	1.5 years [24]	2–5,14	6,14	7,8	7,9–12,21
PFOA (C8)	3.8 years [13]	2–5,14	6,14,15	7,8,16,17,19	7,9–12,18,21
PFNA (C9)	4.3 years [24]	2–5,14	6,14,15	7,8,16,19	7,9–12,18,21
PFDA (C10)	12 years [24]	2–5,14	14,15	7,8,16,17,19,20	7,9,11,12,18,21
PFUnDA (C11)	12 years [24]	2–5,14	14,15	7,16,17,19,20	7,9,12,18,21
PFDoDA (C12)	Unknown	3,5,14	6,14	7,8,16,17,19,20	7,9,12,18,21
PFTrDA (C13)	Unknown	5	6	7,16,17,19	9,12,21
PFTeDA (C14)	Unknown			7,16,19,20	9,12,21

HFPO-DA (GenX, PFPrOPrA)	Unknown	7	7,25	7,11
6:2 FTS	Unknown	26	20	9,18
FOSA	Unknown	2–5,26	16,17,19	9,12,18
MetFOSA	Unknown			9,12
EtFOSA	Unknown	5		9,12,18

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Supplemental Table 2. PFAS information used for hepatocytes treatments

PFAS	Chemical name	CAS #	Purity (%)	Catalog# (Lot)
PFBS (C4s)	Perfluorobutane sulfonic acid	375-73-5	100	S-74451 (27461)
PFHxS (C6s)	Potassium perfluorohexanesulfonate	3871-99-6	98	S-17292M (21279)
PFOS (C8s)	Potassium perfluorooctanesulfonate	2795-39-3	98	PFOS-002N (24187)
PFBA (C4)	Perfluorobutanoic acid	375-224	98	S-17292K (21247)
PFPeA (C5)	Perfluoropentanoic acid	2706-90-3	97.0	S-17292A (21240)
PFHxA (C6)	Perfluorohexanoic acid	307-24-2	97.0	S-17292B (21241)
PFHpA (C7)	Perfluoroheptanoic acid	375-85-9	99.0	S-17292C (21242)
PFOA (C8)	Perfluorooctanoic acid	335-67-1	100.0	PFOA-001N (27462)
PFNA (C9)	Perfluorononanoic acid	375-95-1	97.0	S-17292E (21243)
PFDA (C10)	Perfluorodecanoic acid	335-76-2	98.0	S-17292F (21244)
PFUnDA (C11)	Perfluoroundecanoic acid	2058-94-8	95.0	S-17292G (21245)
PFDoDA (C12)	Perfluorododecanoic acid	307-55-1	95.0	S-17292H (21246)
PFTrDA (C13)	Perfluorotridecanoic acid	72629-94-8	100.0	S-27319-03 (27641-01)
PFTeDA (C14)	Perfluorotetradecanoic acid	376-06-7	97.0	S-27319-02 (27490-01)
HFPO-DA (GenX, PFPrOPrA)	Perfluoro(2-methyl-3-oxahexanoic) acid	13252-13-6	95.7	S-17292T (28563)
FOSA	Perfluorooctane sulfonamide	754-91-6	95.3	S-72644-05 (27475-01)
MetFOSA	N-Methyl perfluoro-1-octanesulfonamide	31506-32-8	95.9	S-72644-06 (29478)
EtFOSA	N-Ethyl perfluoro-1-octanesulfonamide	4151-50-2	97.1	S-72644-07 (29479)
6:2 FTS	1H, 1H, 2H, 2H-Perfluorooctane sulfonic acid (6:2)	27619-97-2	99.2	S-72644-04 (27468-01)

Complete list of per- and polyfluoroalkyl substances (PFAS) that were purchased from AccuStandard Inc. (New Haven, CT, USA) for use in our assays.

Supplemental Table 3. The details of 36 modeling sets shown by classifications and relevant thresholds.

Endpoint	Thresholds*	Inactives	Actives	Endpoint	Thresholds*	Inactives	Actives
Lipid accumulation	1.25	9	10	FASN	1.2	10	9
NR1l3	1.45	10	9	CYP2B6**	1.5	4	15
ABCA1	1.35	10	9	FABP1	1.02	9	10
CIDEA	1	14	5	CPT1B1	1	12	7
SOD1	1.23	9	10	SLC27A1	1.17	8	11
GSTM3	1.2	11	8	UGT1A1	1.2	10	9
SULT2A1	1.5	7	12	GSTA1	1.1	9	10
SLCO1B1	1.2	10	9	EHHADH	1.25	9	10
MT-RNR2	1	14	5	HMGCS1	1.35	10	9
BDH2	1.16	10	9	ACOT2	1.25	10	9
CYP4A11	1.1	8	11	PCK2	1.3	8	11
LPL	1	8	11	NR1L2	1.27	9	10
CYP7A1	1.1	8	11	SCD	1.4	8	11
SAA1**	1	15	4	MTTP	1.3	10	9
PPAR-α	1.17	9	10	GPAM	1.41	9	10
NFE2L2	1.2	10	9	CD36	1.2	8	11
FABP4	1.2	11	8	PPAR-γ	1.3	9	10
NQO1	1.23	9	10	SREBF1	1.3	9	10

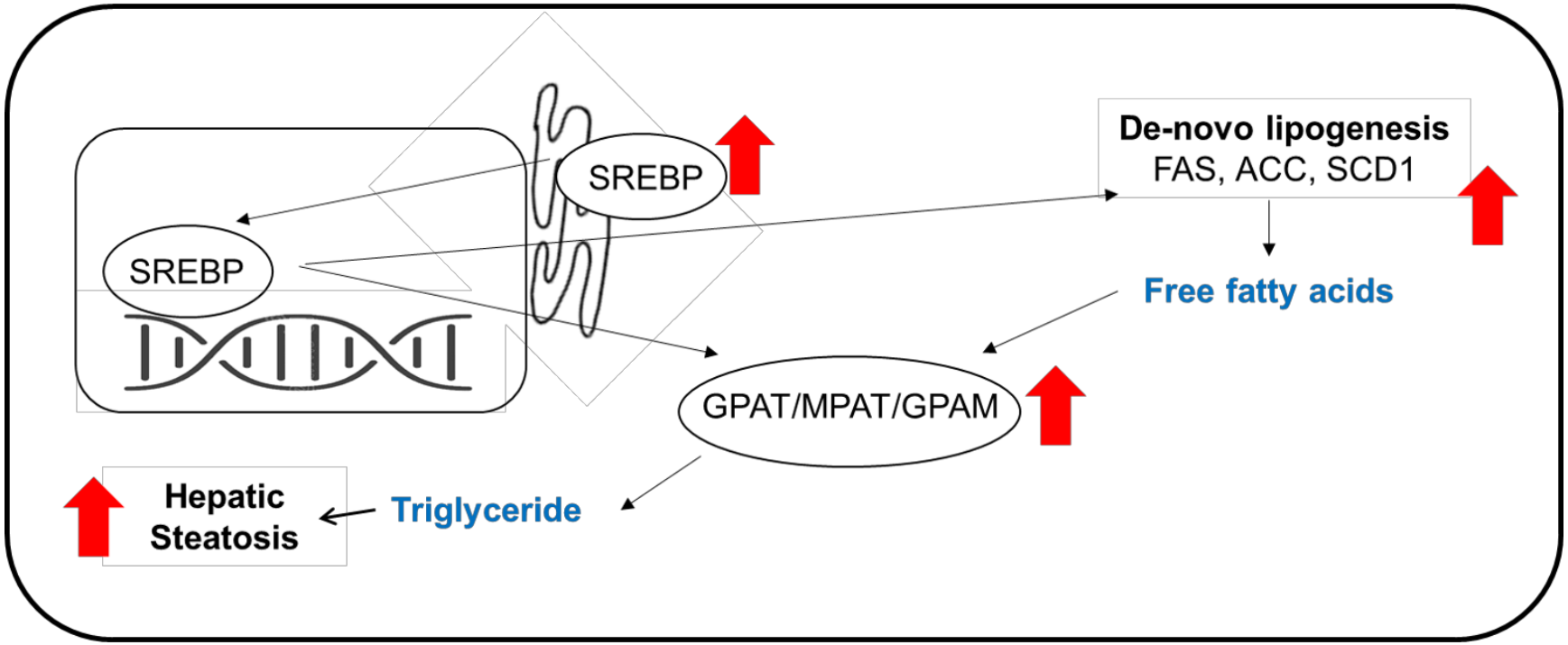
*A PFAS was defined as “inactive” when the fold change of relevant gene expression was less than or equal to the threshold value or defined as “active” when the fold change is larger than the

threshold. The threshold value was defined 1) between 1 and 1.5 for all the endpoints and 2) to balance the two classifications of PFASs. ** For SAA1, the number of actives is less than five, for CYP2B6, the number of inactives is less than five, using the above threshold criterion. Both of these two endpoints were excluded for modeling because the minimum requirement of a classification for modeling is five.

Supplemental Table 4. Model performance for all endpoints using five-fold cross validation.

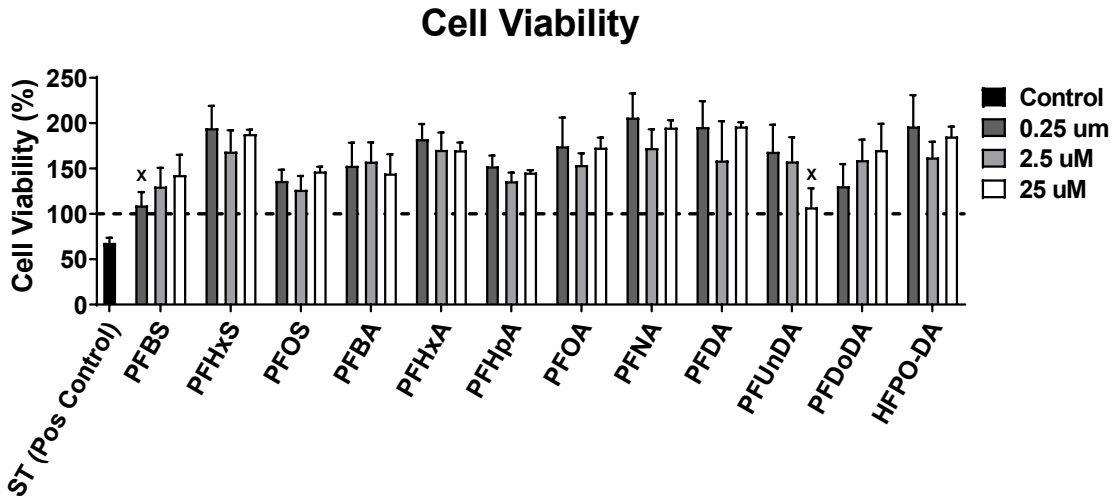
Endpoint	Algorithm	Descriptor	CCR	Endpoint	Algorithm	Descriptor	CCR
Lipid accumulation	SVM	Dragon	0.79	FASN	RF	Dragon	0.68
NR1l3	RF	Dragon	0.79	CYP2B6	-	-	-
ABCA1	SVM	RDKit	0.68	FABP1	SVM	RDKit	0.69
CIDEA	SVM	RDKit	0.66	CPT1B1	SVM	Dragon	0.70
SOD1	*k*NN	Dragon	0.79	SLC27A1	RF	FCFP6	0.63
GSTM3	*k*NN	Dragon	0.77	UGT1A1	SVM	Dragon	0.84
SULT2A1	SVM	RDKit	0.83	GSTA1	*k*NN	Dragon	0.74
SLCO1B1	*k*NN	Dragon	0.84	EHHADH	RF	RDKit	0.74
MT-RNR2	SVM	RDKit	0.66	HMGCS1	SVM	Dragon	0.64
BDH2	*k*NN	Dragon	0.63	ACOT2	SVM	Dragon	0.84
CYP4A11	SVM	Dragon	0.91	PCK2	SVM	Dragon	0.79
LPL	RF	Dragon	0.70	NR1L2	SVM	Dragon	0.78
CYP7A1	SVM	RDKit	0.83	SCD	RF	RDKit	0.71
SAA1	-	-	-	MTTP	SVM	Dragon	0.84
PPAR-α	RF	RDKit	0.68	GPAM	RF	RDKit	0.74
NFE2L2	SVM	Dragon	0.79	CD36	SVM	Dragon	0.63
FABP4	*k*NN	RDKit	0.63	PPAR-γ	SVM	RDKit	0.74
NQO1	SVM	Dragon	0.73	SREBF1	SVM	RDKit	0.79

Supplemental Scheme 1. Map detailing relationship of measured gene targets and hepatic steatosis.



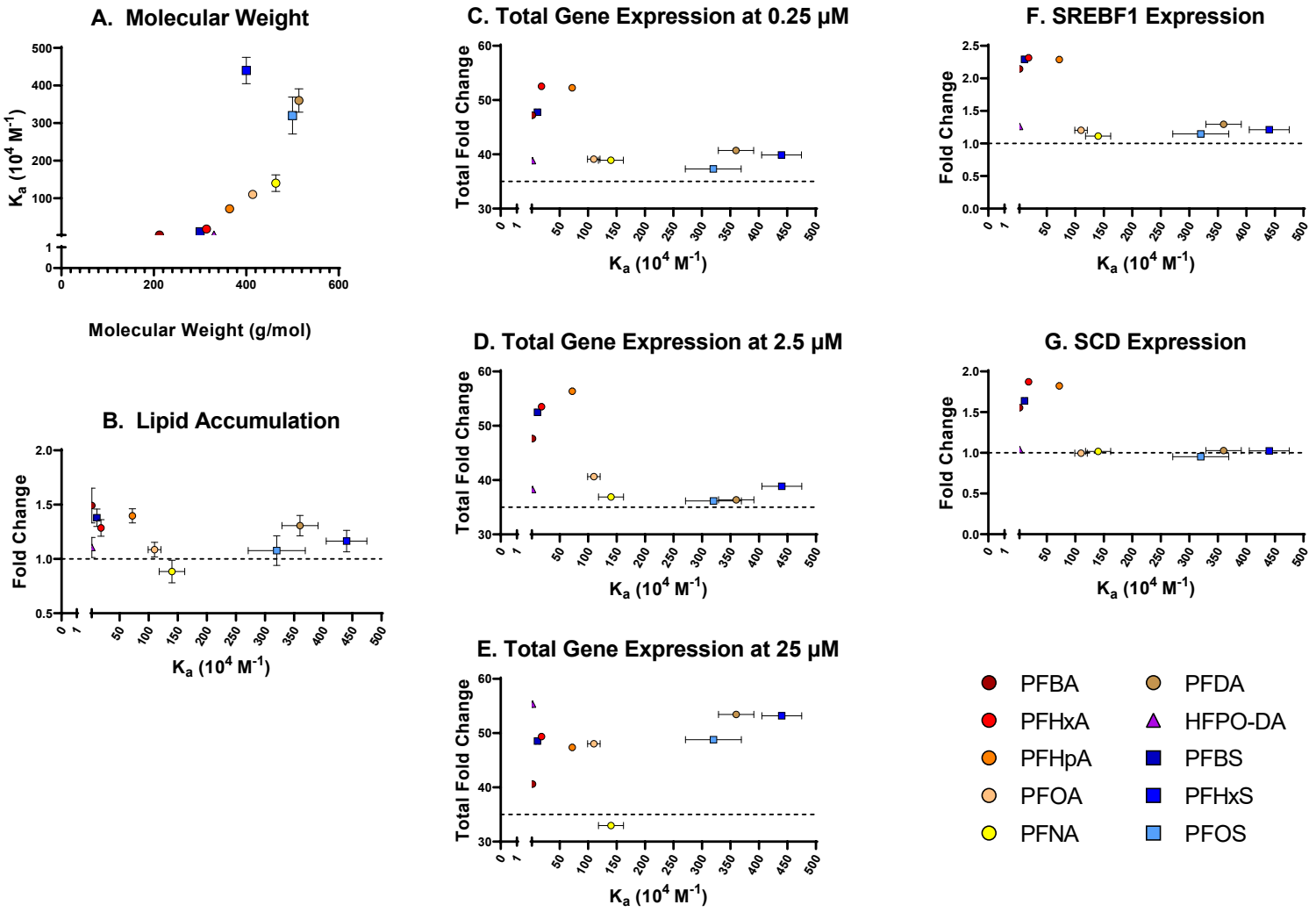
Red arrows indicate hypothesized mechanism of lipid accumulation and hepatic steatosis observed in hepatocytes, by induction of sterol regulatory element-binding protein (SREBP) and lipid synthesis and storage genes.

Supplemental Figure 1. Cell viability assay.



Human hepatocytes were treated with various PFAS (0.25-25 μM) and 1 μM of staurosporine (ST; positive control for cytotoxicity) in 0.1% DMSO in media for 48 hours. The CellTiter-Glo Luminescent Cell Viability Assay reagent (Promega Corporation, Madison, WI) was added to wells according to manufacturer's protocol and luminescence was evaluated on a GloMax 96 microplate luminometer (Promega Corporation, Madison, WI). Percent cell viability was calculated compared to DMSO vehicle controls. Staurosporine reduced cell viability to 68%, while all PFAS treatments, except for 0.25 μM PFBS and 25 μM PFUnDA, significantly increased cell viability ($p<0.05$). This increase in ATP is likely due to induction of peroxisomal beta-oxidation pathway through activation of peroxisome proliferator-activated receptors (PPAR) that has been observed with many of these PFAS compounds. Calculations were done using an ANOVA followed by Fisher's LSD test. All values are means ± SEM; N = 4. "**x**" indicates no significant difference from DMSO controls.

Supplemental Figure 2. Relationship of lipid accumulation and gene expression trends with albumin protein binding



Protein binding association constants (K_a) of several PFAS were determined using am equilibrium dialysis experiment conducted by Allendorf et al. (2019). All trends were calculated using bivariate Pearson correlation and are reported as (Pearson correlation coefficient, and P value). (**A**) As molecular weight was identified the strongest predictor of activity in our descriptor analysis, K_a was plotted and correlated with molecular weight (0.7401, 0.0142*). (**B**) Lipid accumulation at 25 µM plotted against K_a (-0.2962, 0.4060). Fold change of all 35 measured genes at 0.25 µM (**C**; -0.5479, 0.1011), 2.5 µM (**D**; -0.6200, 0.0558), and 25 µM (**E**; 0.2781, 0.4366) were summed and plotted against K_a values. Similarly, fold change of key lipid synthesis gene targets, SREBF1 (**F**; -0.6221, 0.0548) and SCD (**G**; -0.6111, 0.0605), at 25 µM were also plotted against K_a values. K_a values are means ± SD, and all other values are means ± SEM; N = 3-4.

Allendorf, F., Berger, U., Goss, K.U., Ulrich, N., 2019. Partition coefficients of four perfluoroalkyl acid alternatives between bovine serum albumin (BSA) and water in comparison to ten classical perfluoroalkyl acids. Environ. Sci. Process. Impacts 21, 1852–1863. https://doi.org/10.1039/c9em00290a