Environ Health Perspect

DOI: 10.1289/EHP3030

Supplemental Material

Effects of Perinatal Exposure to Dibutyltin Chloride on Fat and Glucose Metabolism in Mice, and Molecular Mechanisms, *in Vitro*

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Figure S4. Effect of the PPARγ inhibitor T0070907 on lipid accumulation in human and mouse MSCs after treatment with DBT. Human **(A)** and mouse **(B)** MSCs were induced to differentiate into adipocytes with the adipogenic cocktail (MDI) and 100 nM DBT with and without 100 nM T0070907 for 14 days. Media with adipogenic cocktail and ligands was replaced every 3 days. Fresh T0070907 was added every 8 hours throughout the experiment. Cells were fixed with 10% formalin and stained with Oil Red O for 30 minutes each. 500 nM ROSI and 50 nM TBT were used as positive controls. DMSO was the vehicle control. Panels show representative pictures after Oil Red O staining. Scale bar: 200μm. DBT: dibutyltin; DMSO: dimethylsulfoxide; h/mMSCs: human/mouse mesenchymal stem cells; ROSI: rosiglitazone; TBT: tributyltin.

Table S1. DBT data from transfection assay

DBT concentration[a]	Luciferase	β-galactosidase[b]	Normalized[c]	Fold induction
1.00E-05	0.05533	0.1342	6.18	0.47
3.33E-06	23.525	0.647	545.76	41.30
1.11E-06	25.169	0.665	568.16	43.02
3.70E-07	3.838	0.755	76.25	5.79
1.23E-07	0.894	0.671	19.99	1.53
4.12E-08	0.655	0.720	13.64	1.04
1.37E-08	0.580	0.689	12.63	0.95
4.57E-09	0.651	0.741	13.18	1.00

[a] DBT concentration in Molar units
[b] Absorbance at 405 nm
[c] Luciferase signal divided by b-galactosidase absorbance multiply by 15 (incubation time in minutes).

Table S2. QPCR Primer list

Gene	Species	Forward	Reverse
36B4	Mouse	AAGCGCGTCCTGGCATTGTCT	CCGCAGGGGCAGCAGTGGT
C/EBPα	Mouse	ACAAGAACAGCAACGAGTACC	GGTCATTGTCACTGGTCAACT
Fabp4	Mouse	GTCACCATCCGGTCAGAGAG	TCGACTTTCCATCCCACTTC
LPL	Mouse	ACAACCAGGCCTTCGAGATT	TCAGGCCAGCTGAAGTAGGA
PPARγ2	Mouse	TGGGTGAAACTCTGGGAGATTC	AATTTCTTGTGAAGTGCTCATAGGC
Fsp27	Mouse	CTGTCGTGTTAGCACCGCAG	GCCATCTTCCTCCAGCACCA
36B4	Human	AACTCTGCATTCTCGCTTCC	ATCCGTCTCCACAGACAAGG
C/EBPα	Human	AGCAAATCGTGCCTTGTCAT	CCCTATGTTTCCACCCCTTT
FABP4	Human	AAAGTCAAGAGCACCATAACC	TTCAATGCGAACTTCAGTCC
LPL	Human	AGGAGCATTACCCAGTGTCC	GGCTGTATCCCAAGAGATGGA
PPARγ2	Human	GCGATTCCTTCACTGATAC	TCAAAGGAGTGGGAGTGGTC
FSP27	Human	CAGACAAGCCCTTCTTCCTG	TTATGGGAGAGGGACAGTGG

C/EBPα: CCAAT/Enhancer Binding Protein Alpha: Fabp4: fatty acid binding protein-4; LPL: lipoprotein lipase; PPARγ2: peroxisome proliferator-activated receptor gamma; Fsp27: fat-specific protein-27.

Table S3. EC50 and maximum activation, calculated from transfection assays reported in Figure 1

		EC_{50}				Maximum activation			
Species	Receptor	4204	ROSI	TBT	DBT	4204	ROSI	TBT	DBT
Mouse	RXR	2.4 nM	N/A	6.7 nM	1.4 μM	3986	N/A	3026	246
Human	RXR	0.65 nM	N/A	9.3 nM	0.47 μM	4864	N/A	4033	545
Mouse	PPARγ	N/A	1.4 μM	0.13 μM	0.44 μM	N/A	1347	160	115
Human	PPARγ	N/A	0.81 μM	0.25 μM	0.48 μM	N/A	754	88	30

To calculate EC_{50} and maximum activations concentrations were transformed to logarithmic scale and a non-linear regression was calculated using the "log(agonist) vs. normalized response – Variable slope" function in GraphPad. Maximum activation represents the highest luciferase absorbance normalized with β-galactosidase absorbance. 4204: IRX194204, DBT: dibutyltin, EC_{50}: Half maximal effective concentration; PPARγ: peroxisome proliferator-activated receptor gamma; RXR: Retinoid X receptor; TBT: tributyltin.

Table S4. Summary of pregnancy efficiency and animal counts for the in vivo study

			Litter size			
			At birth		At weaning	
Set 1	Bred	Pregnant	Mean	SD	Mean	SD
DMSO	8	6	6.2	0.98	6.5	1.00
TBT	8	5	7.4	0.89	7.3	0.96
5 nM DBT	8	6	7.2	1.17	4.8	0.50
50 nM DBT	8	4	6.8	1.50	6.7	1.15
500 nM DBT	8	4	6.5	1.29	6.3	1.26
Set 2						
DMSO	12	5	7.3	1.95	7.3	0.50
TBT	12	7	7.0	0.90	7.0	0.96
5 nM DBT	12	6	5.5	0.84	4.4	0.89
50 nM DBT	12	6	7.0	1.30	6.8	0.96
500 nM DBT	12	9	7.0	0.50	6.8	0.71

DBT: dibutyltin; DMSO: Dimethylsulfoxide; SD: standard deviation; TBT: tributyltin

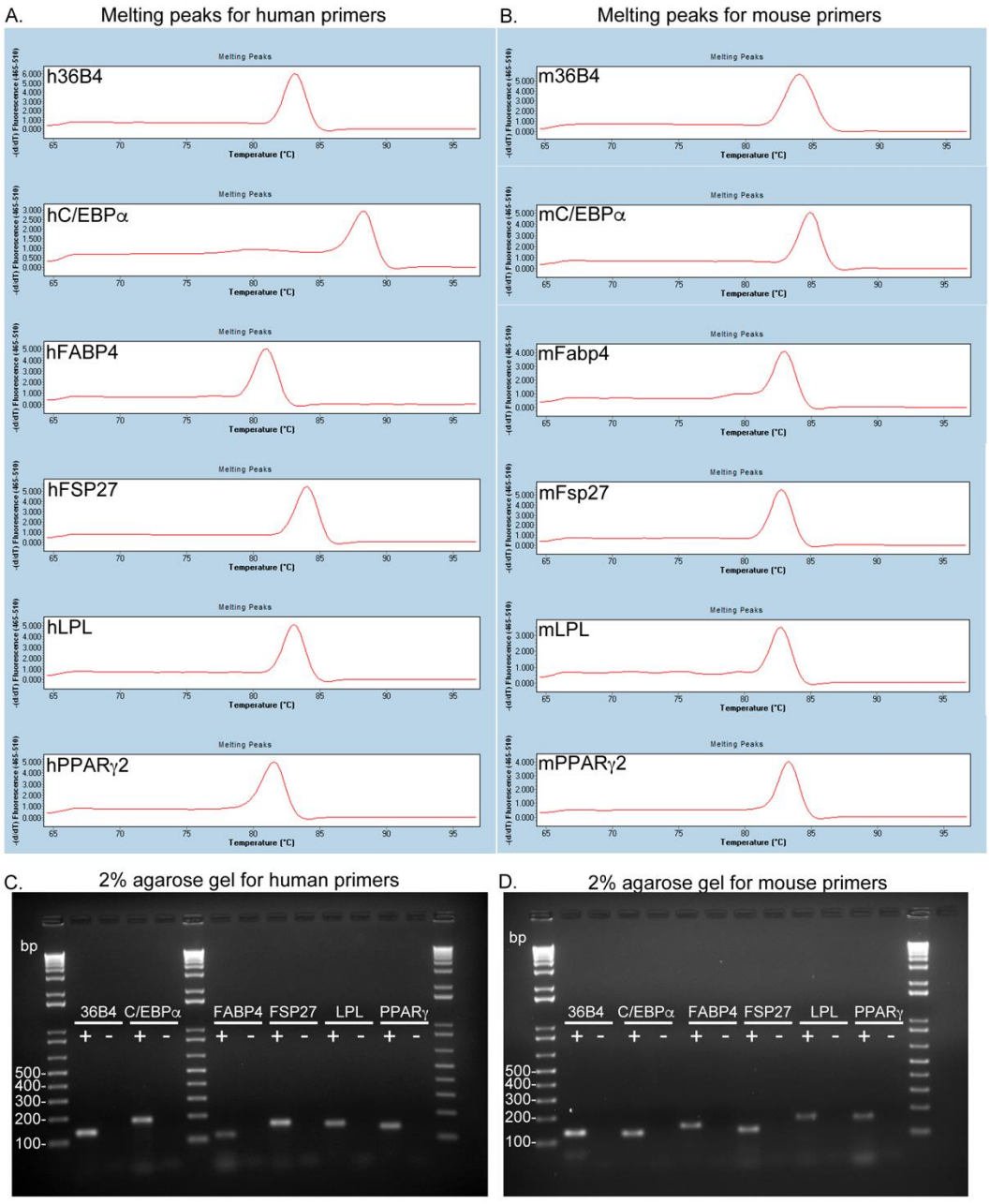


Figure S1. Primer amplification control for human and mouse. Melting peaks for amplicons generated by (A) human and (B) mouse primers. 2% agarose Tris-acetate-EDTA (TAE) gel showing bands for amplicons generated by (C) human and (D) mouse primers. DNAse treated RNA for the template was isolated from human and mouse mesenchymal stem cells induced to differentiate into adipocytes with the adipogenic cocktail and ligands. Reverse transcription reaction was run with (+) and without (-) reverse transcriptase to control for any potential genomic DNA contamination. C/EBPα: CCAAT/Enhancer Binding Protein Alpha; Fabp4: fatty acid binding protein-4; Fsp27: fat-specific protein-27; LPL: lipoprotein lipase; PPARγ2: peroxisome proliferator-activated receptor gamma; h: human; m: mouse; bp: base pairs.

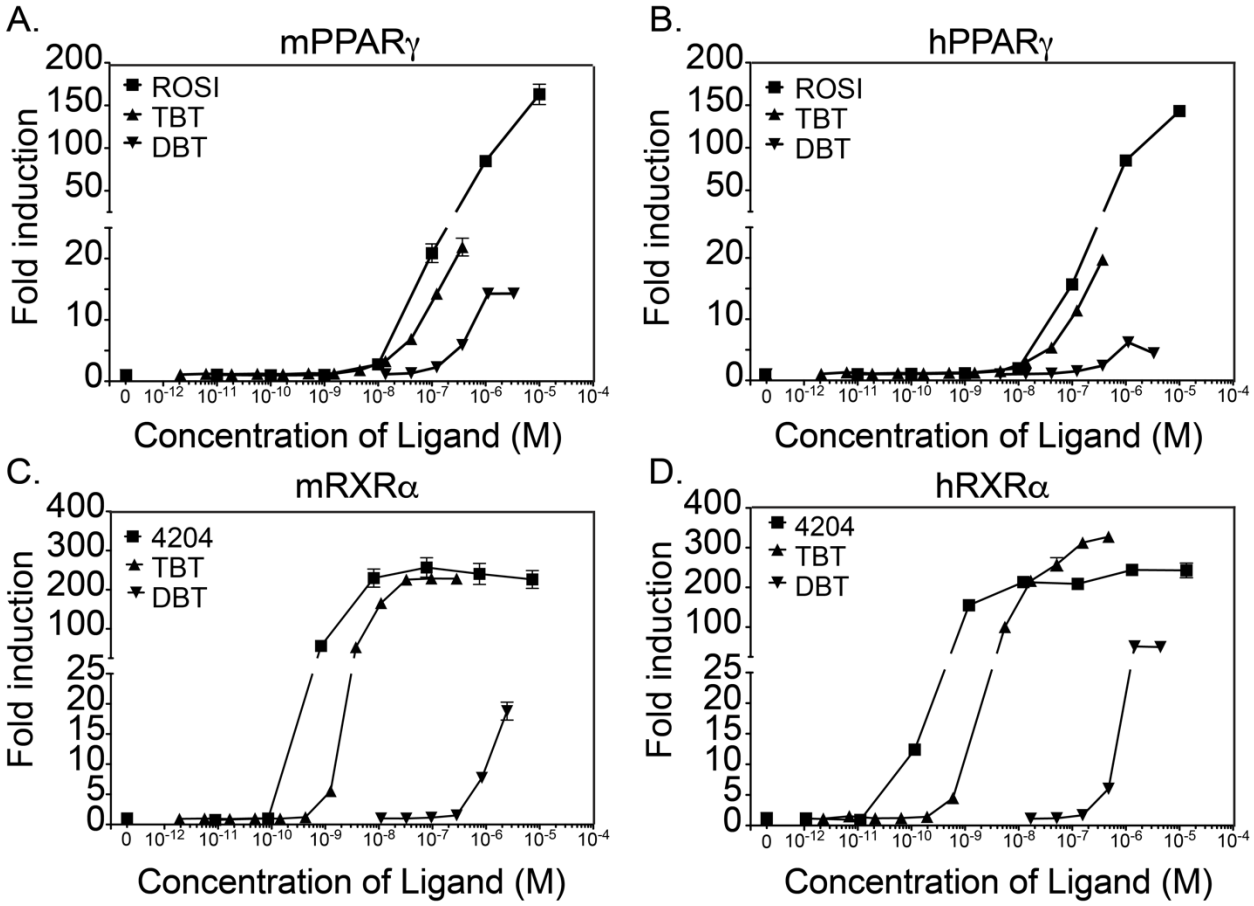
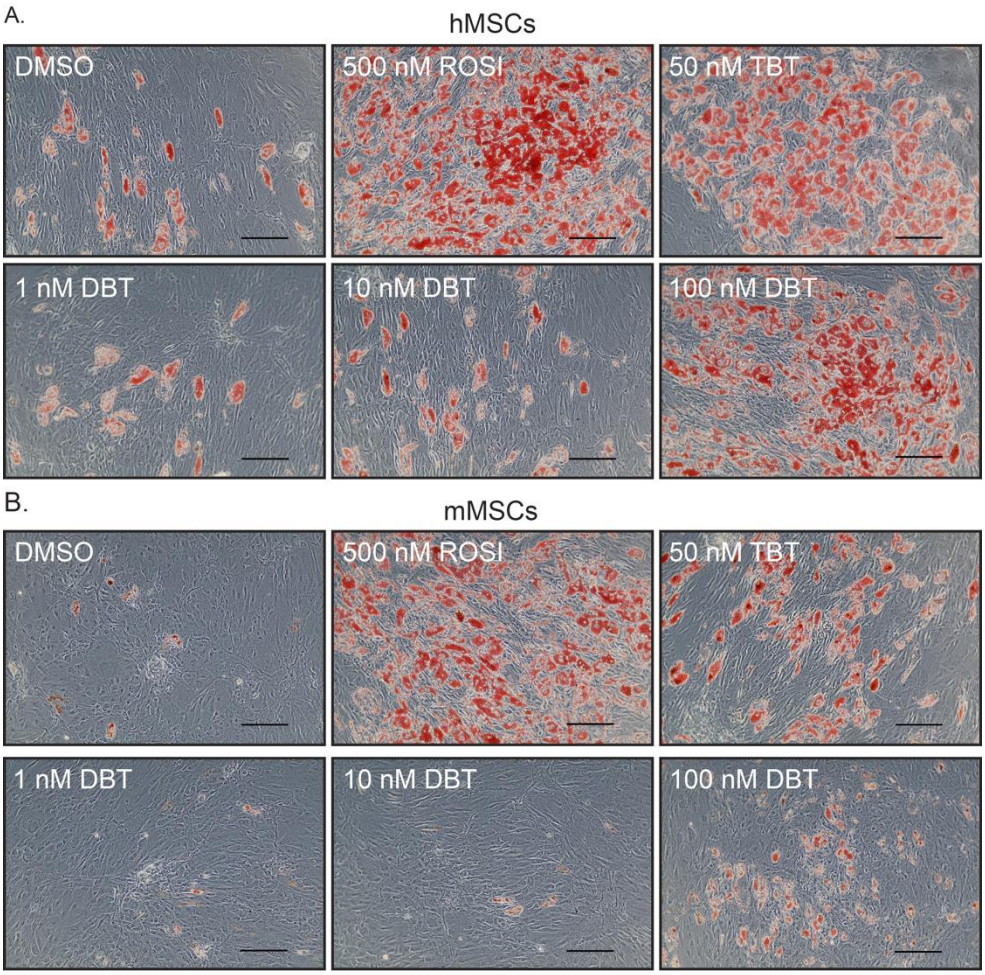


Figure S2. DBT activation of human and mouse PPARγ and RXR. Human and mouse PPARγ **(A-B)** and RXRα **(C-D)** activation by increasing doses of DBT was tested in transiently transfected Cos7 cells. Fold induction was calculated relative to 0.05% DMSO vehicle control. Each data point represents the average of triplicates for each chemical and concentration ± S.E.M. 4204: IRX194204; DBT: dibutyltin; DMSO: dimethylsulfoxide; M: molar; h/mPPARγ: human/mouse peroxisome proliferator-activated receptor gamma; h/mRXR: human/mouse retinoid X receptor; ROSI: rosiglitazone; S.E.M: standard error of the mean; TBT: tributyltin.



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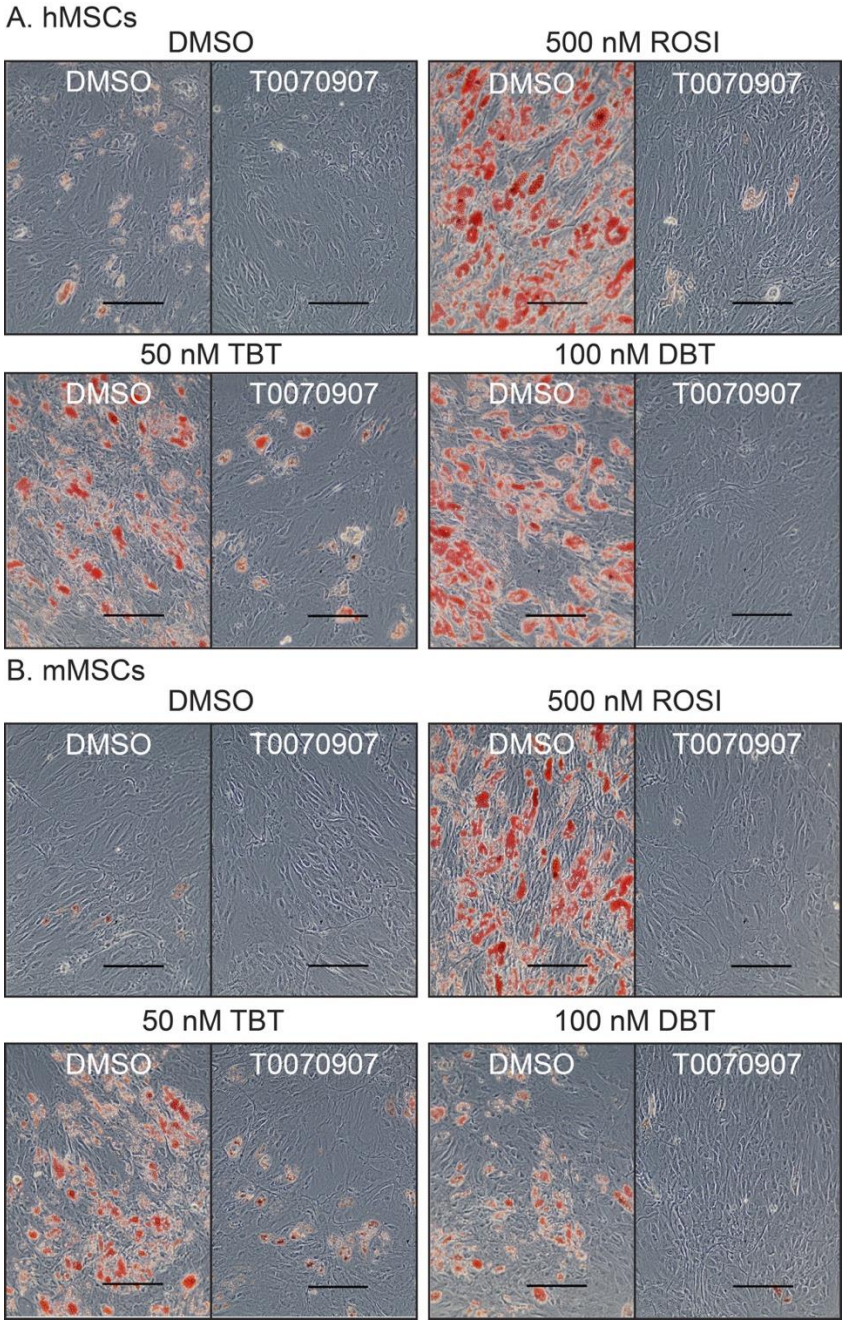

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