

Altered Adipogenesis in Zebrafish Larvae Following High Fat Diet and Chemical Exposure Is Visualised by Stimulated Raman Scattering Microscopy

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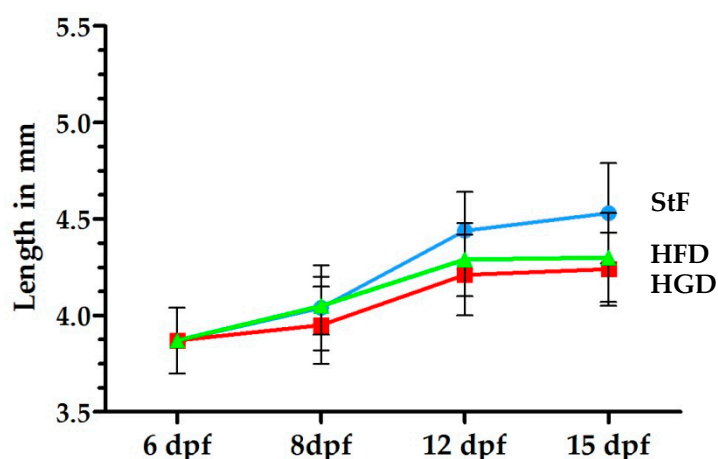


Figure S1. Growth curve from larvae fed with different caloric diets. Standard Length (mm) measurement at selected time points during zebrafish larval development. Larvae were fed with standard diet (StF), high glucose diet (HGD) or high fat diet (HFD). Error bars represent Standard Deviation.

Table S1. Overview of all adipocyte measurements of fish exposed to different diets. All images made with SRS imaging can be found in the supplementary materials.

	average number of adipocytes	average volume per fish in pL
StF	2.0	76
HGD	1.5	14
HFD	5.0	132

FISH ID	number of adipocytes	adipocyte volume in pL	approximate diameter in μm	total volume per fish in pL
StF 1	3	29	38	281
		77	53	
		174	69	
StF 2	2	5	21	45
		40	42	
StF 3	3	1	13	34
		25	36	
		8	25	
StF 4	2	28	38	39
		11	28	
StF 5	2	37	41	39
		2	17	
StF 6	1	82	54	82
StF 7	1	9	26	9

HGD 1	1	26	37	26
HGD 2	1	27	37	27
HGD 3	1	0.1	5	0.1
HGD 4	2	8	25	11
		2	16	
HGD 5	1	0.1	6	0.1
HGD 6	3	0.3	8	20
		2	14	
		19	33	
HFD 1	5	0.3	9	98
		1	13	
		58	48	
		30	39	
		9	26	
HFD 2	3	6	23	13
		3	18	
		3	19	
HFD 3	2	10	26	24
		15	30	
HFD 4	6	3	17	232
		0.1	5	
		128	63	
		6	22	
		90	56	
		6	22	
HFD 5	8	23	36	208
		2	16	
		22	35	
		68	51	
		52	46	
		11	27	
		30	39	
		1	12	
HFD 6	3	7	24	38
		0.7	11	
		30	39	
HFD 7	8	12	28	308
		7	23	
		191	71	
		0.4	9	
		0.04	4	
		67	50	
		28	38	
		3	17	

Table S2. Information about the genes selected for the obesity array.

ENSDARG	GENE code ZFIN	NM	Target gene	Oligo sequence forward	Oligo sequence reverse
ENSDARG00000027740	ZDB-GENE-041010-89	NM_214715.2	adcyap1b	GATGCACCTACAGCGGCTACT	ATCATGTCCAAAAGCCAGGT
ENSDARG00000100086	ZDB-GENE-060825-220	NM_001045425.2	adipob	CCCATAGAGAGCAGCATGTC	GCTCTGATTCTCTCAGTG
ENSDARG00000007490	ZDB-GENE-081022-145	NM_001128689.1	adip1	TCA TCGTA TCTCTGGGTGC	TGCAATTACGACACAGGTCT
ENSDARG00000069089	ZDB-GENE-040817-1	NM_009303347.1	agrp	GGCTGGTTTGTGTGAATGT	TTTCAAGGTGCTCATTTCA
ENSDARG00000040298	ZDB-GENE-030131-1263	NM_00109861.1	apob4b	AATGACAGAGGAACCGTCA	GCTCCCTCAGCATCTCAGTC
ENSDARG0000018817	ZDB-GENE-000412-1	NM_131595.2	bdnf	GGACACTTTCGAGCAGTCA	CTCCAAAAGCACTTGG TTGC
ENSDARG0000036074	ZDB-GENE-020111-2	NM_131885.2	cebpA	GGACACAGCAACCTCTACG	GATCTGCTCAGTCTCTCAG
ENSDARG0000028661	ZDB-GENE-030616-82	NM_001098242.1	cntrf	ACCACTGGCAACATGTGAA	GACGTGCTCTCAGTAGTGG
ENSDARG0000058285	ZDB-GENE-041010-9	NM_001005940.1	cpt1b	TATGACCGTTCACGCGAGA	TACAGGCAGATGCGAGAG
ENSDARG0000038918	ZDB-GENE-070524-2	NM_001135976.2	drd1b	GGAAACACGTTGTGTCTGTC	CAAGGCCACCCAAACA TCG
ENSDARG0000020850	ZDB-GENE-990415-52	NM_131263	ef1a (eef1a11)	TTGAGAGAGAAATCGGTGGTCTG	GGACCGTGTGATTGAGGAAATTC
ENSDARG0000010571	ZDB-GENE-041111-259	NM_001077279.1	erh2	AAATCGGAGAGGGTCTCTGT	TCTGTGGAGCTGAACATGC
ENSDARG0000017299	ZDB-GENE-040912-132	NM_001004682.1	fabp11a	TCAGGACCTTCAAAACA	ACAAGTTTCCGCTTCTCGAT
ENSDARG0000099555	ZDB-GENE-061013-59	NM_001077257.2	foxo1	TGAGCTGGAGTGCATCTTCG	GTGAGGTGGAATCTCAGCCG
ENSDARG0000013721	ZDB-GENE-050309-17	NM_001163806.1	gpcr2	ATAGCCTATCTTGGTGGCT	GGAAATGACGCGCGATGAAG
ENSDARG0000043457	ZDB-GENE-030115-1	NM_001115114	gapdh	GACTCCACCATGGAAGT	TAATGTGGCTGGTCCCTC
ENSDARG0000008840	ZDB-GENE-040426-1375	NM_201154	hmb1a	GTGTGGGAATTGCAACAAGTG	CGAGGGCTGATGATGAGATA TTGC
ENSDARG0000008884	ZDB-GENE-040426-1918	NM_212986	hprt1	CAGCGATGAGGAGCAAGTTATG	GTCCATGATGAGCCCGTAGG
ENSDARG0000099351	ZDB-GENE-021231-1	NM_173283.3	igfbp1a	AGGCCAAAGTCAACGCATA	TTGTCAAGGGCTGTCTGGAG
ENSDARG0000035350	ZDB-GENE-980526-110	NM_131056.1	ins	CCCCAAGAGAGAGCGTTGAGC	CAGCACTGCTCTACAATGCC
ENSDARG0000011948	ZDB-GENE-020503-3	NM_001142672.1	insra	GATTCAGATGCGCGCAGAGA	AGACCTTCCCACCTTTCT
ENSDARG00000071524	ZDB-GENE-020503-4	NM_001123229.1	insrb	GGCCAGGATCAATTTGGGAT	CCATGACAACCAATGTCCGC
ENSDARG00000991085	ZDB-GENE-081001-1	NM_001128576	lepa	TTTTCAGCTCTCCGCTCAAC	TGGTTGTTCAGCGGGAAT
ENSDARG00000070961	ZDB-GENE-080104-1	NM_001309403	lepr	TTGGCTGGAATGGCAATATC	GCTGAATCTCTGCTGTGGT
ENSDARG0000087697	ZDB-GENE-990415-139	NM_131127.1	lpl	GGCCAAATTGTCACTGGT	CATGAGGCCAAGACTGTAA
ENSDARG0000098439	ZDB-GENE-050410-7	NM_00107545	nr1h3 (lxra)	AGACCAAGTCGCCCTACTCA	GCTGCAATCGCTGGGTAT
ENSDARG0000098439	ZDB-GENE-050410-7	NM_00107545.1	mc4r	CTGACCAACCGTGAGAGCAT	TGGTAGCGCAAGAGCTAGAA
ENSDARG0000015515	ZDB-GENE-021223-2	NM_173278.1	ncor1	AGGAGGAATCA TGCAGGCAA	TGGGCTCTCTTTGGCTTTA
ENSDARG0000020482	ZDB-GENE-030131-713	NM_201579	nono	ATGGACACACACCGATGCT	AAATCTCCGAAGCCTTGCCA
ENSDARG0000017180	ZDB-GENE-030131-3161	NM_001243875.1	npcl	CTGAGACCGTGGCCTCTCTT	CCATCCGATTGGCCTCTGT
ENSDARG0000036222	ZDB-GENE-980526-438	NM_131074.2	npv	GTGTGCTGGGGA GTCTCAC	AGCGGTTGACCTTTTCCAT
ENSDARG0000042824	ZDB-GENE-030723-2	NM_182889.1	nr3c1	CG ITTCGCAATGCGCTCAT	GGAATGGAAGTGTGCTGGAT
ENSDARG0000035285	ZDB-GENE-040426-993	NM_200276.2	nfe2l2a (nrf2)	CCATGTCAATTCGATGATGTC	TGAGATCGATGGAAGGTGGA
ENSDARG0000098511	ZDB-GENE-010126-2	NM_001197161.2	ntrk2b	GCACTCCAGGTGTGAGGTAT	CGACTGCAAGTCCATTTTTCG
ENSDARG0000043135	ZDB-GENE-030513-2	NM_181438.3	pomc	ATGTTGTGCTCTGCTGGCT	GGGTAGACGGGGGTTTCATC
ENSDARG0000031777	ZDB-GENE-041210-169	NM_001161333.1	pparaa	CGACGACCTTCACTCTCT	GAGATCCGGATGAGTCTCG
ENSDARG0000054323	ZDB-GENE-990415-211	NM_001102567.1	pparab	GTGACCTCGCACTGTTTGTCT	CTCTCGATTCGCTCGATA
ENSDARG0000044525	ZDB-GENE-990415-212	NM_005168286.2	pparda	ATCGACCTTCTTCAACA	AAGAGCCGCTCTTATCA
ENSDARG0000009473	ZDB-GENE-000112-47	NM_131468.2	ppardb	CGTCAACACGCTACTCTGA	GCCACAGGAGTCCATATCA
ENSDARG0000031848	ZDB-GENE-990415-213	NM_131467.1	pparg	TACGAGACCAACCCCAACA	GTATCTGCTGTGCTCTGTA
ENSDARG0000057737	ZDB-GENE-070314-2	NM_001161551	ppraa	ACCCACTTCTCTACCAA	CGAGTGTACACACCTAGT
ENSDARG0000035819	ZDB-GENE-070112-1762	NM_001080174	sirt3	TCAGACACCAAGTGGAAATC	CGTCAGGTGGGTGGTAAT
ENSDARG0000035559	ZDB-GENE-990415-270	NM_131327.2	tp53	GGGCAATCAGCGAGCAA	ACTGACCTTCTGAGTCTCCA
ENSDARG0000023151	ZDB-GENE-010503-1	NM_199523.2	ucp1	GCCCTCTACGACAAAGCTGAA	TGTATCGTCTGCCCAACC
ENSDARG0000040925	ZDB-GENE-980526-524	NM_178219.2	wnt10b	CCGGATGATGGTCAACAATGCT	TGGCACTTGCACTTCTGCT
ENSDARG00000102464	ZDB-GENE-980526-87	NM_130937.1	wnt5b	CCCTCATCTGCTGCAACTCA	TCTGACCCCTGAGATAGGCC

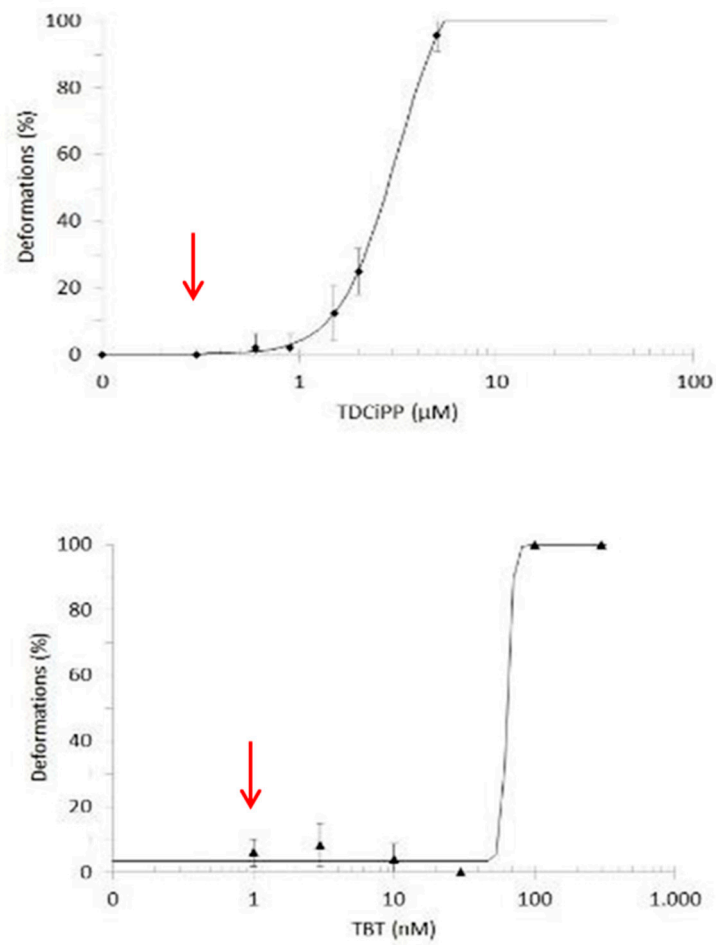


Figure S2. Dose-response curves for selected environmental chemicals, showing percentage of deformed larvae after exposure to (a) TDCiPP, (b) TBT. The red arrows indicate the non-toxic concentrations selected for this study.

Table S3. Overview of all adipocyte measurements of fish exposed to different environmental chemicals. All images made with SRS imaging can be found in the supplementary materials.

	average number of adipocytes	average volume per fish in pL
solvent control	3.0	229
TDCiPP	2.5	25
TBT	4.6	164

FISH ID	number of adipocytes	adipocyte volume in pL	approximate diameter in μm	total volume per fish in pL
solvent 1	1	80	53	80
solvent 2	1	209	74	209
solvent 3	1	90	56	90
solvent 4	3	58	48	160
		100	58	
		1	13	
solvent 5	3	3	17	46
		21	34	
		23	35	
solvent 6	4	71	51	1235
		70	51	
		137	64	
		958	122	
solvent 7	1	4	19	4
solvent 8	10	0.1	6	12
		1	13	
		3	17	
		0.1	6	
		1	14	
		0.3	8	
		0.1	5	
		6	22	
		0.1	5	
		1	12	
TDCiPP 1	1	26	37	26
TDCiPP 2	4	0.1	5	16
		8	25	
		6	22	
		2	17	
TDCiPP 3	1	12	29	12
TDCiPP 4	4	0.6	10	13
		2	16	
		0.03	4	
		10	27	
TDCiPP 5	1	7	24	7
TDCiPP 6	6	3	19	34
		1	14	
		3	17	
		3	18	
		16	31	
		7	24	
TDCiPP 7	1	1	12	1
TDCiPP 8	2	78	53	91
		13	29	
TBT 1	2	177	70	216
		39	42	
TBT 2	6	169	69	550
		280	81	

		39	42	
		43	43	
		11	28	
		7	24	
TBT 3	3	1	12	8
		5	21	
		2	16	
TBT 4	3	61	49	387
		214	74	
		112	60	
TBT 5	14	12	28	61
		0.2	8	
		5	21	
		1	13	
		26	37	
		0.3	9	
		0.4	9	
		0.4	9	
		0.6	10	
		11	28	
		1	13	
		0.05	4	
		0.5	10	
		2	15	
TBT 6	5	0.2	7	28
		0.1	6	
		1	13	
		2	17	
		25	36	
TBT 7	2	0.5	10	48
		47	45	
TBT 8	2	3	18	15
		12	28	