

Supporting Information

Endocrine disruption throughout the hypothalamus-pituitary-gonadal-liver (HPGL) axis in marine medaka (*Oryzias melastigma*) chronically exposed to the antifouling and chemopreventive agent, 3,3'-diindolylmethane (DIM)

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Table S1. Primers used for the amplification of the selected genes in this study.

Gene	Sequence of primers (5'-3')
<i>ERα</i> ¹	F: TCGCCGCTGTTGTGCTGTGATGTT R: TCCTGGATCTGAGTGCAGGGTCCGA
<i>ERβ</i> ¹	F: TGATCCAGGAGGGCTGAGCTCCACGA R: ACCGCTGACGGAGGCTGTTGTGAT
<i>ARα</i> ²	F: TTTGATGAAGTCGGACCTCCTAC R: AACTGGTGCAATTCTCACACC
<i>ChgH</i> ^{1,3}	F: ATGCCGACTATCCTGTGACC R: AGCGTCCAAGAGTCAGAACCC
<i>ChgL</i> ^{1,3}	F: CAAAGTTCGTGTCTCGACCA R: TCAGCATCAAGGGGATAAGC
<i>VTG1</i> ¹	F: TTGGCAGAGATGCAGCAGCGGT R: GGAAATGCAGGACACCCCAGTAGCC
<i>VTG2</i> ¹	F: AAGCCCTCAACCAACTGGCTCCCTAAT R: TGGGCAGCGCCGTTCAAGATGTTGAT
<i>CYP17a1</i> ⁴	F: CAGACTACAGCGACCACGTT R: CTGGATAATGGATCAGGTAGGT
<i>17βHSD</i> ²	F: CGCTACCTCCACAAAGTTGTTGTC R: AGTTCTGCCTCAACAGTTTCACCT
<i>CYP19a</i> ⁵	F: GACACCGTTGTTGGTGACAG R: CATCAAAAAGGGCTCTACGC
<i>CYP19b</i> ¹	F: AGGCACAAAGATCAGGAGGGGAACCA R: GGTCCACAGCCGAAGGGTTGAAAGAA
<i>FSHR</i> ⁵	F: GCGTGTGCGGCTGCTACC R: CGAGATGGCGAAGAAGGGAGATGG
<i>LHR</i> ⁵	F: CCTGGTGGTGTGCTACTGCTAC R: CGGCGGAGATGGCGAAGAAG
<i>3βHSD</i> ⁵	F: TGGTCAACCCCGTCTATGTG R: GGTGTCGTCAGTGGCGAAGT
<i>StAR</i> ²	F: AGAAGGCTATCAGCATCCTCAGTG R: AGGCAGTACCTTACTCAGGACCTT
<i>CYP11a</i> ⁵	F: CGTTGGGTTACTGCTGGACTAC R: GCGGTCAAGCTGGTTGAATATCC
<i>GTHα</i> ⁵	F: TGACACCTGCTGTTCTCTG R: GTTGGGTACGCTCTGGAGAA
<i>FSHβ</i> ⁵	F: ATATGCGAAGGACGGTGCTAC R: AGTACGTGGTTCTGGTGTG
<i>LHβ</i> ⁵	F: CGGGTTGGCAGAGGGATGTTTC R: GGGTCCTTGGTGAAGCAGTGG
<i>sGnRH</i> ⁶	F: CAGAGTGACGGTGCAGGGTGT R: TATGGTCTAAGTCTCTCTGGGT
<i>mGnRH</i>	F: ATGAAAACGTGGATGCTGTG R: CAGGGTGCATTGCTGTTAAG

GnRHR ²	F: GAAGGCTCGGATGAAGACACTGAA R: CCAGATCCCAAGCAGGTAGTATGG
CYP11b ²	F: GCATTGGCCTGTTCTCCTCATCT R: CGGAGGGGTGGTTGTTAGCATT
HCE ⁷	F: TGGCTACGTCCGGATCAACTGGCA R: GGGGGTGTTCAGGTTGTTGGTGTGCG
LCE ⁷	F: GCGTGGAACAAAGTGAACGCAGGT R: ACTGGCCAAGTCTTCCAACGGGA
VTGR	F: GACAACCCGGTCTACCTGAA R: CTGGATTGAAGGAGGGATGA
18s	F: GACAAATCGCTCCACCAACT
(control) ^{3, 5}	R: CCTGCAGCTTAATTGACCC

References

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Table S2. Transcriptional changes of the genes along the HPGL axis after DIM treatment.^a

Tissues	Genes	Male		Female	
		0 µg/L	8.46 µg/L	0 µg/L	8.46 µg/L
Brain	<i>sGnRH</i>	1.0±0.01	1.2±0.07	1.0±0.23	2.4±0.29*
	<i>mGnRH</i>	1.0±0.11	0.6±0.09*	1.0±0.31	3.3±0.34*
	<i>GnRHR</i>	1.0±0.05	1.1±0.14	1.0±0.02	1.4±0.09*
	<i>GTHα</i>	1.0±0.03	1.2±0.11	1.0±0.00	2.0±0.22*
	<i>LHβ</i>	1.0±0.15	1.3±0.04	1.0±0.03	1.6±0.05**
	<i>FSHβ</i>	1.0±0.02	1.1±0.09	1.0±0.28	1.7±0.09*
	<i>CYP19b</i>	1.0±0.10	1.0±0.20	1.0±0.17	0.9±0.04
	<i>ERα</i>	1.0±0.07	1.0±0.11	1.0±0.03	1.3±0.28
	<i>ERβ</i>	1.0±0.17	0.8±0.12	1.0±0.06	1.5±0.08*
	<i>ARα</i>	1.0±0.07	1.0±0.04	1.1±0.35	2.5±0.30*
Gonad	<i>ERα</i>	1.1±0.24	1.4±0.17	1.0±0.17	1.5±0.30
	<i>ERβ</i>	1.0±0.13	1.5±0.22	1.0±0.06	1.8±0.25*
	<i>ARα</i>	1.0±0.21	1.2±0.04	1.0±0.10	1.0±0.16
	<i>LHR</i>	1.0±0.10	1.8±0.29*	1.0±0.11	1.7±0.15*
	<i>FSHR</i>	1.0±0.09	1.7±0.08*	1.0±0.04	1.4±0.08*
	<i>VTGR</i>	1.0±0.03	1.4±0.18	1.0±0.14	2.5±0.52*
	<i>Star</i>	1.0±0.20	1.9±0.53	1.0±0.21	0.4±0.05*
	<i>CYP11a</i>	1.0±0.12	1.7±0.22*	1.0±0.06	0.4±0.15*
	<i>3βHSD</i>	1.0±0.22	1.4±0.06	1.0±0.04	0.6±0.17
	<i>CYP17a1</i>	1.0±0.11	1.6±0.08*	1.0±0.05	1.7±0.24*
Liver	<i>17βHSD</i>	1.0±0.10	2.0±0.15**	1.0±0.09	2.7±0.10**
	<i>CYP19a</i>	1.0±0.08	1.7±0.33	1.0±0.08	0.24±0.09**
	<i>CYP11b</i>	1.0±0.11	3.6±0.21***	1.0±0.08	0.2±0.05**
	<i>ERα</i>	1.0±0.06	1.4±0.49	1.0±0.24	1.5±0.35
	<i>ERβ</i>	1.0±0.04	1.3±0.14	1.0±0.22	1.6±0.24
Placenta	<i>ARα</i>	1.1±0.40	0.9±0.02	1.0±0.15	0.7±0.13
	<i>VTG-1</i>	1.1±0.25	1.2±0.19	1.0±0.07	0.2±0.06**
	<i>VTG-2</i>	1.2±0.47	1.3±0.14	1.0±0.12	0.1±0.11*
	<i>ChGH</i>	1.1±0.30	3.0±0.48*	1.0±0.04	0.2±0.05**
Ovary	<i>ChGL</i>	1.0±0.07	1.5±0.21	1.0±0.04	0.2±0.01***

^a Values represent the mean ± SEM of three replicates.

*P < 0.05, **P < 0.01 and ***P < 0.001 indicate significant difference between the DIM group and the corresponding control group.