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### **Supplemental Material**

#### **Effects of Low-dose Developmental Bisphenol A Exposure on Metabolic Parameters and Gene Expression in Male and Female Fischer 344 Rat Offspring**

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#### **Table of Contents**

ARRIVE Guidelines Checklist.....	4
Table S1. Weight gain, food intake, water intake, and BW for dams at PND22.....	6
Table S2. Concentration of BPA in drinking water, doses aimed for and doses consumed.....	7
Table S3. Genes analyzed with qPCR and their forward and reverse sequences.....	8
Table S4. Primer efficiency.....	11
Table S5. Number of pups and sex ratio.....	13
Table S6. Results for males and females combined.....	14
Table S7. Number of cells per high power field in adipose tissue.....	17
Table S8. Primary analysis: Transcriptional levels in adipose tissue and liver.....	18

## ARRIVE Guidelines Checklist

### Animal Research: Reporting In Vivo Experiments

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	ITEM	RECOMMENDATION	Section/ Paragraph
Title	1	Provide as accurate and concise a description of the content of the article as possible.	Title
Abstract	2	Provide an accurate summary of the background, research objectives, including details of the species or strain of animal used, key methods, principal findings and conclusions of the study.	Abstract
<b>INTRODUCTION</b>			
Background	3	a. Include sufficient scientific background (including relevant references to previous work) to understand the motivation and context for the study, and explain the experimental approach and rationale. b. Explain how and why the animal species and model being used can address the scientific objectives and, where appropriate, the study's relevance to human biology.	Paragraphs 1-3  Paragraph 3 and Discussion,
Objectives	4	Clearly describe the primary and any secondary objectives of the study, or specific hypotheses being tested.	Paragraph 3
<b>METHODS</b>			
Ethical statement	5	Indicate the nature of the ethical review permissions, relevant licences (e.g. Animal [Scientific Procedures] Act 1986), and national or institutional guidelines for the care and use of animals, that cover the research.	Paragraph 2
Study design	6	For each experiment, give brief details of the study design including: a. The number of experimental and control groups. b. Any steps taken to minimise the effects of subjective bias when allocating animals to treatment (e.g. randomisation procedure) and when assessing results (e.g. if done, describe who was blinded and when). c. The experimental unit (e.g. a single animal, group or cage of animals). A time-line diagram or flow chart can be useful to illustrate how complex study designs were carried out.	Paragraph 6  Paragraph 3 & 8.  Paragraph 3
Experimental procedures	7	For each experiment and each experimental group, including controls, provide precise details of all procedures carried out. For example: a. How (e.g. drug formulation and dose, site and route of administration, anaesthesia and analgesia used [including monitoring], surgical procedure, method of euthanasia). Provide details of any specialist equipment used, including supplier(s). b. When (e.g. time of day). c. Where (e.g. home cage, laboratory, water maze). d. Why (e.g. rationale for choice of specific anaesthetic, route of administration, drug dose used).	Paragraph 3 & 5  Paragraph 3 Paragraph 3 Paragraph 3, Institutional Animal Care Paragraph 3
Experimental animals	8	a. Provide details of the animals used, including species, strain, sex, developmental stage (e.g. mean or median age plus age range) and weight (e.g. mean or median weight plus weight range). b. Provide further relevant information such as the source of animals, international strain nomenclature, genetic modification status (e.g. knock-out or transgenic), genotype, health/immune status, drug or test naïve, previous procedures, etc.	Paragraph 3

Housing and husbandry	9	Provide details of: a. Housing (type of facility e.g. specific pathogen free [SPF], type of cage or housing, bedding material, number of cage companions, tank shape and material etc. for fish). b. Husbandry conditions (e.g. breeding programme, light/dark cycle, temperature, quality of water etc for fish, type of food, access to food and water, environmental enrichment). c. Welfare-related assessments and interventions that were carried out prior to, during, or after the experiment.	Paragraphs 3-4  Paragraphs 3-4  Paragraph 3
Sample size	10	a. Specify the total number of animals used in each experiment, and the number of animals in each experimental group. b. Explain how the number of animals was arrived at. Provide details of any sample size calculation used. c. Indicate the number of independent replications of each experiment, if relevant.	Paragraph 6  Paragraph 3, Dams arrived within a 7 week time frame
Allocating animals to experimental groups	11	a. Give full details of how animals were allocated to experimental groups, including randomisation or matching if done. b. Describe the order in which the animals in the different experimental groups were treated and assessed.	Paragraph 3  At sacrifice the offspring
Experimental outcomes	12	Clearly define the primary and secondary experimental outcomes assessed (e.g. cell death, molecular markers, behavioural changes).	Paragraphs 8-12
Statistical methods	13	a. Provide details of the statistical methods used for each analysis. b. Specify the unit of analysis for each dataset (e.g. single animal, group of animals, single neuron). c. Describe any methods used to assess whether the data met the assumptions of the statistical approach.	Paragraphs 13-14, p. 7

## RESULTS

Baseline data	14	For each experimental group, report relevant characteristics and health status of animals (e.g. weight, microbiological status, and drug or test naïve) prior to treatment or testing. (This information can often be tabulated).	Method, Paragraph 3
Numbers analysed	15	a. Report the number of animals in each group included in each analysis. Report absolute numbers (e.g. 10/20, not 50% <sup>2</sup> ). b. If any animals or data were not included in the analysis, explain why.	Method, Paragraph 6  <del>We randomly</del>
Outcomes and estimation	16	Report the results for each analysis carried out, with a measure of precision (e.g. standard error or confidence interval).	Paragraphs 2-9, Figures 1-3 and Table 1A
Adverse events	17	a. Give details of all important adverse events in each experimental group. b. Describe any modifications to the experimental protocols made to reduce adverse events.	No adverse events to report.

## DISCUSSION

Interpretation/scientific implications	18	a. Interpret the results, taking into account the study objectives and hypotheses, current theory and other relevant studies in the literature. b. Comment on the study limitations including any potential sources of bias, any limitations of the animal model, and the imprecision associated with the results <sup>2</sup> . c. Describe any implications of your experimental methods or findings for the replacement, refinement or reduction (the 3Rs) of the use of animals in research.	Throughout  Fewer animals in the high dose group due to fewer dams pregnant. Due
Generalisability/translation	19	Comment on whether, and how, the findings of this study are likely to translate to other species or systems, including any relevance to human biology.	Paragraph 16
Funding	20	List all funding sources (including grant number) and the role of the funder(s) in the study.	Title page

### References:

- Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG (2010) Improving Bioscience Research Reporting: The ARRIVE Guidelines for Reporting Animal Research. *PLoS Biol* 8(6): e1000412. doi:10.1371/journal.pbio.1000412
- Schulz KF, Altman DG, Moher D, the CONSORT Group (2010) CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials. *BMJ* 340:c332.

## Table S1. Weight gain, food intake, water intake, and BW for dams at PND22

**Table S1.** Weight gain, food intake, water intake, and body weight at PND22 for dams exposed to 0, 0.5 or 50 µg BPA/kg BW/day

	[C] (n=14)	[0.5] (n=11)	[50] (n=9)	One-way ANOVA	Dunnett's post-hoc test
Weight gain (g)	48.7 ± 2.2	48.3 ± 3.4	40.3 ± 3.0	<i>p</i> =0.09	[C]-[0.5]: <i>p</i> =1.0, C-[50]: <i>p</i> =0.08
Total food intake (g/week)	150.7 ± 2.0	145.6 ± 5.7	143.3 ± 3.9	<i>p</i> =0.4	[C]-[0.5]: <i>p</i> =0.6, C-[50]: <i>p</i> =0.3
Food intake GD3.5-birth (g/week)	92.1 ± 1.4	96.2 ± 1.7	89.3 ± 3.9	<i>p</i> =0.2 <sup>#</sup>	[C]-[0.5]: <i>p</i> =0.4, C-[50]: <i>p</i> =1.0
Food intake birth-PND22 (g/week)	209.3 ± 3.5	195.0 ± 11.2	198.3 ± 6.3	<i>p</i> =0.3	[C]-[0.5]: <i>p</i> =0.3, C-[50]: <i>p</i> =0.4
Total water intake (ml/day)	31.9 ± 0.9	29.8 ± 0.7	29.7 ± 0.7	<i>p</i> =0.12	[C]-[0.5]: <i>p</i> =0.2, C-[50]: <i>p</i> =0.2
Water intake GD3.5-birth (ml/day)	22.2 ± 1.1	20.1 ± 0.6	20.0 ± 0.9	<i>p</i> =0.1	[C]-[0.5]: <i>p</i> =0.2, C-[50]: <i>p</i> =0.2
Water intake birth-PND22 (ml/day)	41.1 ± 1.0	36.8 ± 2.7	39.1 ± 1.0	<i>p</i> =0.2	[C]-[0.5]: <i>p</i> =0.1, C-[50]: <i>p</i> =0.6
Bodyweight (g)	197.6 ± 3.2	201.6 ± 3.1	198.8 ± 6.2	<i>p</i> =0.8	[C]-[0.5]: <i>p</i> =0.7, C-[50]: <i>p</i> =1.0

<sup>#</sup>: Data not normally distributed and Kruskal-Wallis *p*-value and post hoc tests shown. Pups of one [0.5] dam was transferred to other [0.5] dams at PND4. Results are shown as mean ± SEM

## Table S2. Concentration of BPA in drinking water, doses aimed for and doses consumed

**Table S2.** Exposure of BPA, doses aimed for and actual doses that the dams consumed. Dams (F344) were given either water or bisphenol a (BPA) - 0.0025 or 0.25 mg/L.

Exposure	[C] (n=14)	[0.5] (n=11)	[50] (n=9)
Dose in drinking water	0	0.0025 mg/L	0.25 mg/L
Dose aimed for	0	0.5 µg/kg bw/day	50 µg/kg bw/day
Actual dose (GD3.5-PND22)	0	0.404 µg/kg bw/day	40.1 µg/kg bw/day
Actual dose (GD3.5-till birth)	0	0.272 µg/kg bw/day	26.9 µg/kg bw/day
Actual dose (birth-PND22)	0	0.530 µg/kg bw/day	52.7 µg/kg bw/day

Note: Pups of one [0.5] dam was transferred to other [0.5] dams at PND4. [C]: Control, [0.5]: 0.5 µg BPA/kg bodyweight/day, [50]: 50 µg BPA/kg bodyweight/day, GD: Gestational day, PND: Postnatal day.

### Table S3. Genes analyzed with qPCR and their forward and reverse sequences

Table S3. Genes analyzed in adipose and liver, or only adipose tissue using qPCR and their forward and reverse sequences.

Gene	Forward sequence	Reverse sequence	Tissue measured in	Main gene function
<i>ACC</i>	TCCCGGAGCTACTCTTAAAAAATG	CCCCAACGCCACATG	Iwat, gWAT, Liver, iscpBAT	Lipogenesis
<i>Adiponectin</i>	TGGTCACAATGGGATACCG	CCCTTAGGACCAAGAACACCT	Iwat, Gwat, iscpBAT	Hormonal signaling
<i>AdipoR1</i>	AGCACCGGCAGACAAGAG	CCCTTAGGACCAAGAACACCT	iWAT, gWAT, iscpBAT	Hormonal signaling
<i>AdipoR2</i>	ATGTTTGCCACCCCTCAGT	GATTCCACTCAGACCCAAGC	iWAT, gWAT, Liver, iscpBAT	Hormonal signaling
<i>Ahr</i>	CTTCAGATGCCGGCTGAG	CCTCCCTTGGAATTCATTG	iWAT	Hormonal signaling
<i>CEBP-<math>\alpha</math></i>	AGTTGACCAGTGACAATGACCG	TCAGGCAGCTGGCGGAAGAT	iWAT, gWAT, Liver, iscpBAT	Pro-lipogenic TF
<i>ESR<math>\alpha</math></i>	GATGGGCTTATTGACCAACC	TGGAGATTCAAGTCCCCAAA	iWAT	Hormonal signaling
<i>FABP4</i>	AATGTGCGACGCCTTTGT	TGATGATCAAGTTGGGCTTG	iscpBAT	Fatty acid transport

<i>FABP1</i>	CCTCTCCGGCAAGTACCAAG	CGCAGCCGCAAATGC	Liver	Fatty acid transport
<i>FASN</i>	CTCTGGAAGTGCATGCTGTAAGA	GGTAGATGTCATTTGCGAAAGGT	gWAT, Liver	De novo lipogenesis
<i>GATA2</i>	AATCGGCCGCTCATCAAG	TCGTCTGACAATTTGCACAACA	iWAT, gWAT, Liver, iscpBAT	Anti-adipogenic TF
<i>GPER1</i>	TTCATCAACCTGGCAGCGGCTG	TGCAGAGCACGGCGATATCGT	iWAT, gWAT	Hormonal signaling
<i>IGF1</i>	TCAGTTCGTGTGTGGACCAG	TCACAGCTCCGGAAGCAAC	iWAT	Hormonal signaling
<i>Leptin</i>	GGTGGCTGGTTTGTCTCTGT	TATGTGGCTGCAGAGGTGAG	iWAT, gWAT, iscpBAT	Hormonal signaling
<i>LPL</i>	CAGAGAAGGGGCTTGGAGAT	TTCATTCAGCAGGGAGTCAA	iWAT, gWAT, Liver, iscpBAT	Fatty acid uptake
<i>MTPP</i>	ATGCAAATTGAGAGGTCCG	TTGCTTCCCAGGTACCATTC	Liver	Fatty acid uptake
<i>PGC1<math>\alpha</math></i>	CTGCCATTGTTAAGACCGAGAA	AGGGACGTCTTTGTGGCTTTT	iWAT, gWAT, Liver, iscpBAT	Lipid catabolism
<i>PPAR<math>\alpha</math></i>	TGGAGTCCACGCATGTGAAG	CGCCAGCTTTAGCCGAATAG	gWAT, Liver	Lipid catabolism
<i>PPAR<math>\gamma</math></i>	CTGACCCAATGGTTGCTGATTAC	GGACGCAGGCTCTACTTTGATC	iWAT, gWAT, iscpBAT	Adipogenesis
<i>Pref1</i>	CTGCACTGACCCCATTTGTCT	TTCCCCCGGTTTGTCAACA	iWAT, Liver, iscpBAT	Anti-adipogenic factor
<i>PXR</i>	TGCACACAGGTTCTGTTCTGA	GGGGTGCGTGTCTGGATGC	iWAT	Sensor of toxins
<i>RXR<math>\alpha</math></i>	ACATGCAGATGGACAAGACG	GGGTTTGAGAGCCCCTTAGA	iWAT, gWAT	Lipogenesis

<i>SCD1</i>	CAACACCATGGCGTTCCA	GCGTGTGTCTCAGAGAACTTGTG	iWAT, gWAT, Liver, iscpBAT	Lipogenesis
<i>SREBP-1c</i>	CATCGACTACATCCGCTTCTTACA	GTCTTTCAGTGATTTGCTTTTGTGA	iWAT, gWAT, Liver, iscpBAT	Lipogenesis
<i>ThRβ</i>	CTCTGTCGTCTTTCAACCTGGAT	TGGGCGATCTGAAGACATCA	iWAT	Hormonal signaling
<i>UGT2B1</i>	GCTGCTTCCAGGAACCTG	TGAGGTCCCAACGCTGTCTT	Liver	Detoxification
<i>36B4</i> *	TTCCCACTGGCTGAAAAGGT	CGCAGCCGCAAATGC	iWAT, gWAT, Liver	Housekeeping gene
<i>Gusb</i> *	CTCTGGTGGCCTTACCTGAT	CAGACTCAGGTGTTGTCATCG	iWAT, gWAT, Liver	Housekeeping gene

\* : housekeeping gene, gWAT: Gonadal white adipose tissue, iscpBAT: interscapular brown adipose tissue, iWAT: inguinal adipose tissue, TF: Transcription factor



## Table S4. Primer efficiency

Table S4. Primer efficiency calculations

Gene	k	R <sup>2</sup>	E= 10 <sup>(-1/slope)</sup> -1
ACC	-3.3	0.999	100.7 %
Adiponectin	-3.5	0.999	94.8 %
AdipoR1	-3.3	0.999	99.1 %
AdipoR2	-3.8	0.999	97.6 %
Ahr	-3.8	0.998	82.3 %
CEBP $\alpha$	-3.7	0.998	87.8 %
ESR $\alpha$	-3.2	0.997	105.4 %
FABP4	-3.6	0.993	90.3 %
FABP1	-3.3	0.998	98.5 %
FASN	-3.4	0.988	97.7 %
GATA2	-3.5	0.999	92.2 %
GPER1	-3.0	0.992	114.5 %
IGF1	-3.3	0.997	102.6 %
Leptin	-3.6	0.995	84.5 %
LPL	-3.8	0.991	82.9 %
MTTP	-3.4	0.999	101.3 %
PGC1 $\alpha$	-3.5	1	92.7 %
PPAR $\alpha$	-3.8	0.997	83.9 %
PPAR $\gamma$	-3.4	0.995	97.2 %
Pref1	-3.1	0.994	110.7 %
PXR	-3.8	0.999	83.9 %
RXR $\alpha$	-3.6	0.985	88.2 %
SCD1	-3.8	1	84.2 %
SREBP-1C	-3.2	0.991	105.9 %
ThR $\beta$	-3.6	0.995	90.8 %
UGT2B1	-3.4	0.991	102.9 %
36B4*	-3.3	0.999	105.5 %
Gusb*	-3.4	0.999	97.1 %

Note: Primer efficiency ( $10^{(-1/\text{slope})-1}$ ) based on standard curves from qPCR on serial dilutions of cDNA adipose tissue.  $k$  = The slope based on a plot of the number of quantification cycles versus the nucleic acid input level for each primer pair.  $R^2$  = The correlation coefficient of the line, accepted if  $\geq 0.985$ .

## Table S5. Number of pups and sex ratio

Table S5. Litter details.

Treatment	Dams without litters/total number of dams per dose group, Number and (%)	Number of pups/dam with pups, Mean and (Median)	Male pups/total number of pups (%)
[C]	4/18 (22.2)	7.1(8)	54
[0.5]	0/12 (0)	5.6 (6)	59
[50]	6/15 (40)	6.2 (5.5)	61
One-way ANOVA	*	$p=0.5$	$p=0.7$

\*: Chi-2 test was performed: [C]-[0.5]:  $p=0.13$ , [C]-[50]:  $p=0.45$ , [0.5]-[50]:  $p=0.02$ .

Note: Dams without litters/total number of dams per dose group, number of pups per dam with pups and sex ratio of five-week-old male and female F344 rats developmentally exposed to BPA. [C]: Control, [0.5]: 0.5  $\mu\text{g}$  BPA/kg bodyweight/day, [50]: 50  $\mu\text{g}$  BPA/kg bodyweight/day.

## Table S6. Results for males and females combined

**Table S6.** Relationship between BPA and different parameters, sexes analyzed together.

	Number of animals	[C] (%)	[0.5] (% of [C]) ± SE	[50] (% of [C]) ±SE	Factorial ANOVA	One-way ANOVA	Dunnett's post-hoc test [C]-[0.5], [C]-[50]
Heart weight (g) <sup>a</sup>	[C]: n= 26 [0.5]: n=21 [50]: n=16	0.37 ± 0.008 <sup>a</sup>	0.35 ± 0.006 <sup>a</sup>	0.37 ± 0.01 <sup>a</sup>	<i>p</i> =0.9	<i>p</i> =0.04	[C]-[0.5]: <i>p</i> =0.03, C-[50]: <i>p</i> =0.9
HSI <sup>a#</sup>	[C]: n= 26 [0.5]: n=21 [50]: n=16	0.47 ± 0.008 <sup>a</sup>	0.44 ± 0.004 <sup>a</sup>	0.47 ± 0.01 <sup>a</sup>	<i>p</i> =0.6	<i>p</i> =0.008 <sup>#</sup>	[C]-[0.5]: <i>p</i> =0.03, C-[50]: <i>p</i> =0.9
ACC (gWAT)	[C]: n= 21 [0.5]: n=23 [50]: n=13	100	71.3 ± 5.3	78.8 ± 8.6	<i>p</i> =0.1	<i>p</i> =0.002	[C]-[0.5]: <i>p</i> =0.002, C-[50]: <i>p</i> =0.05
GATA2 (gWAT) <sup>#</sup>	[C]: n= 21 [0.5]: n=23 [50]: n=13	100	84.8 ± 8.2	139.8 ± 25.7	<i>p</i> =0.2	<i>p</i> =0.04	[C]-[0.5]: <i>p</i> =0.002, C-[50]: <i>p</i> =0.05
SREBP-1c (gWAT)	[C]: n= 21 [0.5]: n=23 [50]: n=13	100	74.2 ± 6.1	75.1 ± 7.1	<i>p</i> =0.8	<i>p</i> =0.002	[C]-[0.5]: <i>p</i> =0.3, C-[50]: <i>p</i> =0.8
Adiponectin (iscpBAT)	[C]: n= 26 [0.5]: n=21 [50]: n=16	100	110.4 ± 8.7	83.2 ± 3.4	<i>p</i> =0.8	<i>p</i> =0.02	[C]-[0.5]: <i>p</i> =0.4, C-[50]: <i>p</i> =0.1
Triglycerides (mmol/L)	[C]: n= 26 [0.5]: n=21 [50]: n=16	0.6 ± 0.02	0.8 ± 0.05	0.7 ± 0.05	<i>P</i> =0.1	<i>P</i> =0.001	[C]-[0.5]: <i>p</i> =0.0007, C-[50]: <i>p</i> =0.04
Plasma adiponectin (µg/ml) <sup>b</sup>	[C]: n= 26 [0.5]: n=20 [50]: n=16	9.3 ± 0.5	11.5 ± 1.0	11.5 ± 1.0	<i>p</i> =0.8	<i>p</i> =0.09	[C]-[0.5]: <i>p</i> =0.1, C-[50]: <i>p</i> =0.1
Weaning BW (g)	[C]: n= 26 [0.5]: n=21 [50]: n=16	39.8 ± 0.7	38.2 ± 0.7	37.4 ± 0.9	<i>P</i> =0.8	<i>P</i> =0.9	[C]-[0.5]: <i>p</i> =0.4, C-[50]: <i>p</i> =0.06

Final BW (g)	[C]: n= 26 [0.5]: n=21 [50]: n=16	78.9 ± 1.1	78.1 ± 1.5	78.9 ± 2.1	<i>P</i> =0.7	<i>P</i> =0.9	[C]-[0.5]: <i>p</i> =0.9, C-[50]: <i>p</i> =1.0
Weight gain, week 3 - 5 (g)	[C]: n= 26 [0.5]: n=21 [50]: n=16	39.1 ± 0.7	39.6 ± 1.1	41.6 ± 1.7	<i>P</i> =0.2	<i>P</i> =0.3	[C]-[0.5]: <i>p</i> =0.9, C-[50]: <i>p</i> =0.2
Gonadal fat pad (g)	[C]: n= 26 [0.5]: n=21 [50]: n=16	0.06 ± 0.006	0.07 ± 0.008	0.07 ± 0.07	<i>P</i> =0.8	<i>P</i> =0.7	[C]-[0.5]: <i>p</i> =0.8, C-[50]: <i>p</i> =0.7
Inguinal fat pad (g)	[C]: n= 26 [0.5]: n=21 [50]: n=16	0.3 ± 0.01	0.4 ± 0.02	0.3 ± 0.02	<i>P</i> =0.6	<i>P</i> =0.8	[C]-[0.5]: <i>p</i> =0.8, C-[50]: <i>p</i> =1.0
Retroperitoneal fat pad (g)	[C]: n= 26 [0.5]: n=21 [50]: n=16	0.07 ± 0.006	0.08 ± 0.006	0.06 ± 0.008	<i>P</i> =0.7	<i>P</i> =0.3	[C]-[0.5]: <i>p</i> =0.3, C-[50]: <i>p</i> =0.5
Interscapular WAT (g)	[C]: n= 26 [0.5]: n=21 [50]: n=16	0.1 ± 0.03	0.1 ± 0.007	0.08 ± 0.01	<i>P</i> =0.5	<i>P</i> =0.5	[C]-[0.5]: <i>p</i> =0.9, C-[50]: <i>p</i> =0.4
Interscapular BAT (g)	[C]: n= 26 [0.5]: n=21 [50]: n=16	0.2 ± 0.006	0.2 ± 0.009	0.2 ± 0.01	<i>P</i> =0.5	<i>P</i> =0.7	[C]-[0.5]: <i>p</i> =0.9, C-[50]: <i>p</i> =0.9
Spleen (g)	[C]: n= 26 [0.5]: n=21 [50]: n=16	0.2 ± 0.004	0.2 ± 0.005	0.2 ± 0.007	<i>P</i> =1.0	<i>P</i> =0.2	[C]-[0.5]: <i>p</i> =0.1, C-[50]: <i>p</i> =0.7
Liver weight (g)	[C]: n= 26 [0.5]: n=21 [50]: n=16	3.3 ± 0.06	3.3 ± 0.09	3.2 ± 0.1	<i>P</i> =0.5	<i>P</i> =0.9	[C]-[0.5]: <i>p</i> =0.9, C-[50]: <i>p</i> =1.0
LSI	[C]: n= 26 [0.5]: n=21 [50]: n=16	4.4 ± 0.04	4.2 ± 0.05	4.1 ± 0.06	<i>P</i> =0.5	<i>P</i> =0.3	[C]-[0.5]: <i>p</i> =0.5, C-[50]: <i>p</i> =0.8
Liver fat (%)	[C]: n= 26 [0.5]: n=21 [50]: n=16	0.08 ± 0.01	0.09 ± 0.02	0.08 ± 0.02	<i>P</i> =0.8	<i>P</i> =1.0	[C]-[0.5]: <i>p</i> =1.0, C-[50]: <i>p</i> =1.0
Body length (cm)	[C]: n= 26 [0.5]: n=21	15.1 ± 0.1	15.2 ± 0.1	15.1 ± 0.2	<i>P</i> =0.7	<i>P</i> =0.9	[C]-[0.5]: <i>p</i> =1.0, C-[50]: <i>p</i> =0.9

AGD (mm)	[50]: n=16 [C]: n= 26 [0.5]: n=21	11.9 ± 0.6	12.3 ± 0.6	12.3 ± 0.7	<i>P</i> =0.8	<i>P</i> =0.9	[C]-[0.5]: <i>p</i> =0.9, C-[50]: <i>p</i> =0.9
AGDi (mm/ <sup>3</sup> √g BW)	[50]: n=16 [C]: n= 26 [0.5]: n=21	0.2 ± 0.007	0.2 ± 0.007	0.2 ± 0.008	<i>P</i> =0.9	<i>P</i> =0.8	[C]-[0.5]: <i>p</i> =0.8, C-[50]: <i>p</i> =0.8
HDL (mmol/L)	[50]: n=16 [C]: n= 26 [0.5]: n=21	0.8 ± 0.02	0.8 ± 0.02	0.8 ± 0.02	<i>P</i> =0.2	<i>P</i> =0.3	[C]-[0.5]: <i>p</i> =0.2, C-[50]: <i>p</i> =0.3
LDL (mmol/L)	[50]: n=16 [C]: n= 26 [0.5]: n=21	0.2 ± 0.005	0.2 ± 0.007	0.2 ± 0.007	<i>P</i> =0.7	<i>P</i> =0.6	[C]-[0.5]: <i>p</i> =0.7, C-[50]: <i>p</i> =0.9
Total cholesterol (mmol/L)	[50]: n=16 [C]: n= 26 [0.5]: n=21	2.6 ± 0.07	2.8 ± 0.05	2.7 ± 0.06	<i>P</i> =0.08	<i>P</i> =0.1	[C]-[0.5]: <i>p</i> =0.08, C-[50]: <i>p</i> =0.4
Plasma leptin (ng/mL)	[50]: n=16 [C]: n= 26 [0.5]: n=20	0.6 ± 0.4	0.7 ± 0.3	0.7 ± 0.5	<i>P</i> =0.5	<i>P</i> =0.3	[C]-[0.5]: <i>p</i> =0.2, C-[50]: <i>p</i> =0.4
Gonadal WAT (number of cells/HPF)	[50]: n=16 [C]: n= 4 [0.5]: n=4	81.8 ± 5.4	87.0 ± 3.5	83.3 ± 3.2	<i>P</i> =0.6	<i>P</i> =0.7	[C]-[0.5]: <i>p</i> =0.6, C-[50]: <i>p</i> =0.9
Interscapular BAT (number of cells/HPF)	[50]: n=16 [C]: n= 4 [0.5]: n=4	119.3 ± 9.1	137.9 ± 11.7	121.2 ± 6.7	<i>P</i> =0.6	<i>P</i> =0.3	[C]-[0.5]: <i>p</i> =0.3, C-[50]: <i>p</i> =1.0

Note: Sexes were analyzed together since no significant interaction was seen between sex and BPA dose for these relationships (Factorial ANOVA (Interaction term) *p*>0.05). #: Data not normally distributed and Kruskal-Wallis *p*-value and post hoc tests shown, <sup>a</sup>: Values are reported as mean ± SEM, gWAT: Gonadal white adipose tissue, HPF: High power field, iscpBAT: Interscapular brown adipose tissue, SEM: Standard error of the mean

## Table S7. Number of cells per high power field in adipose tissue

Table S7. Number of adipocytes per high power field in iWAT, gWAT and iscpBAT of rat offspring.

Adipose tissue depot	Number of animals	Females				Males			
		Control	[0.5]	[50]	ANOVA <i>p</i> -value	Control	[0.5]	[50]	ANOVA <i>p</i> -value
iWAT	[C]: n= 6 [0.5]: n=6 [50]: n=6	55.9 ± 1.5	68.2 ± 4.4 <sup>a</sup>	55.3 ± 2.9 <sup>bc</sup>	0.02	57.3 ± 2.5	54.0 ± 3.4 <sup>d</sup>	69.9 ± 5.1 <sup>ef</sup>	0.02
gWAT	[C]: n= 4 [0.5]: n=4 [50]: n=4	78.2 ± 1.0	83.4 ± 1.0 <sup>g</sup>	84.8 ± 1.0 <sup>hi</sup>	0.9 <sup>#</sup>	85.3 ± 5.0	90.5 ± 6.8 <sup>j</sup>	81.8 ± 2.8 <sup>kl</sup>	0.5
IscpBAT	[C]: n= 4 [0.5]: n=4 [50]: n=4	129.6 ± 14.1	137.5 ± 9.3 <sup>m</sup>	118.9 ± 9.5 <sup>no</sup>	0.5	108.9 ± 10.9	138.3 ± 23.4 <sup>p</sup>	123.6 ± 10.7 <sup>qr</sup>	0.6 <sup>#</sup>

<sup>#</sup>: Data not normally distributed and Kruskal-Wallis *p*-value and post hoc tests shown, <sup>a</sup>: [C]-[0.5]: *p*=0.03, <sup>b</sup>: [C]-[50]: *p*=1.0, <sup>c</sup>: [0.5]-[50]: *p*=0.04, <sup>d</sup>: [C]-[0.5]: *p*=1.0, <sup>e</sup>: [C]-[50]: *p*=0.1, <sup>f</sup>: [0.5]-[50]: *p*=0.03, <sup>g</sup>: [C]-[0.5]: *p*=1.0, <sup>h</sup>: [C]-[50]: *p*=1.0, <sup>i</sup>: [0.5]-[50]: *p*=1.0, <sup>j</sup>: [C]-[0.5]: *p*=1.0, <sup>k</sup>: [C]-[50]: *p*=1.0, <sup>l</sup>: [0.5]-[50]: *p*=0.8, <sup>m</sup>: [C]-[0.5]: *p*=1.0, <sup>n</sup>: [C]-[50]: *p*=1.0, <sup>o</sup>: [0.5]-[50]: *p*=0.8, <sup>p</sup>: [C]-[0.5]: *p*=0.8, <sup>q</sup>: [C]-[50]: *p*=1.0, <sup>r</sup>: [0.5]-[50]: *p*=1.0, iscpBAT: interscapular brown adipose tissue, WAT: White adipose tissue. Results are shown as mean ± SEM.

## Table S8. Primary analysis: Transcriptional levels in adipose tissue and liver

**Table S8.** Transcriptional levels in adipose tissue and liver with one-way ANOVA and post hoc tests.

	Sex	[0.5] (n=10-11) (% of [C]) $\pm$ SEM	[50] (n=5-10) (% of [C]) $\pm$ SEM	One-way ANOVA	Dunnett's post-hoc test [C]-[0.5], [C]-[50]
<i>ACC</i> (gWAT)	M	71.6 $\pm$ 7.1	65.3 $\pm$ 10.6	$p=0.003$	[C]-[0.5]: $p=0.011$ , [C]-[50]: $p=0.005$
<i>AdipoR2</i> (gWAT)	M	76.5 $\pm$ 7.7	73.5 $\pm$ 10.0	$p=0.022$	[C]-[0.5]: $p=0.039$ , [C]-[50]: $p=0.034$
<i>LPL</i> (gWAT)	M	79.9 $\pm$ 6.7	76.1 $\pm$ 11.4	$p=0.047$	[C]-[0.5]: $p=0.073$ , [C]-[50]: $p=0.061$
<i>SCD1</i> (gWAT)	M	77.7 $\pm$ 8.8	63.5 $\pm$ 11.4	$p=0.031$	[C]-[50]: $p=0.022$ , [C]-[50]: $p=0.14$
<i>GATA2</i> (gWAT)	M	66.5 $\pm$ 7.5	152.7 $\pm$ 41.9	$p=0.023$	[C]-[50]: $p=0.35$ , [C]-[50]: $p=0.13$
<i>AdipoR1</i> (iWAT)	M	63.5 $\pm$ 6.9	82.7 $\pm$ 7.6	$p=0.040$	[C]-[0.5]: $p=0.036$ , [C]-[50]: $p=0.091$
<i>SCD1</i> (iWAT)	M	63.7 $\pm$ 11.1	71.4 $\pm$ 9.7	$p=0.018$	[C]-[0.5]: $p=0.015$ , [C]-[50]: $p=0.059$
<i>CEBP<math>\alpha</math></i> (Liver)	M	88.5 $\pm$ 5.6	74.9 $\pm$ 4.8	$p=0.040$	[C]-[0.5]: $p=0.35$ , [C]-[50]: $p=0.023$
<i>AdipoR1</i> (gWAT)	F	99.5 $\pm$ 4.1	130.9 $\pm$ 4.2	$p=0.0001$	[C]-[50]: $p=0.0001$ , [C]-[50]: $p=0.99$
<i>SCD1</i> (gWAT)	F	65.1 $\pm$ 9.5	115.6 $\pm$ 13.0	$p=0.032$	[C]-[50]: $p=0.075$ , [C]-[50]: $p=0.66$
<i>SREBP-1c</i> (gWAT)	F	73.8 $\pm$ 6.5	77.9 $\pm$ 4.4	$p=0.022$	[C]-[0.5]: $p=0.016$ , [C]-[50]: $p=0.11$

Note: Non-significant results are not shown. *ACC*: acetyl-CoA carboxylase, *AdipoR*: Adiponectin receptor (1 and 2), *CEBP $\alpha$* : CAAT enhancer binding protein alpha, F: Females, gWAT: Gonadal white adipose tissue, *GATA2*: binding protein 2, iWAT: Inguinal white adipose tissue, *LPL*: Lipoprotein lipase, M: Males, *SCD1*: Stearoyl-CoA Desaturase, SEM, Standard error of the mean, *SREBP-1c*: Sterol regulatory element binding protein-1c.