

Supplementary Materials

Dimova et al “Gestational oxidative stress protects against adult obesity and insulin resistance”

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Fig. S1. Schematic representation of the experimental design.

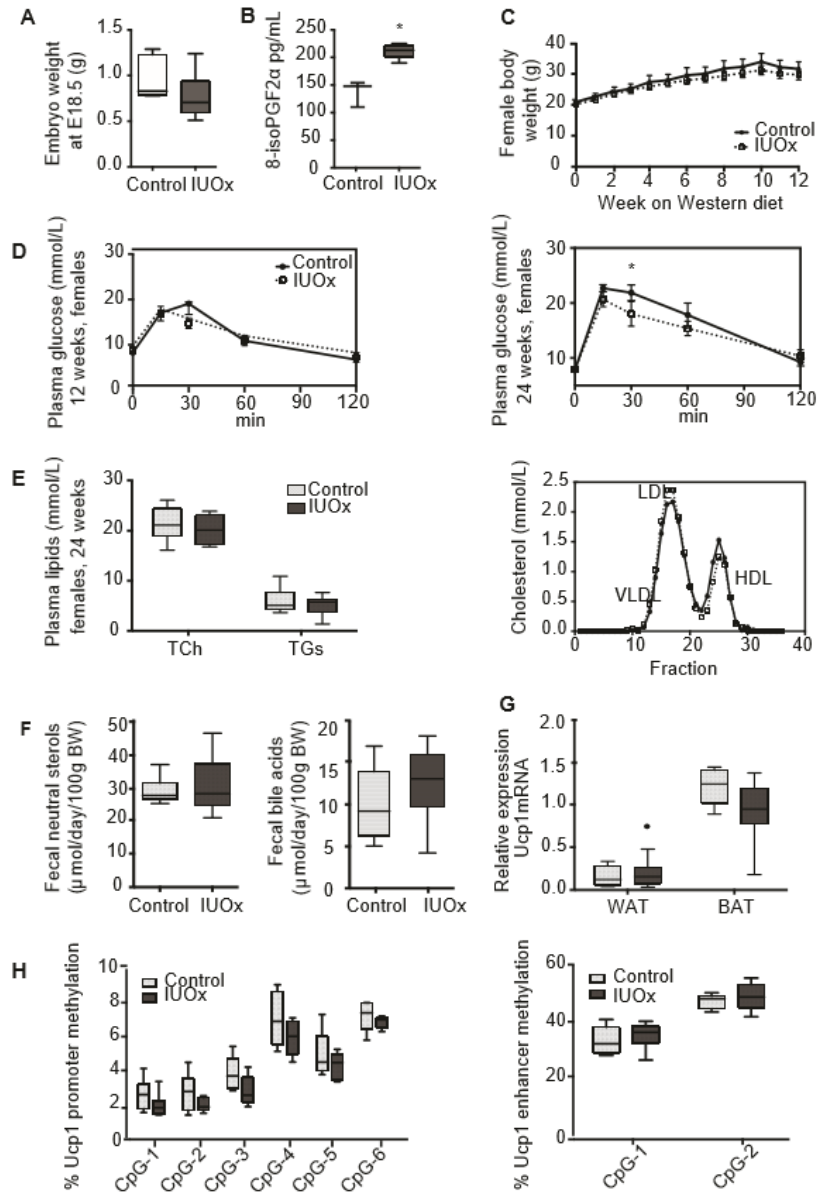
Fig. S2. IUOx does not induce substantial metabolic changes in female mice after 12 weeks of Western diet challenge.

Fig. S3. IUOx results in altered Ucp1 expression in WAT by a Ppar- γ and Pcg1 α -independent mechanism

Table S1. Bisulfite-specific primers encompassing the Ucp1 upstream regulatory sequences.

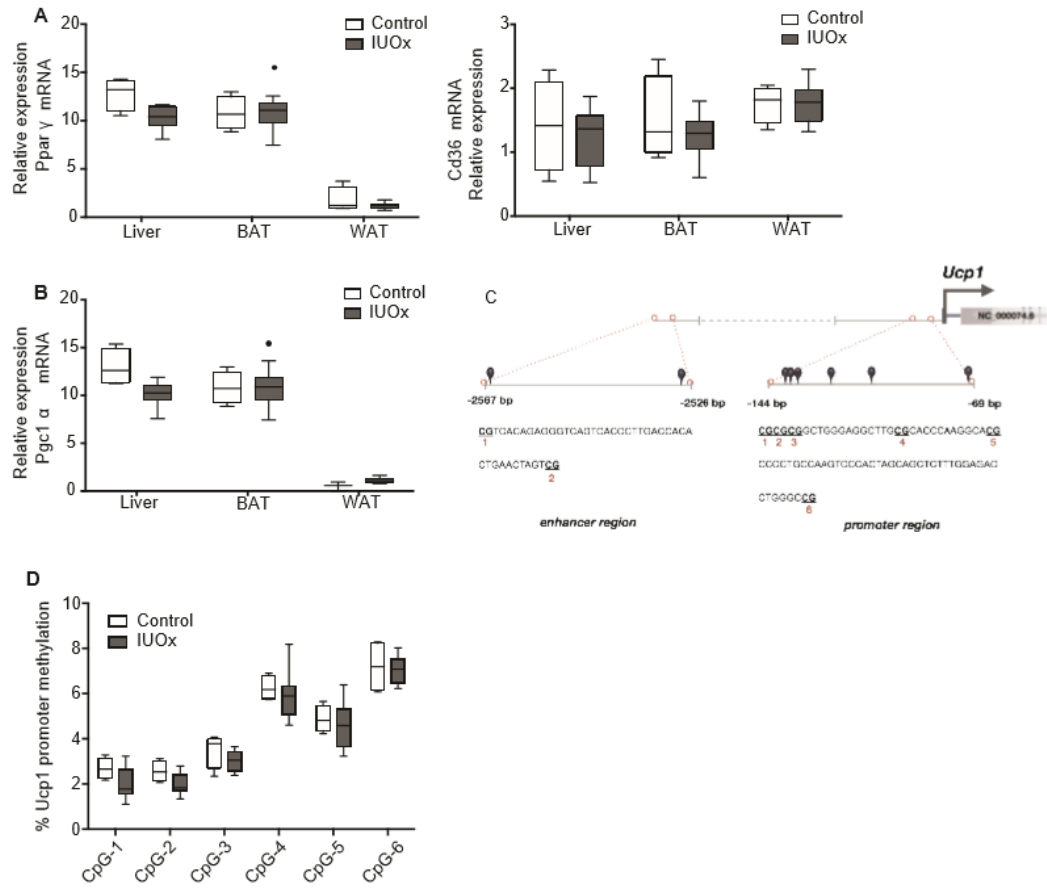
Table S2: Diet composition sheet

Supplementary figure 1



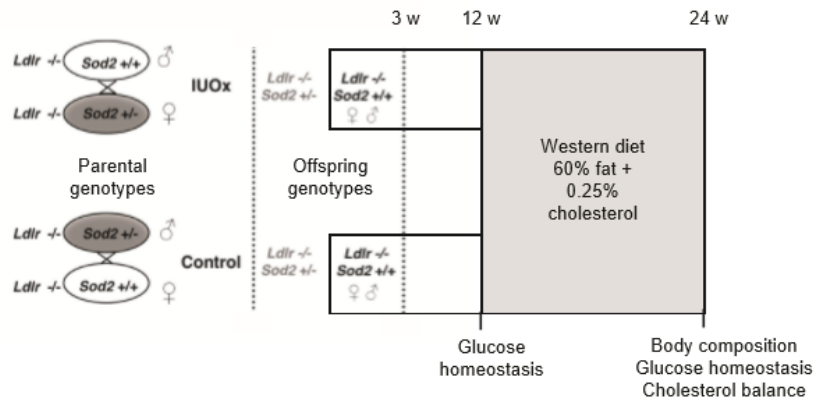
Supplementary figure 1: I/Ox does not induce substantial metabolic changes in female mice after 12 weeks of Western diet challenge. A) Embryonic weight of female *Ldlr*^{-/-} *Sod2*^{+/+} offspring at 18.5 days (n=9/group) B) Oxidative stress marker 8-isoPGF2 α in plasma of female embryos at day E18.5. Data are median and interquartile range; Mann-Whitney U-test. C) Body weight change in female mice during 12 weeks of Western diet feeding. D) Glucose tolerance test before (left) and after (right) Western diet challenge. E) Total cholesterol (TCh) and triglycerides (TG) in plasma (left) and cholesterol in lipoprotein subfractions (right) after FPLC separation of pooled plasma (n=6/group) from female mice after 12 weeks of Western diet. F) Fecal excretion of neutral sterols (left) and bile acids (right). G) Relative expression (qPCR) of Ucp1. H) Ucp1 promoter (left) and enhancer (right) CpG methylation state. Data are presented as median and interquartile range; n=6/group. Mann-Whitney U-test, * p<0.05.

Supplementary figure 2



Supplementary figure 2: IUX does not affect PPAR- γ or Pgc1 α expression or Ucp-1 promoter methylation in male offspring at 24 weeks. A) qPCR-based gene expression of Ppar- γ and its model transcriptional target Cd36 in liver, white (WAT) and brown adipose (BAT) tissue after 12 weeks of Western diet. B) Pgc1 α -expression levels. mRNA expression levels are normalized to the respective expression of 36b4 in the corresponding tissue sample. C) Methylation state of CpG pairs associated with the Ucp1 promoter region. Data are presented as median and interquartile range; n=4-5/group. Mann-Whitney U-test, * p<0.05.

Supplementary figure 3



Supplementary figure 3: Schematic representation of the experimental design. The background of all mice was *Ldlr*-knockout. To obtain a model for isolated intra-uterine oxidative stress (IUOx) *Sod2*^{+/-} dams were crossed with *Sod2*-wild-type *Sod2*^{+/+} males. *Sod2*^{+/+} IUOx offspring were then compared with *Sod2*^{+/+} offspring from crosses of *Sod2*^{+/+} females with *Sod2*^{+/-} males (Control). The littermates heterozygous for the *Sod2*-mutation were not considered in this study due to potential overriding effects of increased oxidative stress caused by their own genotype. At 12 weeks of age all offspring were allowed ad-libitum Western diet containing 60% fat and 0.25% cholesterol. Before and after provision of the diet glucose tolerance testing was performed. Following 12 weeks on Western diet body composition was measured via pDEXA and cholesterol metabolism characterized.

Supplementary table 1: Bisulfite-specific primers encompassing the Ucp1 upstream regulatory sequences.

Enhancer Region -2567 to -2526 bp upstream of Ucp1 TSS			
PCR	5'-AAGTTTGTTTTATAATGGAGAGAGTAG-3'		
Sequencing Primer	5'-GAAGAGTGGAAAGGT-3'	Analyzed sequence	GAYGATTAGTTTAGTGTGGTTAAGGGTGATT GATTTTTTGTGAYGTTGTAGAGGAGTGATAG TAAGTTTTATTTATTTAGTGTTYGTTTTTTT TTTAAGATGTAGAAT
PCR	5'-Biotin-AATACCCTATAAATAATATTCTACATCTT-3'		
Promoter Region -144 to -69 bp upstream of Ucp1 TSS			
PCR	5'-TGGGTATAATTAGGAATTGGTGTTAAA-3'		
Sequencing Primer	5'-GGGTTTTGGGAGTGA-3'	Analyzed sequence	YGYGYGGTTGGGAGGTTTGYGTATTTAAGGT AYGTTTTTGTAAAGTTTTATTAGTAGTTTTTG GAGATTTGGGTYGGTTAGTTATTTTTTTAG TTTTT
PCR	5'-Biotin-CTAACCTAAAAAATCTATATAACCCCTTAC-3'		

Supplementary table 2: Diet composition

Supplementary table 2: Diet composition HFD 60 kcal% Fat, 0.25% added cholesterol (D14010701, Research Diets, Inc.)

<i>D14010701</i>		
	<u><i>gm%</i></u>	<u><i>kcal%</i></u>
Protein	26	20
Carbohydrate	26	20
Fat	35	60
kcal	5.2	
<i>Ingredient</i>	<i>gm</i>	<i>kcal</i>
Casein	200	800
L-Cystine	3	12
Corn Starch	0	0
Maltodextrin 10	125	500
Sucrose	68.8	275
Cellulose, BW200	50	0
Soybean Oil	25	225
Lard	245	2205
Mineral Mix, S10026	10	0
DiCalcium Phosphate	13	0
Calcium Carbonate	5.5	0
Potassium Citrate, 1 H ₂ O	16.5	0
Vitamin Mix, V10001	10	40
Choline Bitartrate	2	0
Cholesterol	1.94	0
Total	775.79	4057