

Supplemental Material

**A Model of Neglect During Postnatal Life Heightens Obesity-Induced
Hypertension in Adult Male and Female C57BL/6 Mice**

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Short title: Early life stress and cardiometabolic risk

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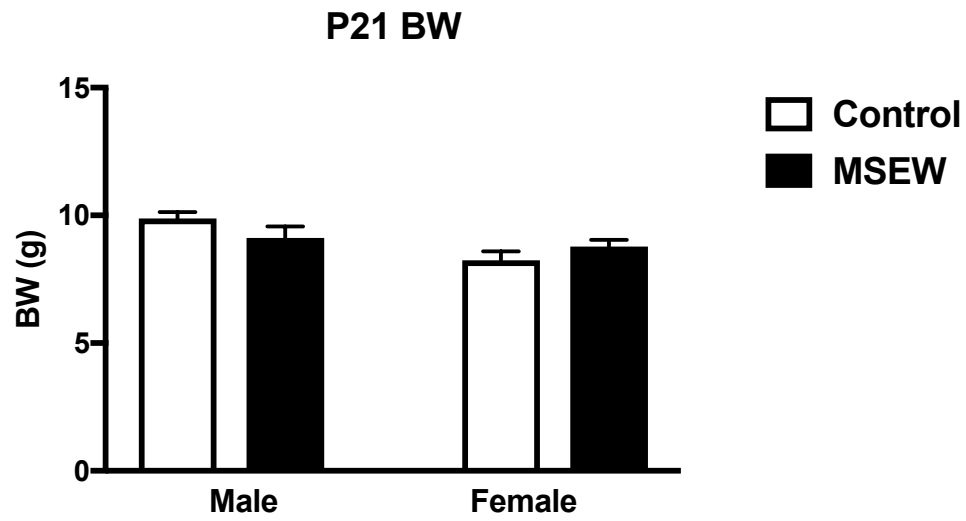
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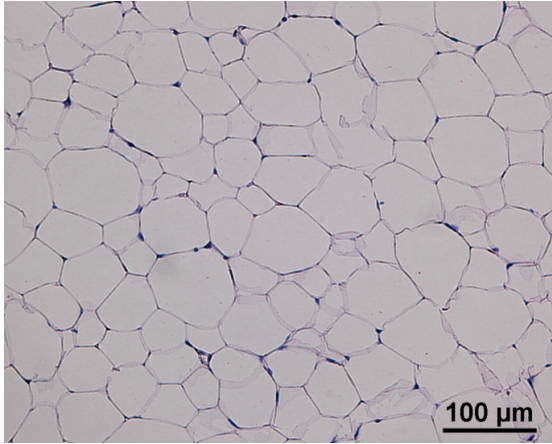
Magnetic Resonance Imaging Analysis

Female mice fed a HF diet for 9 weeks were imaged on a 7T CliniScan MRI (Bruker, Ettlingen, Germany) using a 2 point Dixon technique and a 72 mm diameter circularly polarized transmit receive coil at the Magnetic Resonance Imaging and Spectroscopy Center at the University of Kentucky. Anesthesia was induced and maintained using 1-2 % isoflurane in oxygen at a rate of 1.0 L/min. Respiration and temperature was monitored. Temperature was maintained between 36-37 °C using a water bath and tubing. Image data sets including a fat only and water only image were obtained using a dual echo Flash sequence with TR 12ms, TE 2ms and 3.5ms, flip 15°, FOV 80mm x 57.5mm x 31.7, and resolution 0.18mm x 0.18 mm x 0.18 mm. FSL software (<http://www.fmrib.ox.ac.uk/fsl>) was used for image analysis. The fat image was resampled to 1.8mm x 0.18mm x 0.18mm using FSL. Both the visceral cavity and total body cavity were segmented out manually. The total fat volume was calculated by taking a histogram from the fat only image of the visceral cavity and including voxels that were greater than 80% of the fat peak. 80% was chosen to include adipose tissue and exclude voxels from the liver. For subcutaneous analysis, the visceral cavity was subtracted from the total body cavity and total fat volume was calculated in similar fashion for visceral fat.

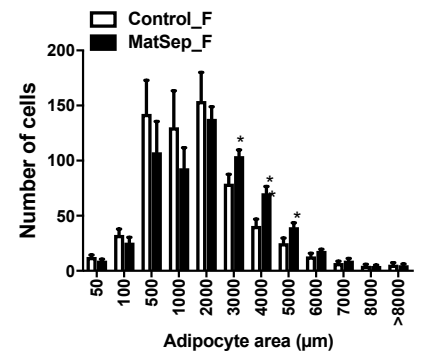
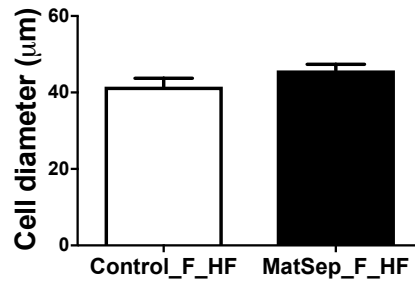
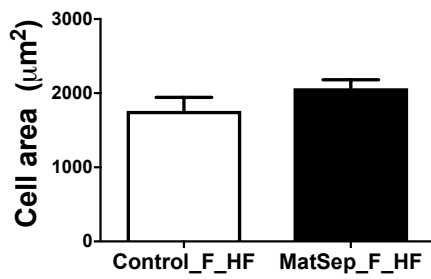
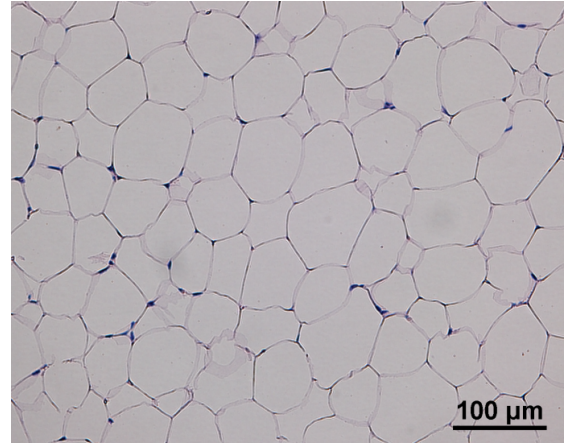


Supplemental figure 1. Body weight in male and female mice at postnatal day 21. MSEW does not affect BW between groups. N=6-8 each group.

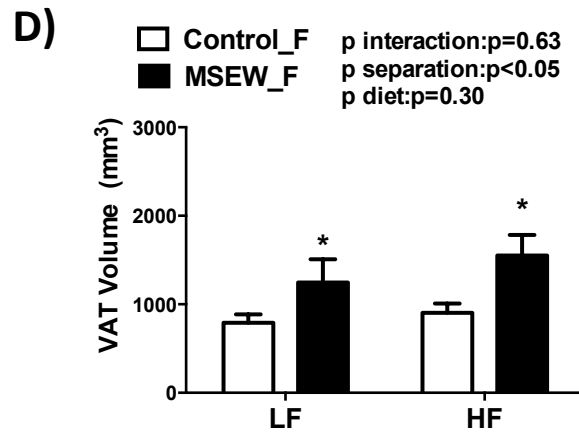
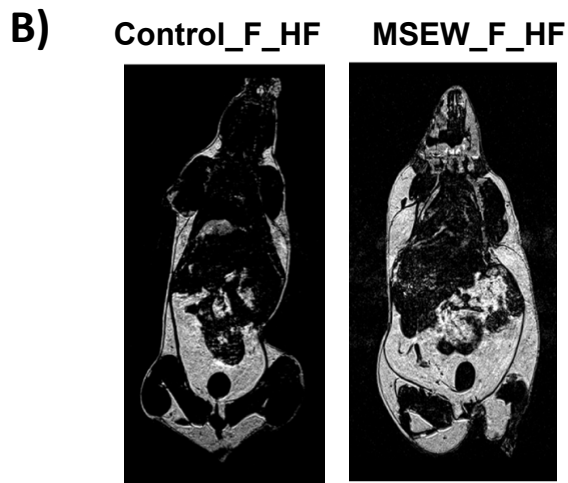
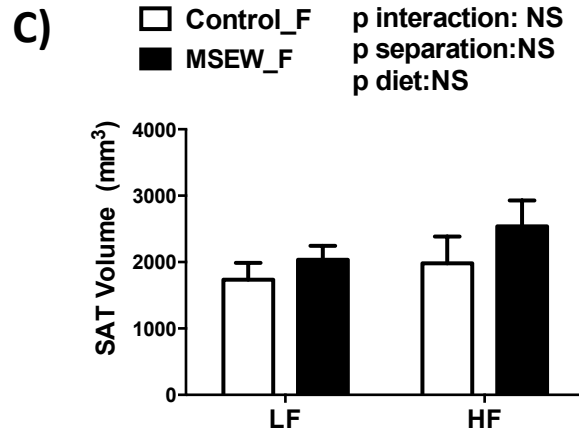
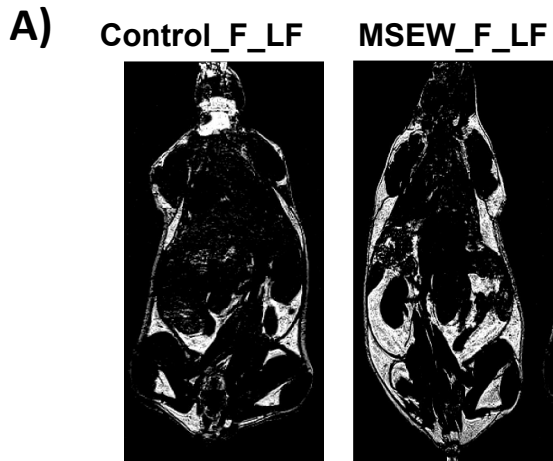
Control_F_HF



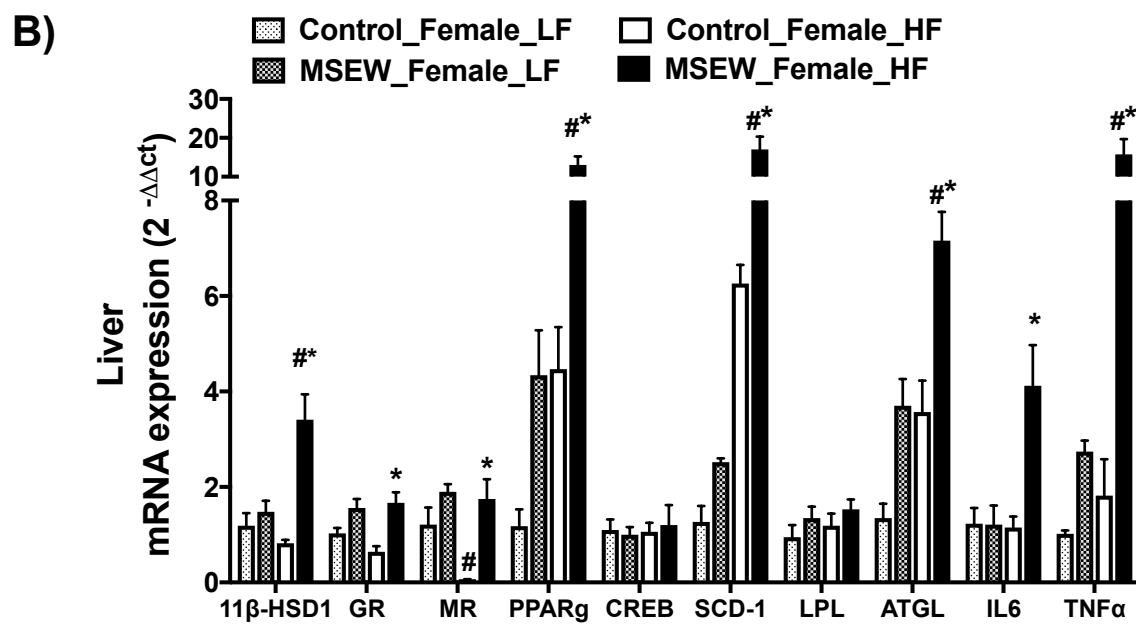
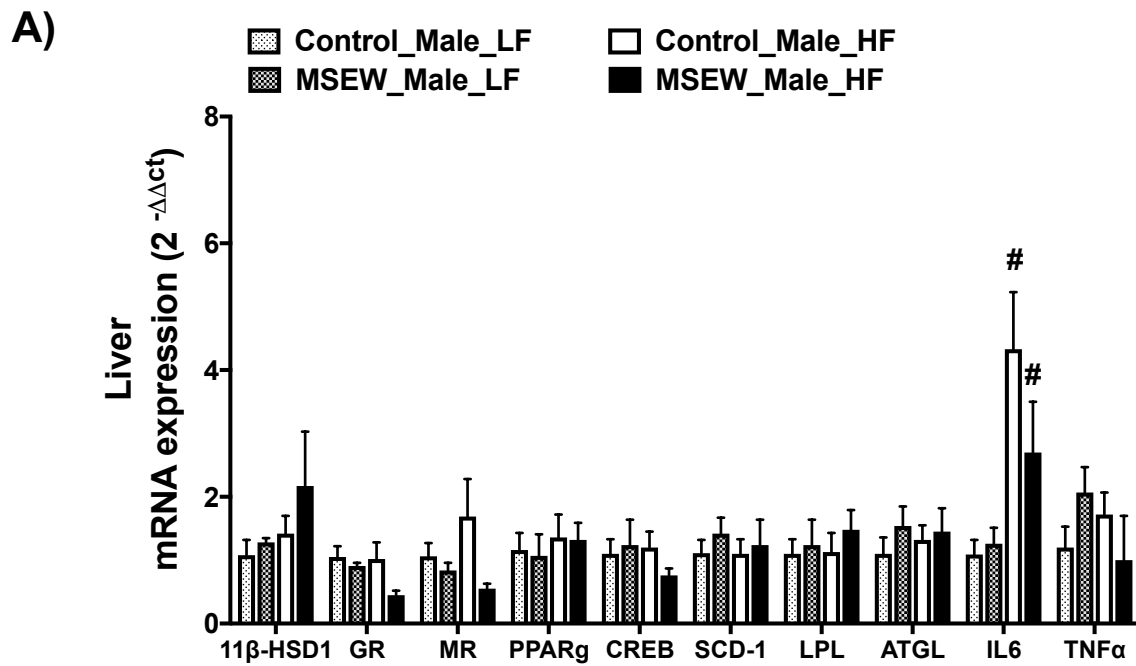
MSEW_F_HF



Supplemental figure 2. Adult female mice fed a HF diet do not show significant changes in scWAT. Representative H&E staining images from scWAT sections showing similar cell area and diameter in control and MatSep mice. Scale bar for bottom panels, 100 μm. N=5 per group.



Supplemental figure 3. MSEW effect on fat distribution in female mice fed LF or HF diet for 9 weeks. A) Representative MRI images in LF diet fed mice B) Representative MRI images in HF diet fed mice C) SAT volume (mm³) D) VAT volume (mm³). N=3-5 per group.



Supplemental figure 4. Gene expression in liver after 16 weeks on LF or HF diet. N=5-8 each group.

Table S1. Tissue weights in male and female mice fed during 16 weeks on LF or HF diet, g=gonadal, pr=perirenal, sc=subcutaneous, WAT=white adipose tissue, BAT=brown adipose tissue, LV/H= left ventricle/heart ratio, FI= food intake. N=6-8 per group. * P separation <0.05, # P diet <0.05, P interaction=NS.

		Control LF	MSEW LF	Control HF	MSEW HF
LV/H ratio	Male	0.78±0.05	0.71±0.02	0.75±0.01	0.71±0.01
	Female	0.81±0.06	0.78±0.05	0.71±0.01	0.69±0.09
Liver (g)	Male	1.27±0.09	1.37±0.05	1.32±0.13	1.51±0.43#
	Female	1.1±0.08	1.20±0.12	1.01±0.06	1.27±0.07*
Kidney (mg)	Male	320±20	300±10	340±10	350±16
	Female	240±10	250±20	280±20	250±10
Adrenal (mg)	Male	7.6±1.0	6.8±1.3	5.7±0.5	6.2±0.1
	Female	9.5±0.8	9.9±1.2	11.8±1.6#	10.2±1.5#
Spleen (mg)	Male	100±10	100±10	90±10	100±10
	Female	160±10	140±7	110±10#	100±5#
gWAT (mg)	Male	1270±220	1258±160	2020±230#	2350±200#
	Female	440±50	500±20	1560±410#	1940±340#*
pr WAT (mg)	Male	410±90	470±60	1020±100#	1140±100#
	Female	300±30	230±50	660±70#	840±50#
sc WAT (mg)	Male	490±90	490±40	1210±200#	1490±170#
	Female	260±20	350±50	650±140#	1120±340#*
BAT (mg)	Male	150±20	180±40	330±50#	410±25#
	Female	70±10	80±20	150±30#	160±10#
FI (g/day)	Male	4.3±0.1	4.6±0.6	3.07±0.23#	3.51±0.15#
	Female	3.2±0.1	3.4±0.2	2.28±0.09#	2.53±0.22#
FI (g/day)	Male	4.3±0.1	4.6±0.6	3.07±0.23#	3.51±0.15#
	Female	3.2±0.1	3.4±0.2	2.28±0.09#	2.53±0.22#

Table S2. Food intake in male and female mice fed during 7 or 14 weeks on LF or HF diet, measured for 5 consecutive days. FI= food intake. N=6-8 per group. # P diet <0.05.

		Control LF	MSEW LF	Control HF	MSEW HF
FI week 7 (g/day)	Male	2.8±0.3	2.4±0.7	2.1±0.3#	1.9±0.6#
	Female	2.2±0.4	2.0±0.2	1.6±0.1#	1.3±0.5#
FI week 14 (g/day)	Male	4.3±0.1	4.6±0.3	3.1±0.2#	3.5±0.1#
	Female	3.2±0.1	3.4±0.2	2.3±0.1#	2.5±0.2#

Table S3. mRNA levels of the 18S housekeeping gene by RT-PCR in gWAT and liver.

18S (2-ddct)		Control LF	MSEW LF	Control HF	MSEW HF
gWAT	Male	14.0±0.3	14.4±0.6	13.4±1.1	14.2±1.5
	Female	15.4±1.2	14.3±0.5	14.9±0.5	15.3±0.2
Liver	Male	11.7±0.2	11.8±0.2	12.0±0.4	11.5±0.2
	Female	17.2±0.1	17.4±0.2	17.3±0.2	17.6±0.3

Table S4. RT-PCR primers sequence.

	5' Forward 3'	5' Reverse 3'
11β-HSD1	GGAAGGTCTCCAGAAGGTAGTGTC	GAGGCTGCTCCGAGTTCAAG
GR	CGGGACCACCTCCCAA	CCCATAATGGCATAACCGAA
MR	GTGGACAGTCCTTTCACTACCG	TGACACCCAGAAGCCTCATCTC
PPARγ	GCCCTTTGGTGACTTTATGGA	GCAGCAGGTTGTCTTGGATG
CREB	ACCAGCAGAGTGGAGATGCT	ATGGCAATGTACTGCCACT
SCD-1	CTGTACGGGATCATACTGGTTC	GCCGTGCCTTGTAAGTTCTG
LPL	ACTCGCTCTCAGATGCCCTA	TTGTGTTGCTTGCCATTCTC
ATGL	TGTGGCCTCATTCTCTAC	TCGTGGATGTTGGTGGAGCT
IL6	TAGTCCTTCTACCCCAATTTCC	TTGGTCCTTAGCCACTCCTTC
TNFα	CCCACTCTGACCCCTTTACTC	TCACTGTCCCAGCATCTTGT
House-keeper		
18S	AGTCGGCATCGTTTATGGTC	CGAAGCATTGCCAAGAAT

Table S5. Fasting glucose in mice fed a LF or HF for 16 weeks. N=8-14 per group. # P <0.05 vs. LF.

		Control LF	MSEW LF	Control HF	MSEW HF
Glucose (mg/dl)	Male	108.4±5.2	107.4±3.8	117.1±5.0	125.1±4.3 #
	Female	104.7±4.5	102.3±5.3	103.8±7.2	119.5±5.8 #