

# Maternal Exercise Mediates Hepatic Metabolic Programming via Activation of AMPK-PGC1 $\alpha$ Axis in the Offspring of Obese Mothers

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## 1. Supplementary Figure S1

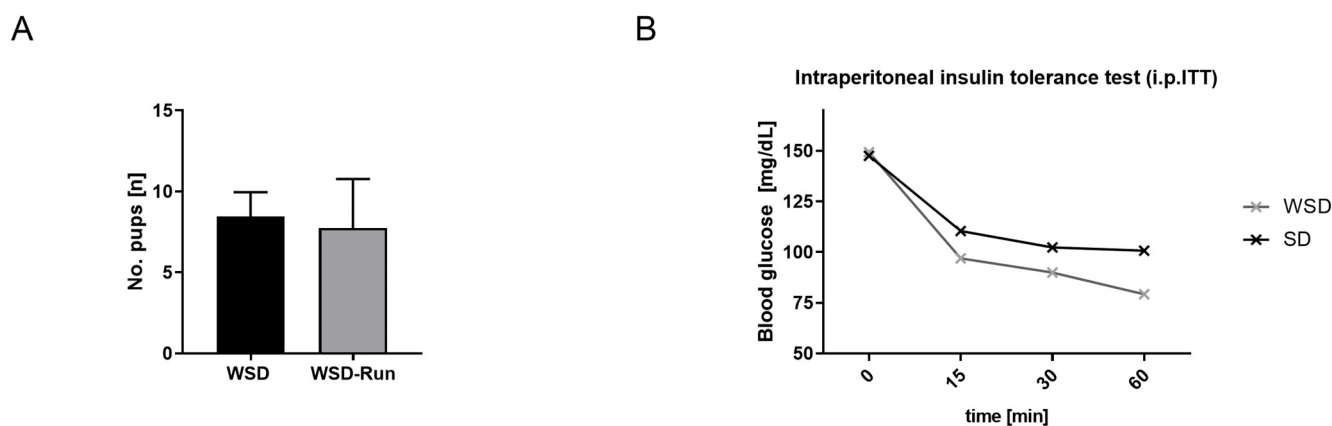
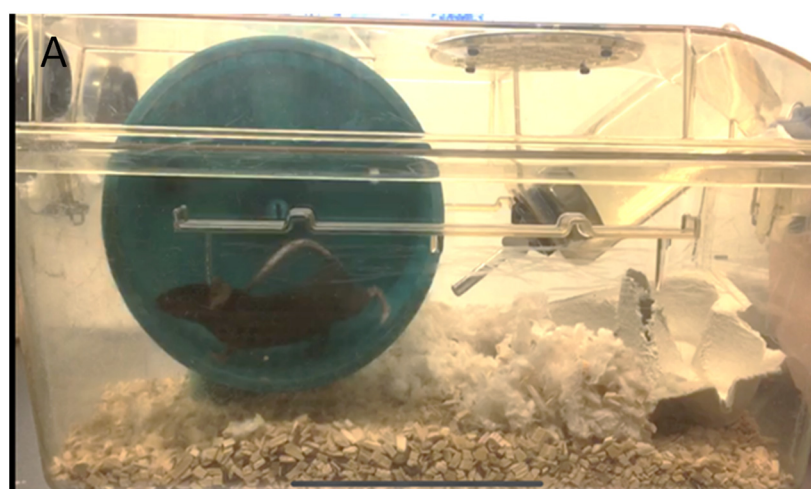
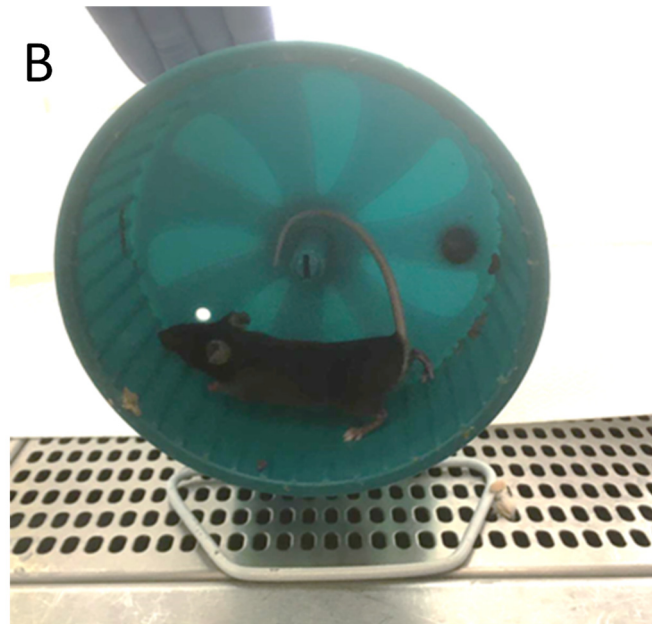


Figure S1. (A) Litter size at birth (Mean litter size WSD group: 8.4 pups/dam; mean litter size WSD-

## 2. Supplementary Figure S2–Animal Model





**Figure 2.** Illustration of the experimental setting and the running wheel provided to the mice for voluntary-wheel-running exercise. Representative image inside the cage (A) and for a better overview outside the cage (B).

Voluntary wheel running (VWR) was used to examine the mechanisms by which maternal exercise confers improvement on both dams and offspring metabolic health. As illustrated, female C57BL/6N mice were housed individually in cages equipped with a running wheel (WSD-Run group). The running wheel was equipped with a tachometer measuring distance (km), average speed (km/h) and time (h:m). Using this equipment an accurate recording of physical activity for each individual animal was possible. Control animals remained sedentary (WSD group).

### 3. Supplementary Table S1 (Primer List, Real Time RT-PCR)

**Table S1.** List of primers used for real-time RT-PCR.

Gene	Primer	Primer sequence 5'-3'
Acaca <sup>1</sup> (Acetyl-CoA carboxylase 1)	for	GAAGTCAGAGCCACGGCACA
	rev	GGCAATCTCAGTTCAAGCCAGTC
Acacb <sup>1</sup> (Acetyl-CoA carboxylase 2)	for	CTACAAGACGGCGCAGGTCA
	rev	AGGCGCCAAACTTCAGCATC
Acox1 <sup>1</sup> (Acyl-CoA Oxidase 1)	for	GAGCCTTTGGACCTTCACTTGG
	rev	CGCATAAGTGCCCGTGATCT
Cpt1a <sup>1</sup> (Carnitine Palmitoyltransferase 1A)	for	CATTACAAGGACATGGGCAAGTT
	rev	CCGTAGTGCAGGAGCGTACA
Fasn <sup>1</sup> (Fatty acid synthase)	for	CCATGGAGCGTATATGTGAACAG
	rev	AATGCCCACGTCACCAATG
Gapdh	for	ATGTGTCCGTCGTGGATCTGA
	rev	TGCCTGCTCACCACCTTCT
Gusb	Probe (FAM, TAMRA)	CCGCCTGGAGAAACCTGCCAAGTATG
	for	CGCTGAGAGTAATCGGAAACAA
	rev	CGCAAAATAAAGGCCGAAGT
	Probe (FAM, TAMRA)	ATCTTCACTCGCCAGAGACAGCCCA
G6pc <sup>1</sup> (Glucose-6-phosphatase)	for	TGTCTGTGATTGCTGACCTG
	rev	GTAGAAGTGACCATAACATAG
Il6 (Interleukin 6)	for	ACAAGTCGGAGGCTTAATTACACAT
	rev	AATCAGAATTGCCATTGCCCAA

	Probe (FAM, TAMRA)	TCTTTTCTCATTTCACGATTTCACAGAGAA
Il1b <sup>1</sup> (Interleukin 1 beta)	for rev	TGACAGTGATGAGAATGACCTGTTC GGACAGCCCAGGTCAAAGG
Insr <sup>1</sup> (Insulin receptor)	for rev	GCAAACAGATGCCACTAATCCTT GGGCTTCCACTTTAAGATAATCTGA
Irs-1 <sup>1</sup> (Insulin Receptor Substrate 1)	for rev	CCAGAGTCAAGCCTCACACA CCCAACTCAACTCCACCACT
Mcp-1 (Monocyte chemoattractant protein 1)	for rev	GGCTCAGCCAGATGCAGTTAAC CTTGGTGACAAAACTACAGCTTCTT
Nfkb1 <sup>1</sup> (Nuclear Factor Kappa B Subunit 1)	for rev	CATCCCGGAGTCACGAAATC GCACAATCTTTAGGGCCATTTT
Nfkb2 <sup>1</sup> (Nuclear Factor Kappa B Subunit 2)	for rev	TCTAGCCACAGAGATGGAGGAGTT AGGTCCGGGCATTACATTA
Pck <sup>1</sup> (Phosphoenolpyruvate carboxykinase, Pepck)	for rev	CCACAGCTGCTGCAGAACAC GAAGGGTCGCATGGCAAA
Ppara (Peroxisome Proliferator Activated Receptor $\alpha$ )	for rev	CCAGTACTGCCGTTTTTACAAGT GCTTTTTTTCAGATCTTGGCATTCTT
Pprag (Peroxisome Proliferator Activated Receptor $\gamma$ )	for rev	CCCAATGGTTGCTGATTACAAA GCCTGTTGTAGAGCTGGGTCTT
Ppargc1a (Peroxisome proliferator-activated receptor gamma coactivator 1- $\alpha$ )	for rev	TCGAAAAAGAAGTCCCATACACAA TTCCACACTTAAGGTTTCGCTCAATA
Srebp1c <sup>1</sup> (Sterol regulatory element-binding protein 1c)	for rev	CATCGACTACATCCGCTTCTTG GTGATTGCTTTTGTGTGCACTTC
Tnfa <sup>1</sup> (Tumor necrosis factor- $\alpha$ )	for rev	AGGGATGAGAAGTTCCCAAATG GCTTGTCACTCGAATTTTGAGAAG

<sup>1</sup>Genes were measured by the SYBR-Green method. No probe was needed. Abbreviations: FAM, 6-carboxyfluorescein; TAMRA, tetramethylrhodamine.

#### 4. Supplementary Table S2 (Antibody List, Immunoblots)

**Table S2.** Blots were probed with the following antibodies.

Primary antibody	Description	Purchased from
phospho-ACC (Ser79)	Monoclonal rabbit anti-phosphorylated ACC, 1:1000	Cell Signaling, Danvers, MA, USA; catalog no. 3661
ACC	monoclonal rabbit anti-total ACC. 1:2000	Cell Signaling, Danvers, MA, USA; catalog no. 3662
phospho-AMPK- $\alpha$ (Thr172)	Monoclonal rabbit anti-phosphorylated AMPK $\alpha$ , 1:1000	Cell Signaling, Danvers, MA, USA; catalog no. 2535
AMPK- $\alpha$	monoclonal rabbit anti-total AMPK $\alpha$ , 1:2000	Cell Signaling, Danvers, MA, USA; catalog no. 2603
phospho-AKT (Ser473)	monoclonal rabbit anti-phosphorylated AKT; 1:1000	Cell Signaling, Danvers, MA, USA; catalog no. 4058

AKT	monoclonal rabbit anti-total AKT, 1:2000	Cell Signaling, Danvers, MA, USA; catalog no. 9272
PGC1 $\alpha$	monoclonal rabbit anti-PGC1 $\alpha$ , 1:2000	Invitrogen, Carlsbad, CA, USA; catalog. no. PA5-38021

Secondary antibody:

Anti-rabbit IgG (horseradish peroxidase-linked; Cell Signaling Technology, Danvers, MA, USA catalog no. 7074) was used as secondary antibody.

Abbreviations: ACC, acetyl-CoA-carboxylase; AMPK $\alpha$ , AMP-activated protein kinase alpha subunit; PGC1 $\alpha$ , Peroxisome proliferator-activated receptor gamma coactivator 1-alpha, IgG immunoglobulin G.

## 5. Supplementary Table S3 (Experimental Diets)

**Table S3.** Information on crude nutrients and energy density of the experimental diets.

SD			WSD
Name	complete feed for rats & mice		DIO – 45 kJ% fat (Lard)
Company	Ssniff		Ssniff
Order number	V1534 - R/M-Maintenance		E15744-344
Additional information	complete feed for rats & mice		HF diet for rodents with lard (& soybean oil) correspond to D12451 Research Diets
Metab. Energy	kcal/kg	3225	4615
Metab. Energy	MJ/kg	13.5	19.30
Gross Energy	MJ/kg	16.2	22.50
Fat	kJ%	9	45.00
Protein	kJ%	24	20.00
Carbohydrates	kJ%	67	35.00
Sugar	kJ%	8.8	19.1
Crude Fat	g/kg	33	236
Crude Protein	g/kg	190	220
Crude Fibre	g/kg	50	57
Crude Ash	g/kg	64	53
N free extracts	g/kg	546	400
<b>Sugar</b>			
Sucrose	g/kg	-	211.0
Monosaccharides	g/kg	54.0	-
Dissacharides	g/kg		-
Sugar (total)	g/kg	54	211.0
<b>Carbohydrates</b>			
Dextrin	g/kg	-	108.0
Polysaccharides	g/kg	359	-
Starch	g/kg	-	68
Carbohydrates (total)	g/kg	413	387
Cellulose powder	g/kg	-	57
Cholesterol	mg/kg	-	-

<b>Fat Sources</b>				
soybean oil	%	n/a		2.8
butter fat	%	n/a		-
corn oil	%	n/a		-
pork lard	%	n/a		20.8
palm oil	%	n/a		-
<b>Fatty Acids</b>				
butanoic acid	C 4:0	mg/kg	-	-
caproic acid	C 6:0	mg/kg	-	-
caprylic acid	C 8:0	mg/kg	-	-
capric acid	C-10:0	mg/kg	-	-
lauric acid	C-12:0	mg/kg	-	500
myristic acid	C-14:0	mg/kg	0.01	2900
palmitic acid	C-16:0	mg/kg	0.45	53,300
margaric acid	C-17:0	mg/kg	-	-
stearic acid	C-18:0	mg/kg	0.09	29,200
arachidic acid	C-20:0	mg/kg	0.01	70
palmitoleic acid	C-16:1	mg/kg	0.01	6200
oleic acid	C-18:1	mg/kg	0.62	94,200
linoleic acid	C-18:2	mg/kg	1.66	34,600
alpha-linolenic acid	C-18:3	mg/kg	0.23	3700
eicosanoic acid	C-20:1	mg/kg	-	-
eicosadienoic acid	C-20:2	mg/kg	-	-
arachidonic acid	C-20:4	mg/kg	-	-
<b>Minerals</b>				
Calcium		g/kg	10.00	92.00
Phosphorus		g/kg	7.00	64.00
Magnesium		g/kg	2.20	23.00
Sodium		g/kg	2.40	20.00
Potassium		g/kg	9.10	97.00
Sulfur		g/kg	n/a	n/a
Chlorine		g/kg	n/a	n/a
Ca/P		%	1.43:1	1.44:1
<b>Trace Elements</b>				
Iron		mg/kg	189	168
Manganese		mg/kg	68	95
Zinc		mg/kg	91	65
Copper		mg/kg	15	13
Iodine		mg/kg	2.1	1.2
Molybdenum		mg/kg	-	n/a
Fluorine		mg/kg	-	n/a
Selenium		mg/kg	0.3	0.2
Cobalt		mg/kg	-	n/a
Mineral & trace element mix	%	n/a		6
<b>Vitamins</b>				
Vitamin A		I.E./kg	15,000	15,000
Vitamin D3		I.E./kg	1100	1500
Vitamin E		mg/kg	110	150
Menachinone		mg/kg	7	20
Thiamin		mg/kg	18	25

Riboflavin	mg/kg	22	16
Vitamin B6	mg/kg	21	16
Vitamin B12	mg/kg	0,1	30
Nicotinic acid	mg/kg	115	47
Pantothenic acid	mg/kg	40	55
Folate	mg/kg	7	16
Biotin	mg/kg	0.51	300
Choline	mg/kg	1370	920
Vitamin C	mg/kg	not added	n/a
Choline chloride	mg/kg	not added	0,2
Vitamin premix	%	not added	1
<b>Amino acids</b>			
Lysine	mg/kg	10,000	18,300
Methionine	mg/kg	3300	7800
Cystine	mg/kg	3500	3900
Met+Cys	mg/kg	6800	11,800
Threonine	mg/kg	7100	9700
Tryptophan	mg/kg	2500	2900
Arginine	mg/kg	11,900	8600
Histidine	mg/kg	4400	6700
Valine	mg/kg	9000	15,400
Isoleucine	mg/kg	7900	12,500
Leucine	mg/kg	13,900	21,900
Phenylalanine	mg/kg	8800	11,400
Phe+Tyr	mg/kg	14,900	23,100
Glycine	mg/kg	8800	4700
Glutamic acid	mg/kg	41,000	49,700
Aspartic acid	mg/kg	17,900	16,400
Proline	mg/kg	12,900	25,300
Serine	mg/kg	9900	13,200
Alanine	mg/kg	8200	6600
<b>Antioxidant</b>			
Butylated hydroxytolu- ene = E321	%	n/a	n/a