Supplementary Material and Methods

Serum Biochemistry

Serum glucose was measured using glucose oxidase method (Synermed, Westfield, IN). Serum triglycerides and NEFA levels were estimated using commercially available reagents (triglycerides, Synermed, Westfield, IN and NEFA C reagents, Wako Chemicals, Richmond, VA). Insulin and leptin concentrations in serum were assayed using ELISA (Linco Research, St. Charles, MO). Serum FGF21 concentrations were assayed using commercially available radioimmunoassay (Phoenix Pharmaceuticals, Burlingame, CA). Adiponectin and resistin were assayed using commercially available ELISA (B-Bridge International, Sunnyvale, CA).

Immunoblotting

Total lysates from livers of offspring at PND21 (N = 6 per group) were prepared in RIPA buffer (25 mM Tris-HCl, 150 mM NaCl, 1% NP-40, 1% deoxycholic acid, 0.1% SDS and 2 mM EDTA) containing 1 mM PMSF and protease inhibitors (Sigma Chemicals). Nuclear proteins were prepared using NE-PER reagents (Thermo-Fisher, Rockford, IL). Hepatic mitochondrial protein was prepared using Mitochondrial Isolation Kit (Thermo Fisher). Proteins were resolved by SDS-PAGE and transferred to PVDF membranes. Immunoblotting was carried out using standard procedures. Membranes were incubated with rabbit or mouse anti-AMPK, pAMPK Thr_{172}, ACC, IR-β, IRS-1, pAKT Ser_{473}, AKT (Cell Signaling Technologies, Danvers, MA); pAKT Thr_{308} (Millipore); ERK1/2, pERK1/2 (Santa Cruz Biotech, Santa Cruz, CA); FASN (AbCam, Cambridge, MA); GAPDH, Lamin A (Sigma); or SREBP-1 (Novus Biologicals) antibody, overnight in TBST containing either 5% non-fat milk or BSA at 4 °C. Anti-CPT1a antibody was kindly provided by Dr. Victoria Esser (University of Texas Southwestern Medical

Center, Dallas, TX). Following incubation with HRP-conjugated secondary, membranes were washed and proteins visualized using ECL-plus reagent (GE Healthcare Biosciences, Piscataway, NJ). Densitometric quantitation was performed using Quantity One software (Biorad).

Chromatin Immunoprecipitation Assay

The ChIP-IT Enzymatic™ kit (Active Motif) was used with minor modifications for *in vivo* samples and described previously. Briefly, triplicate pooled liver samples (with each pool representing 2-3 separate animals) were cross-linked in 37% formalin for 10 min. Cross-linking was stopped by adding glycine stop-fix solution (3 ml 10X glycine buffer; 3 ml 10X phosphate-buffered saline and 24 ml water). Nuclei were isolated by homogenizing the fixed liver tissue, followed by centrifugation at 1500 *g* for 15 min at 4 °C. Enzymatic shearing was carried out using recommended conditions and consistency of shearing was ascertained by electrophoresis on purified DNA on 1.5% agarose. Aliquots of sheared chromatin were incubated with 5 μg of either anti-SREBP-1 (Novus Biologicals), anti-PPAR-α (Abcam) or anti-rabbit or anti-mouse IgG, respectively. Chromatin-bound transcription factor immune complexes were collected using magnetic protein G beads. The yield of specific promoter target sequences was analyzed by PCR. SREBP-1 recruitment was assessed by amplifying the 353 bp region (from -288 bp to +65 of the transcription start site) of the FASN promoter (forward primer, 5'-GAGATGAGGGCGTCGGGAT-3'; reverse primer, 5'- TGGAGGCAGACGACAAGCGA-3'). PPAR-α recruitment to the FGF21 promoter was monitored by amplifying the 112 bp region (from -1434 bp to -1322 of the transcription start site) of the FGF21 promoter (forward primer, 5'- AGCACAGGACCTGAATGCTAAAC-3'; reverse primer, 5'-GCTGCATCAGCCTCCTGTAAG-3') enclosing the distal PPRE.

LEGENDS FOR SUPPLEMENTARY TABLES AND FIGURES

Sup Table 1: Primers Sequences for Real-time RT-PCR Analyses Gene specific primers were designed using Primer Express™ Software (Applied Biosystems, Foster city, CA). Real-time PCR reactions were carried out according to manufacturer's instructions for 2X SYBR green master mix and monitored on a ABI Prism 7000 sequence detection system (Applied Biosystems, Foster city, CA) as described under methods.

Sup Table 2: Maternal Overweight-Induced Changes in Hepatic Gene Expression of Offspring Liver at Weaning. Gene expression was assessed in offspring liver at PND21 using Rat Genome 230 2.0 microarrays (Affymetrix). Microarray data analyses were carried out using GeneSpring version 7.3X software. The normalized data was utilized to generate list of differentially expressed genes between offspring of OW and lean dams at PND21. Genes were filtered based on minimum ±1.8-fold change (OW vs. lean) and P value ≤ 0.05 using Student's t-test. Abbreviations for gene symbols can be queried from the NetAffx™ Data Analyses Center (http://www.affymetrix.com/analysis/index.affx).

Sup Fig 1. (A) Body weights of female rats fed liquid diets via total enteral nutrition (TEN) at 155 Kcal/$kg^{3/4}$/d (Lean) or 220 Kcal/$kg^{3/4}$/d (overweight, OW, N = 18 per group) for 3 wk. Infusion of diets was carried out for 23 h/d via computer controlled syringe pumps. **(B)** Body composition analyses (N = 8 per group) of rats estimated non-invasively by NMR analyses (ECHO MRI) at 3 wk. Fat mass and lean mass expressed as percentage of body weight. **(C)** Body weight of female rats through gestation fed liquid diets via TEN. Data are expressed as

means ± SEM. Statistical differences were determined using a Student's t-test. Differing superscripts or * indicates $p < 0.05$.

Sup Fig 2. Hierarchical Clustering of Gene Expression Profiles Global condition clustering of all transcripts on the Rat 230 2.0 GeneChip showing distinct clustering among offspring based on maternal phenotype (lean or OW).

Sup Fig 3. (A) Pie chart of gene ontology based on molecular function of 147 differentially expressed transcripts in liver of offspring from lean or OW dams at PND21. Genes were filtered based on minimum ±1.8-fold change (OW vs. Lean) and P value ≤ 0.05 using Student's t test. Numbers represent the number of transcripts in each category.

SUPPLEMENTARY TABLE 1 Primers Sequences for Real-time RT-PCR Analyses

Gene Name	Forward primer (5'- 3')	Reverse primer (5'- 3')
ACC-α	GTCTCTTTCCGGACCTTTGAAG	ATAGAGTGAAGTGTGACCGGACTCT
ACLY	GTCAGCCAAGGCAATTTCAGAG	TAACCCGGGCATACTTGAACC
Acot-1	GAATTGGGCTGCTTGGGATT	CACGGAGCCATTGATGACAAC
Adiponutrin	GGGAGAACGTGCTGGTGTCT	AGGAGGGATTAGGCCAGAGAAG
Adiponectin	GCGTCACTGTCCCCAATGTT	CGGAATGTTGCAGTGGAATTT
AdipoR1	CTGACTGGCTGAAAGACAATGACT	TTCTGTGTGGATGCGGAAGAT
AdipoR2	TCCCAAGAAGATGAAGGGTTTATG	CGACCTTCCCACACCTTACAA
Cyp4a	CACTCCCGTGTGAGGAACATC	TGATCATGGGCAAGTTGACAA
Cpt-1a	AGCGTTCTTCGTGACGTTGG	TCCATGCAGCAGGGATTTG
Cyclophilin A	AAGCATACAGGTCCTGGCATCT	TGCCATCCAGCCACTCAGT
ELOVL6	AACGAGAACGAAGCCATCCA	ATCAGATGCCGACCACCAAA
FABP5	GCCAAACCAGACTGCATCATT	TTCTCTCCCAAGGTGCAAGAA
FASN	CGCCGACCAGTATAAACCCAA	TCACCCTCAATGATGTGCACA
FGF21	CACACCGCAGTCCAGAAAGTC	TGGTTGTTGGCAAAGAAACCTA
G6PD	GAGACTTCAGTTCCGCGATGT	TGGTGTATACCGCCTCATTGG
Insig-1	AGAGAGAGTGGGCCAGTGTCA	CAGGGACAGCTGCACATTATTG
Malic enzyme	TGCAATTGGTGGTGCTTTCA	TCAGCTTTGCTGGTCGGATTA
Mte1	GAATTGGGCTGCTTGGGA	CACGGAGCCATTGATGACAAC
PPAR-α	ACGATGCTGTCCTCCTTGATG	CAGAATGGCTTCCTCAGGTTCT
PPAR-γ2	AAACTCTGGGAGATCCTCCTGTT	GCATCTCTGTGTCAACCATGGT
Pxmp4	CTGCAGGCCATTCTGAAAGC	CACCTTGCACATGGGACTGTA
SREBP-1	CAAGTGCTGCAGGAAACTG	GGCTGGATTCCACCTTTCTGT
Trib3	GCCTATCAGCCTCTGCTCGAT	CCTCCCTCAACCAGGGATGTA
Vnn1	ATCCTGCCTAAAGTCACCCTGTTA	GCTGCTGATAAGATCGCTCCTT

Gene specific primers were designed using Primer Express™ Software (Applied Biosystems, Foster city, CA). Real-time PCR reactions were carried out according to manufacturer's instructions for 2X SYBR green master mix and monitored on a ABI Prism 7000 sequence detection system (Applied Biosystems, Foster city, CA) as described under methods.

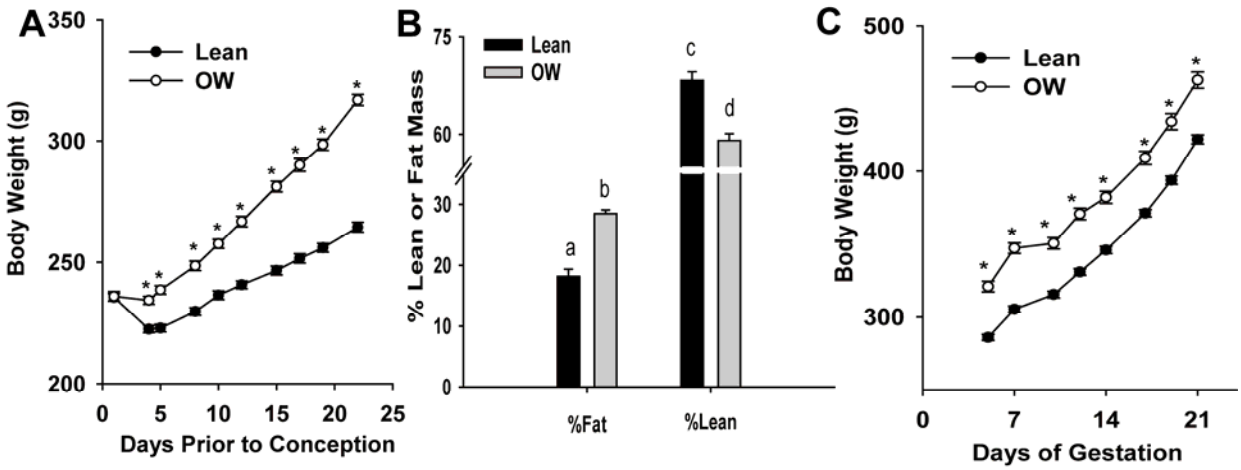
SUPPLEMENTARY TABLE 2 Maternal Overweight-Induced Changes in Hepatic Gene Expression of Offspring Liver at Weaning via Microarray Analyses

Identifier	Gene Symbol	Description	Ratio OW vs lean
Carbohydrate Metabolism			
1391544_at	Adpn	Similar to adiponutrin	**18.39**
1367854_at	Acly	ATP citrate lyase	**3.74**
1370281_at	Fabp5	fatty acid binding protein 5, epidermal	**2.58**
1387643_at	Fgf21	fibroblast growth factor 21	**0.24**
1367856_at	G6pd	glucose-6-phosphate dehydrogenase	**2.01**
1387312_a_at	Gck	glucokinase	**0.36**
1367894_at	Insig1	insulin induced gene 1	**2.44**
1370870_at	Me1	malic enzyme 1	**2.04**
1387670_at	mtGPDH	glycerol-3-phosphate dehydrogenase 2	**0.52**
1371646_at	PGD	phosphogluconate dehydrogenase	**2.50**
1387263_at	Pklr	pyruvate kinase, liver and RBC	**2.26**
1368460_at	Slc2a5	solute carrier family 2, member 5	**1.96**
1370694_at	Trib3	tribbles homolog 3 (Drosophila)	**0.44**
Lipid Biosynthesis			
1387538_at	Acaca	acetyl-coenzyme A carboxylase alpha	**4.02**
1373778_at	Acc2	Acetyl-Coenzyme A carboxylase beta	**1.93**
1372462_at	Acat2	similar to acetyl CoA transferase-like	**3.38**
1368177_at	Acsl3	acyl-CoA synthetase long-chain 3	**1.88**
1375944_at	Acss	Acyl-CoA synthetase short-chain 2	**2.25**
1388108_at	Elovl6	ELOVL family member 6	**2.65**
1375782_at	Fads1	Fatty acid desaturase 1	**1.89**
1367708_a_at	Fasn	fatty acid synthase	**5.11**
1371104_at	Srebf1	sterol regulatory element binding factor 1	**2.70**
1387852_at	Thrsp	thyroid hormone responsive protein	**2.98**
Lipid Metabolism			
1398250_at	Cte1	cytosolic acyl-CoA thioesterase 1	**0.31**
1371886_at	Crat	carnitine acetyltransferase	**0.50**
1386946_at	Cpt1a	carnitine palmitoyltransferase 1, liver	**0.47**
1370397_at	Cyp4a14	cytochrome P450, 4a14	**0.41**
1368934_at	Cyp4a10	cytochrome P450, 4a10	**0.39**
1367659_s_at	Dci	dodecenoyl-coenzyme A delta isomerase	**0.42**
1386885_at	Ech1	enoyl coenzyme A hydratase 1, peroxisomal	**0.46**
1388210_at	Mte1	mitochondrial acyl-CoA thioesterase 1	**0.51**
1377049_at	Ntel1	NTE-related protein	**0.51**
1371012_at	Phyh2	2-hydroxyphytanoyl-Coenzyme A lyase	**0.46**
Electron Transport			
1370241_at	Cyp2c7	cytochrome P450, 2c7	**0.45**
1369424_at	Cyp2a2	cytochrome P450, 2a1	**2.70**
1369666_at	Gpd2	Glycerol-3-phosphate dehydrogenase 2	**0.48**
1384355_at	Plxna2_predicted	plexin A2	**1.83**

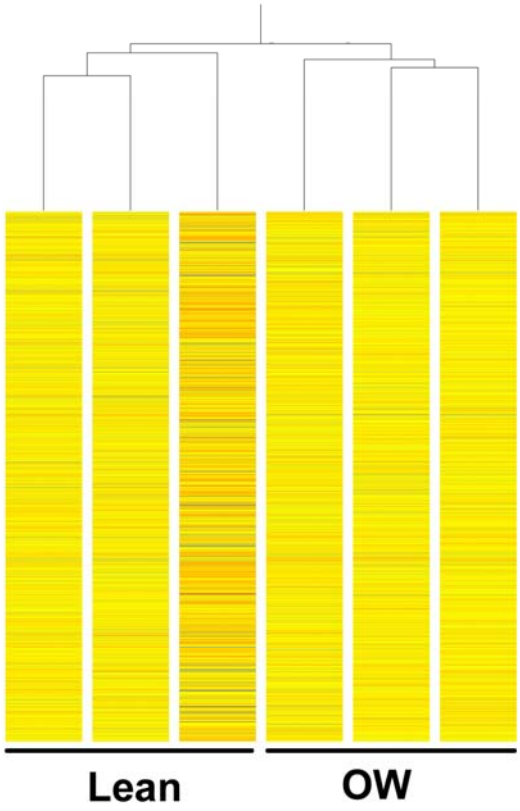
Identifier	Gene Symbol	Description	Ratio OW vs Lean
Cholesterol Metabolism & Transport			
1369455_at	Abcg5	ATP-binding cassette, G5	**0.50**
1387020_at	Cyp51	cytochrome P450, subfamily 51	**1.80**
1368458_at	Cyp7a1	cytochrome P450, 7a1	**0.39**
1368435_at	Cyp8b1	cytochrome P450, 8b1	**0.45**
1368189_at	Dhcr7	7-dehydrocholesterol reductase	**2.69**
1368086_a_at	Lss	lanosterol synthase	**1.99**
1367667_at	Fdps	farensyl diphosphate synthase	**2.05**
1387233_at	Hsd17b7	17-β Hydroxysteroid dehydrogenase 7	**1.88**
1388872_at	Idi1	Isopentenyl-diphosphate delta isomerase	**2.12**
Metabolic Processes			
1368826_at	Comt	catechol-O-methyltransferase	**2.30**
1388210_at	Mte1	mitochondrial acyl-CoA thioesterase 1	**0.51**
1371697_at	Pnpla2_predicted	similar to RIKEN cDNA 0610039C21 (predicted)	**0.50**
1383117_at	Pxmp4	peroxisomal membrane protein 4	**0.31**
1377037_at	RGD1564089_predicted	peroxisomal long chain acyl-CoA thioesterase Ia	**0.45**
1379361_at	Pex11a	peroxisomal biogenesis factor 11A	**0.50**
1377672_at	Sult1c2	sulfotransferase family, 1c2, cytosolic	**0.47**
1370943_at	Sult1c2a	sulfotransferase family, 1c1, cytosolic	**0.53**
1368733_at	Ste	sulfotransferase, estrogen preferring	**3.05**
1372120_at	Ube1dc1	ubiquitin-activating enzyme E1-domain containing 1	**0.51**

Supplementary Figure 1

Body weight and Body Composition of Female Rats Prior to and during Gestation



Supplementary Figure 2
Condition Clustering of Gene Expression Profiles



Supplementary Figure 3
Condition Clustering of Gene Expression Profiles