Supplemental Results and Discussion

Gender Analysis

Gender effects were analyzed first in regards to phenotypic variables, and then in regards to analytes. In phenotypic variables there were no differences in male versus female for maternal 2nd trimester nutrients (including FFA) or for offspring BW or %FM in the total cohort. While the NW and OB cohorts separately had no differences in these parameters either, in regard to gender, the OB cohort alone had 15% higher ppBMI in the mothers of females (Mean 40.8±3.95 kg/m^2) versus males (34.3±1.95 kg/m^2, p=0.009). This is of unclear significance.

In metabolite analysis we found several significant gender-stratified correlations, however none were sufficient to attribute gender as a primary driver in our results. In uMSC-myocytes neonatal %FM in offspring of OB, but not NW, mothers initially correlated with elevations in a large number of LCAC and LCOH species indicating incomplete beta-oxidation. In males only we found positive correlations with C12:1 (p=0.05, r=0.82) and C18:1OH (p=0.04, r=0.84). In females only we found positive correlations with C16:1 (p=0.04, r=0.96), C12:1OH (p=0.04, r=0.96), and C16:1OH (p=0.009, r=0.99). Neither gender separately demonstrated positive correlation for other VLCAD and LCHAD associated species found in the total OB-only cohort including C12, C14:2, C14:1, C14, C18:2, C14:1OH, or C14OH (see Table S2A).

Compensatory increase in omega-oxidation, as evident by dicarboxylic species, was more prominently found in males of OB mothers in relation to %FM. This included positive correlations with C5DC (p=0.002, r=0.96) and total DCAC (p=0.04, r=0.84). C6DC was found in the total OB cohort, and not gender specific. For maternal FFA, which also initially correlated with elevations in DCAC in the OB cohort, demonstrated positive correlations with C5DC (p=0.03, r=0.99), C6DC (p=0.03, r=0.99), and Total DCAC (p=0.05, r=0.99) primarily in females from the OB cohort, as well as lower free carnitine levels (C0, p=0.04, r=0.99) which are also negatively correlated with established obesity. These FFA results from OB cohort females only should be taken with caution however, since there were only an n=3 in this subcohort, potentially falsely skewing gender specific results. DCAC and FFA positive correlations from the total OB cohort remain much stronger by p-value and r-value with both genders combined.

Finally, in relation to %FM, amino acids associated with anaplerosis demonstrated gender-specificity, but the same pathway of glutamine/aspartate/alanine metabolism remained enriched. Glutamine remained negatively correlated with %FM only in male offspring of OB, but not NW, mothers (p=0.04, r=-0.84), while Aspartate (p=0.01, r=0.99), Asparagine (p=0.01, r=0.99), and Alanine (p=0.01, 0.99) were all positively correlated with %FM in female offspring of OB, but not NW, mothers. Maternal FFA correlated with Aspartate in a non-gender dependent manner. Alanine was positively correlated with male gender (p=0.005, r=0.73), specifically in the NW cohort (p=0.03, r=0.85). Only in females from the OB cohort did we find correlations with 1-carbon metabolites including Glycine (p=0.003, r=-0.99), Methionine (p=0.01, r=0.99), and Cystathionine (p=0.008, r=-0.99). Results should also (as above) be considered in light of the lower n=3 in females from the OB cohort. In only males of OB mothers Phenylalanine (p=0.03, r=0.81), Tyrosine (p=0.03, r=0.81), and total Aromatic Amino Acids (p=0.03, r=0.81) were also positively correlated with FFA. This is a stronger, but similar correlation to the total OB cohort. These 1-Carbon and Aromatic Amino Acids are associated with established obesity, and patterns found in children at risk for obesity. Taken together, in uMSC-myocytes, this indicates some gender specific differences but an unchanged overall outcome of beta-oxidative dysfunction at VLCAD and LCHAD with compensatory omega-oxidation and amino acid metabolism, largely dependent on maternal obesity, FFA, and neonatal %FM.

In uMSC-adipocytes where we originally found very few analyte correlations with %FM and FFA, we found weak negative correlations between FFA and medium chain acylcarnitines species C8 (0.03 r=-0.76), C8OH (p=0.02, r=-0.80), C10 (p=0.02, r=-0.78), C12 (p=0.05, r=-0.71), and C12OH (p=0.05, r=-0.70) in cells from female offspring but not males (total female cohort, n=9). No other significant pathway enriching correlations between analytes, %FM, FFA, and gender were found.

Supplemental Figures and Legends

Transcriptomic Analysis: uMSC-Myocytes and uMSC-Adipocytes

Cell lysate RNASeq analysis (n=14)

↓

Dataset cleaning (see methods)

↓

Entire RNASeq Dataset

Student t-test (NW vs OB, RPG vs LPG)

STRING Analysis for p<0.05

Analyze for enrichment in Interaction Score>0.9 (p<0.05, FDR<0.25)

↓

Curated RNASeq Dataset: Energy Metabolism Pathways

GSEA Analysis for all phenotypic variables (p<0.05, FDR<0.25)

Analyze significant results by core gene enrichment and leading edge analyses

↓

Curated RNASeq Dataset: Energy Pathway Subanalysis by Functional Pathway Group

GSEA Analysis for targeted phenotypic variables (p<0.05, FDR<0.25)

Analyze significant results in physiologic context

Metabolomics: uMSC-Myocytes and uMSC-Adipocytes

Acylcarnitine and amino acid analysis (n=22)

↓

Student t-test (NW vs OB, all metabolites)

Pearson Correlation Analysis (all continuous phenotypic variables, all metabolites)

↓

FDR Analysis by KEGG defined Pathways/Enzymes

Significance: p<0.05, FDR<0.25 (see methods)

↓

Highly targeted RNA Expression analysis: KEGG genes of interest based on metabolite patterns

Supplementary Figures 1&2: Workflow diagrams for metabolomic (a) and transcriptomic (b) analyses in uMSC differentiated towards myocytes and adipocytes.

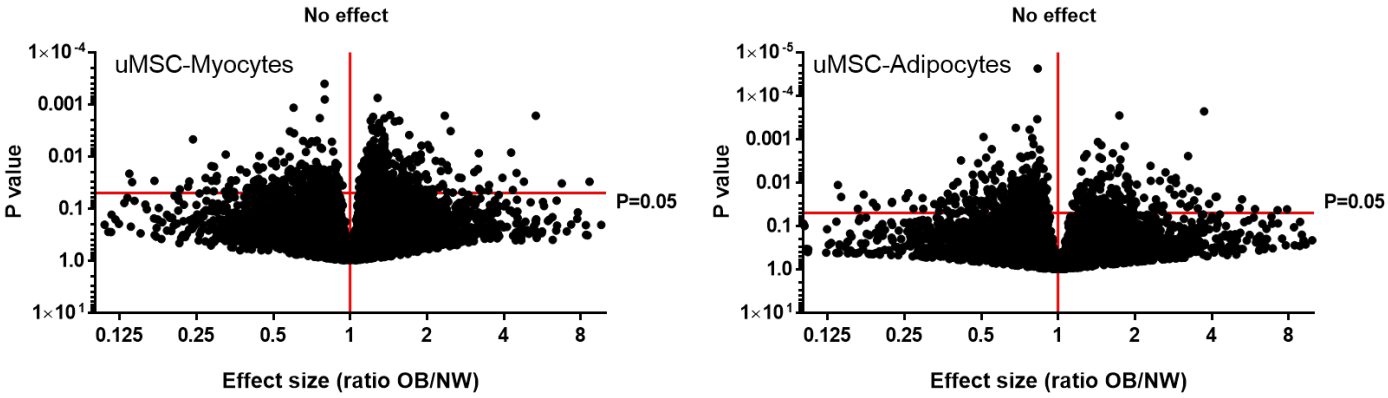

Figure S3&4: Volcano plot of differentially expressed genes (n=7 obese (OB) vs n=7 normal weight (NW)) found through RNA-Seq analysis of uMSC-myocytes (a) and uMSC-adipocytes (b). Protein coding genes with p≤0.05 were entered into STRING to agnostically evaluate pathway enrichment.

Supplemental Tables

	Phenotype	NW (N=12)	OB (N=12)	p-value
Maternal	Age at Delivery	28.92±5.36	27.92±6.79	0.71
	Pre-pregnancy BMI	**21.12±1.16**	**36.35±4.19**	**7.49E-11**
	Free Fatty Acids (27wks)	420.25±101.57	495±171.37	0.23
	Glucose (27wks)	79.58±10.52	78.91±5.20	0.86
	Insulin (27wks)	13.42±9.41	14.64±3.31	0.70
	HOMA-IR (27wks)	2.82±2.43	2.87±0.75	0.95
	Triglycerides (27wks)	151±35.52	161.3±42.35	0.60
Neonate	Infant Sex (M/F)	6/6	8/4	0.68
	Gestational Age	39.86±0.88	39.38±0.59	0.17
	Birth Weight	3367.25±376.13	3240.83±290.26	0.39
	Fat Mass	0.28±0.11	0.33±0.15	0.42
	Fat Free Mass	2.91±0.25	2.76±0.25	0.18
	Body Mass	3.19±0.30	3.09±0.31	0.45
	% Fat Mass	8.74±2.97	10.45±4.07	0.28
	% Fat Free Mass	91.26±2.97	89.55±4.07	0.28

Table S1: Categorical comparison of Maternal and Neonatal phenotypes in offspring of obese (NW) versus obese (OB) mothers.

Table S2			
Phenotype	**Pathway**	**Analyte**	**p-value (r-value)**
ppBMI	His	3-Methylhistidine	**0.0199 (-0.4924)**
	1C&Sulf	Cysta:2ABU	0.0462 (0.4293)
	Ala/Asp/Glu	Aspartic acid	0.0254 (0.4752)
FFA (Total)	Ala/Asp/Glu	Alanine	**0.0027 (0.6202)**
		Aspartic acid	**0.0211 (0.4997)**
	His	1-Methylhistidine	**0.0484 (0.4357)**
FFA (OB)	Ala/Asp/Glu	Alanine	**0.012 (0.7528)**
	Phe/Tyr	Phenylalanine	**0.051 (0.6298)**
		Total Aromatic	**0.0536 (0.6246)**
	His	1-Methylhistidine	**0.0495 (0.633)**
	LCAC	C14	0.044 (0.6452)
	SCOH/MCOH	C6OH	**0.0343 (0.6694)**
	DCAC/MCOH	C5DC	**0.0091 (0.7707)**
		C6DC	**0.006 (0.7949)**
		Total DCAC	**0.0203 (0.7143)**
FFA (NW)	Arg&Pro	Beta-Alanine	0.0392 (-0.6263)
	LCOH	C16OH	0.0514 (-0.5993)
Triglycerides	Ala/Asp/Glu	Gln:Asp	**0.0283 (0.5162)**
		Gln:Glu	**0.0409 (0.486)**
		Glutamine	**0.0379 (0.4924)**
		Asp:Asn	**0.0055 (0.5836)**
Insulin	Arg&Pro	Ornithine	0.0502 (0.4325)
	CPT	C0	**0.0127 (0.5338)**
Glucose	Ala/Asp/Glu	Asp:Asn	**0.0095 (0.5521)**
	DCAC/MCOH	C6DC	**0.039 (-0.4533)**
	1C&Sulf	Cys:AABU	0.0472 (0.4377)
	Arg&Pro	Arginine	0.0451 (-0.4415)
Birth Weight	1C&Sulf	Met:AABU	0.0509 (0.4212)
Neonatal %FM (Total)	Ala/Asp/Glu	Alanine	**0.0134 (0.5303)**
		Aspartic acid	**0.0176 (0.5122)**
		Gln:Asp	**0.0045 (-0.5938)**
		Gln:Glu	**0.0248 (-0.4879)**
		Glutamine	**0.0192 (-0.5061)**
	Arg&Pro	Arg:Orn	0.0495 (-0.4338)
	LCAC	C12:1	**0.0461 (0.4396)**
		C12	**0.0372 (0.4573)**
		C14:1	**0.0136 (0.5295)**
		C14	**0.0271 (0.4814)**
		C16:1	**0.0034 (0.6093)**
		Long (all+OH)	**0.0311 (0.4711)**
	LCOH	C14:1OH	**0.0014 (0.6513)**
		C14OH	**0.0137 (0.5287)**
		C16:1OH	**0.0002 (0.7326)**
		LCHAD (all 12-18 OH)	**0.0018 (0.6383)**
	SCOH/MCOH	C4OH	**0.0247 (0.4883)**
Neonatal %FM (OB)	Ala/Asp/Glu	Alanine	**0.0015 (0.8578)**
		Gln:Asp	**0.0331 (-0.6725)**
	LCAC	C12:1	**0.007 (0.7865)**
		C12	**0.018 (0.7235)**
		C14:2	**0.0093 (0.7693)**
		C14:1	**0.0022 (0.8421)**
		C14	**0.0464 (0.6397)**
		C16:1	**0.0199 (0.7157)**
		C18:2	**0.0454 (0.6419)**
		Long Chain (C12-18)	**0.0395 (0.6558)**
		Long (all+OH)	**0.0135 (0.7448)**
	LCOH	C14:1OH	**0.0214 (0.7101)**
		C14OH	**0.0257 (0.6948)**
		C16:1OH	**0.001 (0.8709)**
		LCHAD (all 12-18 OH)	**0.0064 (0.7911)**
	SCOH/MCOH	C4OH	**0.0398 (0.655)**
	DCAC/MCOH	C5DC+C10OH	**0.0042 (0.8139)**
		C6DC	**0.0445 (0.6438)**
		Total DCAC	**0.0049 (0.8056)**
Neonatal %FM (NW)	None	None	None

Table S3			
Phenotype	**Pathway**	**Analyte**	**p-value (r-value)**
ppBMI	Arg&Pro	Cit:Arg	**0.0228 (-0.494)**
		Citrulline	**0.0224 (-0.4955)**
	CPT	C16	**0.042 (-0.4474)**
	LCAC	Long Chain (C12-18)	**0.033 (-0.4666)**
FFA (Total)	Ala/Asp/Glu	Glu:Gln	**0.0262 (-0.4958)**
FFA (OB)	None	None	None
FFA (NW)	LCAC	C14:2	0.0263 (-0.6625)
Triglycerides	LCOH	C16:1OH	**0.0149 (0.5788)**
		C16OH	**0.029 (0.5289)**
	MCAC	C8:1	**0.0195 (0.5597)**
	MCAC	C10:1	**0.0445 (0.4926)**
	DCAC/MCOH	C5DC+C10OH	**0.0422 (0.4975)**
		Total DCAC	**0.0221 (0.5503)**
Insulin	Ala/Asp/Glu	Asp:Asn	**0.0196 (-0.517)**
		Asparagine	**< 0.0001 (0.8342)**
	LCAC	C12	0.051 (-0.4421)
Glucose	Ala/Asp/Glu	Asp:Asn	**0.0251 (-0.499)**
		Asparagine	**0.0041 (0.613)**
		Gln:Glu	**0.0422 (-0.4582)**
	Arg&Pro	Arg:Glu	0.0527 (-0.4393)
Birthweight	1C&Sulf	Taurine	**0.0066 (0.5736)**
Neonatal %FM (Total)	CPT	C18	**0.0319 (-0.4806)**
	DCAC/MCOH	C3DC+C8OH	**0.0102 (-0.5601)**
Neonatal %FM (OB)	CPT	C18	**0.0349 (-0.7023)**
	MCAC	C8:1	0.0359 (-0.6996)
	SCOH/MCOH	C4OH	**0.0313 (-0.7122)**
		C5OH	**0.0256 (-0.7299)**
	DCAC/MCOH	C3DC+C8OH	**0.0162 (-0.7655)**
Neonatal %FM (NW)	None	None	None

Table S2&3: Acylcarnitine and Amino Acid Analysis in uMSC-Myocytes (S2) and uMSC-adipocytes (S3) vs maternal and neonatal phenotypes. Total number analyzed n=22. Of note n=2 of our 24 samples did not have metabolomic analysis available. Listed are p-value (r-value), determined by linear regression analysis, for analytes with p-values ≤0.05. Green (positive correlation) and red (negative correlation) represent metabolite changes vs phenotypes if the FDR≤0.25.

Biologic Process (GO)			
#Pathway ID	Pathway Description	Count in Gene Set	False Discovery Rate
GO.0006139	nucleobase-containing compound metabolic process	134	0.00686
GO.0010468	regulation of gene expression	121	0.00686
GO.0034654	nucleobase-containing compound biosynthetic process	100	0.00686
GO.0046483	heterocycle metabolic process	137	0.00686
GO.0051641	cellular localization	74	0.00686
GO.0051649	establishment of localization in cell	65	0.00686
GO.1901360	organic cyclic compound metabolic process	142	0.00686
GO.1901362	organic cyclic compound biosynthetic process	104	0.00846
GO.0006725	cellular aromatic compound metabolic process	135	0.00876
GO.0090304	nucleic acid metabolic process	120	0.00876
GO.0009058	biosynthetic process	137	0.0097
GO.0044271	cellular nitrogen compound biosynthetic process	107	0.013
GO.0010467	gene expression	118	0.0134
GO.0016070	RNA metabolic process	107	0.0134
GO.0034641	cellular nitrogen compound metabolic process	144	0.0154
GO.0046907	intracellular transport	50	0.0154
GO.0051171	regulation of nitrogen compound metabolic process	117	0.0154
GO.1901576	organic substance biosynthetic process	133	0.0154
GO.2000112	regulation of cellular macromolecule biosynthetic process	109	0.0154
GO.0010556	regulation of macromolecule biosynthetic process	110	0.0163
GO.0016197	endosomal transport	16	0.0163
GO.0032774	RNA biosynthetic process	89	0.0163
GO.1902582	single-organism intracellular transport	45	0.0167
GO.0060255	regulation of macromolecule metabolic process	145	0.018
GO.0019219	regulation of nucleobase-containing compound metabolic process	109	0.0229
GO.0009889	regulation of biosynthetic process	114	0.025
GO.0051252	regulation of RNA metabolic process	102	0.025
GO.0008152	metabolic process	231	0.0272
GO.0031323	regulation of cellular metabolic process	149	0.0279
GO.0031326	regulation of cellular biosynthetic process	112	0.0354
GO.0006807	nitrogen compound metabolic process	148	0.037
GO.1902580	single-organism cellular localization	35	0.0398
GO.0034645	cellular macromolecule biosynthetic process	107	0.049
GO.2001141	regulation of RNA biosynthetic process	98	0.0498

Table S4: GO Biologic Processes enriched in differential gene expression in uMSC-Myocytes from offspring of obese (n=7) versus normal weight (n=7) mothers. Analyzed by student t-test, with genes having significant p-values entered into STRING database analysis.

KEGG Pathways			
#Pathway ID	Pathway Description	Count in Gene Set	False Discovery Rate
4810	Regulation of actin cytoskeleton	23	8.93E-06
4722	Neurotrophin signaling pathway	15	0.000138
5205	Proteoglycans in cancer	20	0.000447
4010	MAPK signaling pathway	19	0.00545
4015	Rap1 signaling pathway	17	0.00545
4919	Thyroid hormone signaling pathway	12	0.00545
4152	AMPK signaling pathway	12	0.00623
4510	Focal adhesion	16	0.00834
5211	Renal cell carcinoma	8	0.0144
4014	Ras signaling pathway	16	0.0164
5215	Prostate cancer	9	0.0183
5200	Pathways in cancer	20	0.0221
5131	Shigellosis	7	0.025
4151	PI3K-Akt signaling pathway	20	0.034
5166	HTLV-I infection	16	0.0436
4012	ErbB signaling pathway	8	0.045
5213	Endometrial cancer	6	0.0483

Table S5: KEGG Pathways enriched in differential gene expression in uMSC-Adipocytes from offspring of obese (n=7) versus normal weight (n=7) mothers. Analyzed by student t-test, with genes having significant p-values entered into STRING database analysis.

Phenotype	Pathway Source	Pathway of Interest	p-value	FDR	Enrichment Score	Enrichment Core Genes
ppBMI	KEGG	mTOR Signaling	0	0.003	-0.68	RPTOR, MAPK(1,3,8), TSC(1&2), AKT(1-3), BRAF, EIF4E2, PRKA (A1,CA,CB,R2A,R2B), ULK2, CAB(39&39L)
	KEGG	MAPK Signaling	0	0.007	-0.67	MAPK(1,3,8,9), MAP2K1, MAPK3K7, MKNK1, SOS(1&2), AKT(1-3), PRKA (CA&CB), NRAS, KRAS, BRAF, RAF1, IKBKB, HSPA8, CRK, CRKL
	KEGG	Insulin Signaling	0	0.004	-0.56	IRS1, RPTOR, PRKA(A1,CA,CB,R1A,R2A,R2B), PRKCI, PIK3(CA,CB,CD,R1), GSK3B, MAPK(1,3,8,9), MAP2K1, AKT(1-3), SOS(1&2), RAF1, BRAF, NRAF, CALM3
	KEGG	Adipokine Signaling	0	0.007	-0.55	LEPR, ADIPOQR2, PPARA, CPT2, ACSL1, IRS1, PRKA(A1,G2,R2), CAMKK(1&2), MAPK(8&9), AKT(1-3), IKBKB
	Reactome	Signaling by FGFR	0	0.00007	-0.72	CREB1, PDPK1, PIK3R1, FOXO3, GSK3A, CAMK4, CALM3, NRAS, KRAS, RAF1, AKT(1-3), MAPK(1&3), MAPK2K1, MKNK1, PRKA(CA,CB,R1A,R2A)
	Biocarta	CREB Pathway	0.002	0.01	-0.74	CREB1 ,MAPK(1&3), AKT1, PRKA(CB, R1A, R2A), CAMK2(B&D), GRB2, SOS1
	KEGG	Apoptosis	0.002	0.01	-0.62	EXOG, IKBKB, AKT(1-3), PIK3(CA,CB,CD,R1), PRKA(CA,CB,R2A,R2B)
	Reactome	Immune System	0.004	0.004	-0.61	EP300, CREB1, FOXO(1&3), SMURF(1&2), PARK2, IRS1, CAMK2(B&D), IKBKB, AKT(1-3), PIK3(CA,CB,CD,C3,R1, AP1), MAPK(1,3,8,9,3K7), MAP2K1

	KEGG	Wnt Signaling	0.008	0.02	-0.67	EP300, CREBBP, CAMK2(B&D), PPP2R(1A,5A, 5B, 5D, 5E), PPP2CB, PRKACB, MAPK(8,9,3K7), GSK3B, CCND(1&3)
Free Fatty Acids	KEGG	mTOR Signaling	0.04	0.21	-0.56	RPTOR, MAPK(1&3), TSC(1&2), PIK3(CA,CB,R1,R2), AKT3, ULK(1&2), BRAF, PRKAA2, CAB39L, STRADA
	KEGG	MAPK Signaling	0.03	0.23	-0.58	MAPK(1,3,8), MKNK1, SOS(1&2), AKT(2&3), PRKA (CA&CG), NRAS, KRAS, BRAF, IKBKB, HSPA(1A,1B,6,8), CRK, CRKL
	Reactome	Transcriptional Regulation of White Adipocyte Diffrentiation	0.04	0.19	-0.72	EP300, CREBBP, PPARA
	Reactome	Energy Dependent Regulation of mTOR by LKB1 (STK11) AMPK	0.03	0.2	-0.59	PRKA(A2,B1,B2,G2), TSC(1&2), RPTOR, CAB39L
	KEGG	Phosphatidylinisitol Signaling System	0.02	0.2	-0.57	PIK3(CA, CB, C2A, C2B, R1, R2), INPPL
	Reactome	Integration of Energy Metabolism	0.03	0.19	-0.5	PRKA(A2,B2,G2,CA,CG,R1A,R1B,R2A), ACAC(A&B), FASN, ACLY
	KEGG	Oxidative Phosphorylation	0.02	0.15	0.6	NDUF(A1,A2,A3,A4,A5,A6,A7,A8,A11, AB1,B1,B2,B3,B4,B6,B7,B8,B10,C1,C2, S2,S3,S5,S6,S7,S8,V2), SDH(B,C,D), UQCR(B,FS1,H,Q,10,11), COX(5B,6A1,6A1,6B1,6C,7A2,7A2L,7B, 7C,8A,11),LHPP,PPA(1,2), TCIRG1, ATP5(B,H,D,G1,G3,J,J2,L,T) (note: only Mitochondrial Complex, TCA Cycle, and Fatty Acid Oxidations genes in data set)
Triglycerides	Reactome	Glycogen Breakdown Glycogenolysis	0.004	0.16	-0.58	CALM1, PHKG2, PYG(L&M)
	Reactome	Synthesis of PIP at the Late Endosome Membrane	0.002	0.16	-0.89	PIK3(C2A&R4)

Glucose	None	None	None	None	None	None
Insulin	None	None	None	None	None	None
HOMA	KEGG	Histidine Metabolism	0.002	0.12	0.74	ALDH(1A3, 1B1, 3B2), MAOB, HNMT
Neonatal %FM	Biocarta	Leptin Pathway	0.04	0.24	-0.72	LEPR, CPT1A, PRKA(B2,G1,G2), ACACA
	Reactome	Downregulation of TGF Beta Receptor Signaling	0.03	0.19	-0.71	PPP1C(A,B,C), SMURF(1&2)
	Reactome	CA Dependent Events	0.04	0.24	-0.67	CALM1, CAMK4, PRKA(CB,R1A,R2A), MAPK1
	Reactome	Energy Dependent Regulation of mTOR by LKB1 (STK11) AMPK	0.03	0.21	-0.58	RPTOR, STRADA, TSC(1&2), PRKA(B2, G1, G2)
	KEGG	Phosphatidylinisitol Signaling System	0.008	0.25	-0.59	PIK3(CA,CB,CD,C2A,C3,R1,R2), CALM1
	KEGG	NK Cell Mediated Activity	0.04	0.24	-0.55	MAPK(1&3), PIK3(CA,CB,CD,R1), SOS(1&2), KRAS, NRAS, BRAF, RAF1
	Reactome	Signaling by FGFR	0.04	0.24	-0.57	PRKA(CB,R1A,R2A), MAPK(1&3), PIK3(CA&R1), SOS1, TSC(1&2), AKT3, KRAS, NRAS, BRAF, RAF1, CALM1, CAMK4, CBL, FOXO3, PDPK1, PPP2(CB&R1A)
	Reactome	Integration of Energy Metabolism	0.07	0.21	-0.5	PRKA(B2,CB,CA,G2,R1A,R2A), PPP2(CB,R1A,R5D), ACAC, SLC25A5
	KEGG	Oxidative Phosphorylation	0.008	0.11	0.56	NDUF(A1,A2,A3,A4,A5,A6,A7,A8,A11, AB1,B1,B2,B3,B4,B5,B6,B7,B8,B10,C1, C2,S3,S5,S6,S7,S8), SDH(A&C), UQCR(B,H,Q,10,11), COX(5A,5B,6B1,6C,7A2,7B,7C,8A,17), TCIRG1, LHPP, ATP5(D,E,G1,G3,H,J,J2,L) (note: only

						Mitochondrial Complex and TCA Cycle genes in data set)

Table S6: uMSC-Adipocyte targeted RNA-Seq analysis by GSEA using a curated gene set enriched for nutrient sensing and energy processing pathways. Leading edge analysis of core genes indicates significant overlap between ppBMI, FFA, and Neonatal %FM for genes in MAPK and mTOR signaling

Supplemental Experimental Procedure

uMSC Growth and Conditions

Undifferentiated cells (Pass 2-3) from offspring NW and OB mothers were thawed and grown to confluence in MSC growth media

(MSC-GM; Lonza, Walkersville, MD). Cells were then sub-cultured into experimental plates. Once cells returned to confluence, they were separately exposed to myocyte differentiation media and adipocyte differentiation media. Information regarding stage of differentiation state can be found in Boyle et al, 2016, as well as Janderova et al, 2003 and Gang et al, 2004 from which we based our protocols. The adipogenic differentiating uMSCs were induced for three cycles, with media changes every three days, and myogenic media was changed every 3 to 4 days. Media was harvested by pooling 3x500uL aliquots (500uL per well, 24 wells per plate) for analysis on day 21 of differentiation, following approximately 3 days continuous media exposure. Mean media exposure times at harvest did not differ significantly between NW and OB cohorts for uMSC-Myocytes (3.4±1.0 vs 3.8±0.9 days, p=0.62) and uMSC-adipocytes (3.2±1.0 vs 3.7±0.8 days, p=0.45).

Media Metabolomic Analysis

Using the Biochrom 30+ Amino Acid Analyzer 32 total amino acids were analyzed. Amino acid reaction ratios were calculated, as the ratio of product over substrate of a known amino acid associated reaction. Lower ratios corresponded to a more favorable reaction. Using MS/MS-ESI, 38 primary acylcarnitine species were analyzed, as well as calculated totals of short (C0-C5), medium (C6-C12), dicarboxylic acid (C3DC-C6DC), and long chain species (C14-C18) with and without hydroxyl groups. All analytes were placed in the context of known KEGG pathways. These included the central metabolic pathway for the Citric Acid Cycle (map00020), and input pathways including Ala/Asp/Glu (map00250), Val/Leu/Ile Degradation (map00280), Tyrosine (map00350, which includes Phenylalanine), Cysteine, Methionine, Glycine, Serine, and Threonine (map00270 and 00260, considered 1-Carbon metabolism), Arginine and Proline (map00330), Lysine (map00310), Tryptophan (map00380), and Histidine (map00340) metabolism. Acylcarnitines were associated with pathways of fatty acid oxidation (map00071) which can be further characterized by specific enzymatic reactions defined by acylcarnitine signature

RNA-Seq Analysis

Cells were washed in PBS and lysed. Lysate was flash frozen in liquid nitrogen and stored at -80°C until the RNA was extracted at a later date. RNA extraction and purification was done using the miRNEasy Minikit (Qiagen). Purified RNA samples were sent to the University of Colorado Genomics Core Laboratory for analysis. Mapping and Bioinformatics analysis were supported in part by the Biostatistics Shared Resources of Colorado's NIH/NCI Cancer Center (support grant P30CA046934). Quality control was done to trim reads to Q15 quality score, removing anything that was trimmed to less than 40 base pairs. Reads were mapped to hg19 with GSNAP, using dbSNP to guide mapping. Expression was called using Cufflinks. Genes were removed if mean reads per group (NW or OB) were less than 0.1 FPKM, or there were more than 50% of subjects without reads in either group. Further, in both STRING and GSEA curated dataset analysis only protein coding genes were included.

Statistical Analysis

STRING Database 10.0 was used to analyze for RNA-Seq enrichment in genes with OB vs NW p-values <0.05. Output is a single p-value for protein-protein (or in this case gene-gene) interaction. Go Biological Processes and KEGG pathway enrichment significance is expressed by FDR value calculated in silico by Benjamini-Hochberg, and in all cases for STRING was <0.05. Analysis was stringently limited to only those interactions with confidence values of >0.9. Gene Set Enrichment Analysis (GSEA, http://www.broad.mit.edu/gsea/) was used to analyze continuous variables versus continuous gene expression values in a hand-curated dataset using KEGG defined pathways in nutrient sensing and energy metabolism listed above. Total genes in this gene set were ~1150 after quality control. Subsets of mitochondrial oxidative phosphorylation genes were analyzed separately. GSEA methods and interpretation have been well described.