Supplemental data

Elevated stearoyl-CoA desaturase-1 expression in skeletal muscle contributes to abnormal fatty acid partitioning in obese humans

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Supplemental experimental procedures

Microarray data analysis

Absolute intensity values for each chip were screened for probe saturation using the "Array Data Manipulation" program created by Tanya Teslovich at Children's National Medical Center, Washington, DC. Each chip was scanned twice, once after the first stain, and a second time after the third stain. The saturation program identifies probes that have become saturated in the second scan, after the probe signals are amplified. Probe saturation was common when the PMTs for Affymetrix scanners were calibrated to a higher value (ten times higher than the current settings). Information on scanner PMT settings are available at http://www.affymetrix.com/support/technical/product_updates/scanner_gain_update.pdf. The program considers a probe saturated if its signal in the first scan (before antibody amplification) is greater than 1500 and the (scan 2/scan 1) ratio is less than a user-specified minimum value of 0.8. If the probe was saturated on any array in the experiment, then the 'scan 1' signal intensity is used for that gene across all chips. After the files had been de-saturated, they were loaded into GeneSpring® (http://www.silicongenetics.com) for statistical analysis, graphical visualization of genes, and unsupervised hierarchical clustering. Genes that were absent across all chips were eliminated through filtering. In the first round of analysis each gene for all sixteen chips was normalized to the mean expression value of that gene for the lean condition. A parametric t-test,

not assuming variances between the conditions were equal, was performed to generate a list of genes that had a p-value of less than 0.05 between the three conditions. GeneSpring® was also used to cluster genes by their ontology group.

Primary human adipocyte cultures

Adult Human dermal fibroblast (HDF-a) Cat#2320 from ScienCell Research Laboratories, San Diego, CA were subcultured and grown to confluency as specified by the supplier. Primary adipocytes were isolated from liposuction aspirates of subcutaneous adipose tissue from subjects undergoing elective procedures in local plastic surgical offices. Adipose-derived stem cells were expanded and induced into adipogenesis as previously described (Delany et al., 2005).

Supplemental references

Delany, J., Floyd, Z.E., Zvonic, S., Smith, A., Gravois, A., Reiners, E., Wu, X., Kilroy, G., Lefevre, M. and Gimble, J.M. Proteomic Analysis of Primary Cultures of Human Adipose-derived Stem Cells; Modulation by Adipogenesis. Mol. Cell. Proteomics, *In Press*.

Gene	Affymetrix GeneChip® (Arbitrary units)		
	Lean	**Obese**	**P Value**
Lipid biosynthesis genes			
ACCα	0.35 ± 0.06	0.29 ± 0.07	0.48
ACCβ	0.52 ± 0.05	0.73 ± 0.09	0.09
DGAT	0.48 ± 0.07	0.45 ± 0.01	0.78
GPAT	4.27 ± 0.48	4.07 ± 0.31	0.74
SCD1	0.26 ± 0.13	0.85 ± 0.20	**0.03**
SREBP1c	0.87 ± 0.21	0.86 ± 0.08	0.96
PPAR nuclear receptors			
PPARα	0.42 ± 0.09	0.31 ± 0.03	0.27
PPARδ	0.04 ± 0.01	0.05 ± 0.01	0.58
PPARγ	0.04 ± 0.004	0.08 ± 0.02	0.08
Fatty acid oxidative genes			
CPT1β	2.64 ± 0.46	2.26 ± 0.32	0.51
MCD	2.44 ± 0.50	2.01 ± 0.24	0.45
PDK4	9.27 ± 0.96	7.58 ± 0.87	0.21
Adipocyte-enriched genes			
Leptin	Not Detected	Not Detected	
Adiponectin	2.03 ± 0.31	2.30 ± 0.33	0.65
Adipose differentiation related protein	1.55 ± 0.09	1.63 ± 0.14	0.47
Fatty acid binding protein 4 (AP2)	1.13 ± 0.14	1.16 ± 0.14	0.72
Adipsin	6.12 ± 0.78	5.32 ± 0.42	0.91

Table S1. Normalized raw intensity scores of lipid-regulatory genes

Total RNA was extracted from rectus abdominus muscles of obese (n=8, BMI, 53.8 ± 3.5) and lean control subjects (n=8, BMI, 23.8 ± 0.58) and transcriptional profiling was performed on individual samples using Affymetrix Human Genome chips. Raw intensity scores were normalized to cyclophilin B as an endogenous housekeeping gene and presented as mean ± SEM. Differences between lean and obese were analyzed by independent t-tests. ACC, acetyl CoA carboxylase; DGAT; diacylglycerol acyltransferase 1, GPAT; mitochondrial glycerol-3-phosphate acyltransferase, SREBP1c; sterol regulatory element binding protein 1c, CPT1β; carnitine palmitoyltransferase 1β, MCD; malonyl-CoA decarboxylase, PDK4; pyruvate dehydrogenase kinase 4, PPAR; peroxisome proliferator activated receptor.

Over-Expressed Genes	GeneChip â (Obese vs. Lean)	P Value
Hemoglobin, gamma A (NM_000559)	4.78 ± 1.81	0.05
Stearoyl-CoA desaturase (AB032261)	3.48 ± 1.25	0.05
Serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 5 (J02639)	2.95 ± 0.87	0.05
Retinol dehydrogenase homolog (NM_005771)	2.93 ± 0.72	0.03
Growth factor receptor-bound protein 14 (NM_004490)	2.70 ± 0.33	0.001
Neuropeptide Y receptor Y1 (NM_000909)	2.32 ± 0.32	0.005
602136853F1 NIH_MGC_83 Homo sapiens cDNA clone IMAGE:4273260 5', mRNA sequence (BF674712)	2.16 ± 0.54	0.05
Ribosome binding protein 1 (dog 180kD homolog) (NM_004587)	2.15 ± 0.45	0.05
Ubiquitin carrier protein (NM_014501)	2.15 ± 0.51	0.05
Hypothetical protein FLJ13397 (NM_024948)	2.11 ± 0.30	0.007

Under-Expressed Genes	GeneChip â (Obese vs. Lean)	P Value
Frizzled-related protein (NM_001463)	0.36 ± 0.25	0.0001
Nicotinamide N-methyltransferase (NM_006169)	0.39 ± 0.24	0.0002
Muscle-specific beta 1 integrin binding protein (NM_014446)	0.39 ± 0.05	0.00005
Nicotinamide N-methyltransferase (NM_006169)	0.41 ± 0.09	0.0003
Chloride channel, calcium activated, family member 3 (NM_004921)	0.43 ± 0.11	0.001
Hypothetical protein MGC2663 (NM_024106)	0.45 ± 0.16	0.01
HSPC159 protein (NM_014181)	0.48 ± 0.09	0.0006
Connective tissue growth factor (M92934)	0.49 ± 0.11	0.002
Acetyl LDL receptor; SREC=scavenger receptor expressed by endothelial cells (NM_003693)	0.49 ± 0.08	0.0006
Transforming growth factor beta-activated kinase-binding protein 1 (NM_006116)	0.51 ± 0.09	0.001

Table S2A and 2B. Highest ranking differentially expressed genes between lean and obese humans

Total RNA was extracted from rectus abdominus muscles of obese (n=8, BMI, 53.8 ± 3.5) and lean control subjects (n=8, BMI, 23.8 ± 0.58) and transcriptional profiling was performed on individual samples using Affymetrix Human Genome chips as described in Methods. All

transcripts that were statistically different between lean and obese groups were sorted based on fold difference (obese vs. lean) and the top 10 most up-regulated (Table A) and down- regulated (Table B) transcripts are presented. Data are expressed as fold-difference (obese/lean) and mean ± SEM.

Human Cell Culture	C_T	Relative Expression
pre-adipocytes	27.1 ± 0.07	1.0
adipocytes	22.0 ± 0.01	34.3
fibroblasts	25.0 ± 0.6	4.3
myoblasts (PC)	25.6 ± 0.3	2.8
myoblasts (C)	25.5 ± 0.2	3.0
myotubes	25.2 ± 0.3	3.8

Human Tissue	C_T	Relative Expression
SkM-lean	24.0 ± 0.5	1.0
SkM-obese	22.6 ± 0.3	2.6
adipose tissue	19.5 ± 0.2	23.4

Table S3. SCD1 mRNA expression in human cells and tissues

Total RNA was isolated from primary human pre-adipocytes, differentiated adipocytes, pre-confluent (PC) and confluent (C) myoblasts and mature myotubes (originating from lean donors), rectus abdominus muscle (SkM) and adipose tissue. Human fibroblasts were obtained from ScienCell Research Laboratories. SCD1 mRNA expression levels were quantified by RTQ-PCR using 20 ng cDNA as template. Relative expression levels were normalized to cyclophilin B and data are presented as means ± SEM. The C_T value (threshold cycle) represents

the fractional cycle number during RTQ-PCR at which probe florescence passes a fixed baseline, and is inversely related to mRNA abundance.

Figure S1. Relationship between SCD mRNA levels and body mass index

Muscle samples were obtained from lean and obese humans with a body mass index ranging from 22-60 kg/m^2. Linear regression analysis was performed using gene expression data from Table 1 and body mass index of the donor subject.