

Primers sequences used in Taqman PCR and genotyping

Perilipin	For	TGGACCACCTGGAGGAAAAG
	Rev	TTCGAAGGCGGGTAGAGATG
	Probe	6CGGCTCTTCAATACCCTCCAGAAAA0
AP2	For	CACCGCAGACGACAGGAAG
	Rev	GCACCTGCACCAGGGC
	Probe	6TGAAGAGCATCATAACCCTAGATGGCGG0
LPL	For	TGGAGAAGCCATCCGTGTG
	Rev	TCATGCGAGCACTTCACCAG
	Probe	6TGCAGAGAGAGGACTCGGAGACGTGG0
Glut 4	For	ACTCATTCTTGGACGGTTCCTC
	Rev	CACCCCGAAGATGAGTGGG
	Probe	6TGGCGCCTACTCAGGGCTAACATCA0
PGC1α	For	AACCACACCCACAGGATCAGA
	Rev	CTCTTCGCTTTATTGCTCCATGA
	Probe	6CAAACCCTGCCATTGTTAAGACCGAGAA0
Resistin	For	CCATCGACAAGAAGATCAAACAAG
	Rev	CAGCAATTTAAGCCAATGTTCTTTAT
	Probe	6TTCAACTCCCTGTTTCCAAATGC0
FAS	For	GCCAGACAGAGAAGAGGCA
	Rev	CTGACTCGGGCAACTTCCC
	Probe	6GGAGGAGGTGGTGATAGCCGGTATGTC0
UCP1	For	CCCGCTGGACACTGCC
	Rev	ACCTAATGGTACTGGAAGCCTGG
	Probe	6AAGTCCGCCTTCAGATCCAAGGTGAAG0
UCP2	For	GATCTCATCACTTTCCCTCTGGATA
	Rev	CCCTTGACTCTCCCCTTGG
	Probe	6CGCCAAGGTCCGGCTGCAGA0
UCP3	For	GGACCTCCTCACTTTTCCCC
	Rev	CTGGGTTCTCCCCTTGGACT
	Probe	6CCCACGGCCTTCTACAAAGGATTTGTG0
SREBP1c	For	GCCATGGATTGCACATTTGA
	Rev	GGCCCGGGAAGTCACTG
	Probe	6GACATGCTCCAGCTCATCAACAACCAAG0
PPARα	For	CCTCAGGGTACCACTAGGGAGT
	Rev	GCCGAATAGTTCGCCGAAA
	Probe	6CACGCATGTGAAGGCTGTAAGGGCTT0
PPARδ	For	TGTGTGGAGACCGGCGA
	Rev	CGCAGAATGGTGTCTCTGGA
	Probe	6GCCTCATGAATGTGCCCCAGGTAGAAG0
PPARγ1	For	TTTAAAAACAAGACTACCCTTTACTGAAATT
	Rev	AGAGGTCCACAGAGCTGATTC
	Probe	6AGAGATGCCATTCTGGCCCCACCAACTT0
PPARγ2	For	GATGCACTGCCTATGAGCACTT
	Rev	AGAGGTCCACAGAGCTGATTC
	Probe	6AGAGATGCCATTCTGGCCCCACCAACTT0
PPAR sens (F1)	For	CCAACCAATCTTTTGCAAGACATAGAC
ASC 306 (F2) PPAR antis (R)	For	AATGGCCGCTTTTCTGGATTCATCGAC
	Rev	ACATGCAATTTACCCACACATGAGTG
DR1 5' short arm	For	CATGCCGCCTGCGCCCTATTGATCATG
	Rev	ATTAATGATGTAATTGCCAACTTCTC

Generation of PPAR γ 2 KO mice.

A genomic mouse PPAR γ 2 clone was obtained from a 129 mouse strain P1 Artificial Chromosome (PAC) genomic library (RPCI mouse PAC library 21). A replacement targeting construct was created in which the PPAR γ 2-specific exon B1 was replaced with a IRES β GalMCNeo cassette (consisting of an internal ribosome entry site flanked at the 5' end with an artificially incorporated primer site used for PCR screening (DR1) and linked at the 3' end to a LacZ reporter gene carrying a polyadenylation signal; this was followed by a lox-P flanked promoted neomycin phosphotransferase selectable marker cassette). A fragment (1.17 kb) extending from intron B1 and containing the promoter P2, and a fragment (4.04 kb) that spans exon B1 through intron 1 were subcloned and used for the 5' homology arm and the 3' homology arm respectively. HSV tk genes were situated externally to both arms to allow negative selection against random integrations. The transcriptional orientation of the Neo gene was the same as the PPAR γ 2 gene in the construct.

The linearized targeting vector was electroporated into 129S6/SvEv embryonic stem cells, selected with G418 and resistant clones isolated and expanded followed by genomic DNA extraction for PCR and Southern blot analysis (Fig. 1). Recombinant clones were identified by PCR using the DR1 primer located in the 5' region of the LacZ/Neo cassette, together with a primer external to the 5' short arm. Homologous recombination was verified by Southern blot analysis after digestion with Nsi I, SphI and Nco I enzymes (Fig. 1B). The targeted clone was injected into C57Bl/6 blastocysts. Chimaeric offspring were mated with 129/SvEv mice. Two lines of mice carrying the disrupted exon B1 of PPAR γ 2 were bred and used for analysis. All experiments were performed following the generation of descendants, which were hybrids between the C57Bl/6 and 129/Sv strains. Genotyping was performed by multiplex PCR using standard protocols.

Lipid profiling methodology.

The WAT lipid analysis for normal diet fed mice was performed on 12 mice (6 PPAR γ 2 KO and 6 wild type), using two replicates per sample. Approximately 50 mg of tissue was used per sample. Samples were homogenized in 300 μ l 0.9% NaCl and extracted with 2 ml CHCl₃:MeOH (2:1). 15 μ l of homogenate was used for the LC/MS runs. The liquid chromatography was performed on Waters 2795 HPLC system, with the reverse-phase column C18 (Hypersil BDS, Agilent Technologies: 150x4.6mm, particle size 5 μ m, Temp 35°C). The solvent system used is as described in (1). Solvents used: H₂O, ACN, 2-Propanol, DKM, Am. acetate 1M, HCOOH. Conditions: flow rate 0.8 ml/min and flow to MS 0.15ml/min. The mass spectrometry analyses were performed in Electro Spray Ionisation positive ion mode on a Quattro Micro triple quadrupole mass spectrometer (Micromass): Mass range m/z 250-1200, full scan mode (rate 2.3 s/scan), capillary voltage 4 kV, cone voltage 40 V, source temp. 130 °C, desolvation temp. 250°C, desolvation gas flow 900 l/h. The same conditions were used in tandem MS analyses (MS/MS) for targeted analyses of specific ions, with tissue-specific samples pooled and concentrated. The MS and MS/MS spectra were visually checked using the MassLynx software (Micromass). Details of the data processing methodology will be described elsewhere. In summary, the raw files from full scan MS runs were converted into the netCDF format. Peak detection was based on method described by (2). Each peak was described by its m/z value and retention time. Peak lists were then aligned based on the partial linear fit algorithm (3), modified to allow alignment of peaks described by two indices. Peaks were then filtered based on their profiles and intensity. Selected peaks were calibrated based on a statistical model as previously described for microarray data (4), with the difference that the method was applied to the whole dataset, not the subset of controls. After the data processing, the individual peak profiles were characterized by fold change (calculated as mean peak high

in KO mice vs. mean peak high in WT mice) and the t test statistic comparing means of KO and WT mice, with corresponding p - values. The data processing and statistical analysis methods were implemented using Matlab release 14 software (MathWorks, Inc.). Principal components analysis on normalized data was performed using the PLS_Toolbox 3.0 software (Eigenvector Research, Inc.). Linear correlation networks were used to detect pattern of peaks with similar profiles, and the representative peaks from each cluster, based on statistical criteria and intensity, were selected for targeted MS/MS analyses. Each peak selected was checked against the raw MS data across different samples. For correlation network analysis, linear correlation was calculated between each pair of peak profiles (i.e. peak intensity values across all 12 mice). Each peak was represented as a node in a network. If the correlation value between the two nodes was over the threshold set to $C > 0.90$, they were connected by an edge. So obtained nodes and edges were then visualized as a network. The visualization tool was developed based on Tom Sawyer Visualization toolkit (Tom Sawyer, Inc.).

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