SUPPLEMENTARY DATA

Gene Expression

Total RNA was isolated from human adipose tissue using RNAeasy Lipid Tissue Mini (Qiagen, Valencia, CA) and RNA quantity and quality were verified using an Agilent 2100 Bioanalyser (Palo Alto, CA).Standard curves were constructed from pooled cDNA for each gene and 18S.The data are expressed in arbitrary units, and in graphs in which multiple genes are represented the only comparison that can be made are the two values (e.g. summer and winter) for each gene. Histamine receptor expression was measured in the abdominal fat from the cohort of 55 subjects described in (Kern et al, JCEM 2014) and in 3T3L1 cells.The forward (F) and reverse (R) primer sequences are in Table S1 and S2.

Cell culture

TIB64 cells (P815, ATCC, Manassas, VA) were grown in DMEM (Catalog Number 11885-092; Thermo Fisher Scientific, Grand Island, NY) medium with10% fetal bovine serum (FBS) (Catalog Number 101; Tissue Culture Biologicals, Tulare, CA). 3T3-L1 (CL-173, ATCC) cells were maintained in DMEM with 10% bovine calf serum (Catalog Number 16010; Thermo Fisher Scientific) and differentiated into adipocytes as follows. The cells were plated in 6-well dishes at a density of $1x10^5$ cells/well and 48-hours after they reached confluency, the medium was changed to differentiation medium (DM), which consisted of DMEM, 10% FBS (Tissues Culture Biologicals), 1 μM dexamethasone (Sigma, Saint Louis, MO), 0.5 mM IBMX (Sigma), 0.32ul/mL Insulin (Novo Nordisk, Clayton, NC) for 4-days.The adipocytes were maintained in DM without dexamethasone and IBMX, changing the medium every 2-days.TIB64 cells were grown to a density of $5X10^5$ cells per well and then cold shocked at 30°C for 4-hours followed by recovery at 37°C for the indicated time.Nedachromil (Sigma) was used at a concentration of 10 nM as a degranulation inhibitor.The mast cells and media were harvested at the indicated time during the recovery period.Mast cell conditioned medium was diluted 1:2 with adipocyte medium and then was applied to adipocytes for 4-hours at 37°C.For pharmacological inhibitor studies, adipocyte DM containing 5 μM chlorpheniramine (C3025, Sigma), 50 μM H89 (AG-CR1-0002, Adipogen, San Diego, CA), 10 μM propranolol (P0884, Sigma), or 100 nM SB 202190 (19-134, Millipore) was added to adipocytes 30-minutes before conditioned medium treatment. The medium was replaced with 50% mast cell conditioned medium / 50% adipocyte DM containing the same concentration of inhibitors, and the adipocytes were then harvested 4-hours after incubation at 37°C.For adipocyte-mast cell coculture experiments, 3T3-L1 adipocytes were grown in 6-well plates and differentiated.The cells were then cocultured alone or with 10^4 mast cells in the insert.The system was cold shocked and allowed to recover at 37°C for the indicated times (n=3). To determine the effect of mast cell conditioned media on lipolysis, differentiated 3T3-L1 cells were deprived of insulin for 24-hours and then incubated for 4-hours in mast cell conditioned media (diluted 1:2 in adipocyte differentiation medium without insulin) as indicated.The media was removed and the adipocytes were incubated overnight in potassium ringers containing 2% fatty acid free bovine serum albumin.Free fatty acids were measured with a HR series NEFA kit (Wako, Richmond, VA).

SUPPLEMENTARY DATA

Histochemistry

Slides were prepared for immunohistochemistry by incubation through a xylene alcohol series to deparaffinize and hydrate samples. Antigen retrieval was performed in sodium citrate (10 mM pH 6.5) at 92°C for 12-minutes, followed by gradual cooling to 50°C in a water bath and then cooling to room temperature. Endogenous peroxidase activity was quenched by incubation of slides in 3% H_2O_2 in PBS. Slides were blocked in 2.5% normal horse serum followed by a Streptavidin/Biotin block (#SP-2002, Vector Laboratories, Burlingame, CA). Uncoupling Protein 1 (UCP1) was detected using anti-UCP1 antibody (#ab10983, Abcam, Cambridge.MA) 1:200 in 2.5% normal horse serum at 4°C overnight. Sections were incubated with a goat anti-rabbit biotin-conjugated secondary antibody (1:1,000) (Jackson Immuno Research, West Grove, PA) in 1% Tyramide Signal Amplification (TSA) blocking buffer followed by the streptavidin-horseradish peroxidase (1:200) included as part of the TSA kit (Invitrogen, Carlsbad, CA). TSA-Alexa Fluor 594 (Invitrogen) was used to visualize antibody-binding. Sections were then washed and mounted with Vectashield fluorescent mounting media (Vector Laboratories). Immunocytochemistry on differentiated 3T3-L1 cells was performed as follows. After the 4-hour treatment with histamine, the cells were fixed in the 6-well dishes with 2% paraformaldehyde for 1 hour before staining. UCP1 was then detected using the methods described above.

SUPPLEMENTARY DATA

Supplementary Table S1. Primers for real-time PCR

Gene[1]	Forward	Reverse
RBM3	GAGGGCTCAACTTTAACACCG	GACCACCTCAGAGATAGGTCC
PGC1α	TCTGAGTCTGTATGGAGTGACAT	CCAAGTCGTTCACATCTAGTTCA
UCP1	AGGTCCAAGGTGAATGCCC	TTACCACAGCGGTGATTGTTC
UCP2	GGAGGTGGTCGGAGATACCAA	ACAATGGCATTACGAGCAACAT
UCP3	AAGGTCCGATTTCAGGCCAG	GCGATGGTTCTGTAGGCGTC
MCP1	TCGCCTCCAGCATGAAAGTC	AGGTGACTGGGGCATTGATTG
IL1β	TTCGACACATGGGATAACGAGG	TTTTTGCTGTGAGTCCCGGAG
IL12β	TGCCCATTGAGGTCATGGTG	CTTGGGTGGGTCAGGTTTGA
CD206	CTACAAGGGATCGGGTTTATGGA	TTGGCATTGCCTAGTAGCGTA
IL10	GACTTTAAGGGTTACCTGGGTTG	TCACATGCGCCTTGATGTCTG
CCL1	CTCATTTGCGGAGCAAGAGAT	GCCTCTGAACCCATCCAACTG
CD68	CTTCTCTCATTCCCCTATGGACA	GAAGGACACATTGTACTCCACC
LEP	TGCCTTCCAGAAACGTGATCC	CTCTGTGGAGTAGCCTGAAGC
Foxp3	GTGGCCCGGATGTGAGAAG	GGAGCCCTTGTCGGATGATG
FNDC5	TGGAGGAGGATACGGAGTACA	CCACATGAACAGGACCACGA
PRDM16	AGAGCAGCTGAGGAAGCATTT	TTCGGAAAGGGACAGCATCA
TPSB2	GTGAAGGTCCCCATAATGGAAAA	CACAGCATGTCGTCACGGA
ADIPOQ	AACATGCCCATTCGCTTTACC	TAGGCAAAGTAGTACAGCCCA
FCER1A	CTCCATTACAAATGCCACAGTTG	TCACGCGGAGCTTTTATTACAG
HIF1α	ATCCATGTGACCATGAGGAAATG	TCGGCTAGTTAGGGTACACTTC
METRNL	GGTGCAGTGTTTTGGCCTG	AGCTCGTACTGGAAGCCTGT
IL4	TCCGATTCCTGAAACGGCTC	TGGTTGGCTTCCTTCACAGG
IRF4	GCTGATCGACCAGATCGACAG	CGGTTGTAGTCCTGCTTGC
TNFα	GAGGCCAAGCCCTGGTATG	CGGGCCGATTGATCTCAGC
IL6	CCTGAACCTTCCAAAGATGGC	TTCACCAGGCAAGTCTCCTCA
AMPK	TTTGCGTGTACGAAGGAAGAAT	CTCTGTGGAGTAGCAGTCCCT
CIDEA	ATTGATGTGGCCCGTGTAACG	CAGCAGTGCAGATCATAGGAAA
CPT1A	TCCAGTTGGCTTATCGTGGTG	TCCAGAGTCCGATTGATTTTTGC
CPT1B	CCTGCTACATGGCAACTGCTA	AGAGGTGCCCAATGATGGGA
DIO2	GGCTGACCGCATGGACAATAA	GCTACCCCGTAAGCTATGTTG
TBX15	TTTCAGCTTGGCTAGGACCG	TGCTCTGCTCAGAATCTCTTCC
TMEM26	ATGGAGGGACTGGTCTTCCTT	CTTCACCTCGGTCACTCGC
CPA3	GGGTTTGATTGCTACCACTCTT	GCCAAGTCCTTTATGATGTCTGC

[1] Primer sequences for real time RT-PCR.

SUPPLEMENTARY DATA

Supplementary Table S2. Primers for real-time PCR

Gene[1]	Forward	Reverse
HRH1	AGATGTGTGAGGGCAACAAGA	CAAGCAGATAGTGCTCAGGAC
HRH2	CAGTTCGGGTCGCCATCTC	CTGGTCTCGTTCCTGCTGTTC
HRH3	CCCATACACGCTGCTGATGAT	GGAGGTTTCGTACCAGTAGTCA
HRH4	ATGCTAGGAAATGCTTTGGTCA	AGGAATGGAGATCACACCCAC
mHRH1	CAGACCTGATTGTAGGGGCAG	CATAGAGAGCCAAAAGAGGCAG
mHRH2	AACGAGGTATATGGACTGGTGG	GCTGATGTGGTTGATCCTCTTG
mHRH3	CAAGACGGGCTGTTCGGAA	CGGACAGGTACTCCCAACTCA
mHRH4	CCTCGTGGGTTTGATTTCCAT	AGTAATGAGCCAAAACATGCAGA
mCPA3	TCAGACCATCCAGTCAACCTT	CCTGCCTGCGATTTCATCTTT
mIL4	GGTCTCAACCCCCAGCTAGT	GCCGATGATCTCTCTCAAGTGAT
mPGC1α	TATGGAGTGACATAGAGTGTGCT	CCACTTCAATCCACCCAGAAAG
mTPSB2	CCCAACAAGCCTGGCATCTA	GTTCTTGCCTGACCCTGGAT
mUCP1	CAAAAACAGAAGGATTGCCGAAA	TCTTGGACTGAGTCGTAGAGG
mTNFα	CCCTCACACTCAGATCATCTTCT	GCTACGACGTGGGCTACAG
mRBM3	CTTCGTAGGAGGGCTCAACTT	CTCCCGGTCCTTGACAACAAC

[1] Primer sequences for real time RT-PCR. Mouse genes are indicated by m; all other genes are human.

SUPPLEMENTARY DATA

Supplementary Figure S1. Histamine receptor expression in adipose tissue and 3T3-L1 adipocytes. A-C) Histamine receptor mRNA expression was measured in abdominal subcutaneous adipose tissue in the summer (n=28) and winter (n=27); *:P<0.05; **P=0.01. D-G) Histamine receptor expression was measured in differentiated 3T3-L1 adipocytes treated for 4-hours at 37°C (control) or 30°C (cold) and then treated for 4-hours with 10 nM histamine or 1 nM mouse IL4 as indicated. The data were analyzed by two-way ANOVA, and the P-values for interaction, cold, and treatment are indicated.