iScience, Volume *23*

Supplemental Information

Differential Expression of Ormdl Genes in the Islets of Mice and Humans with Obesity

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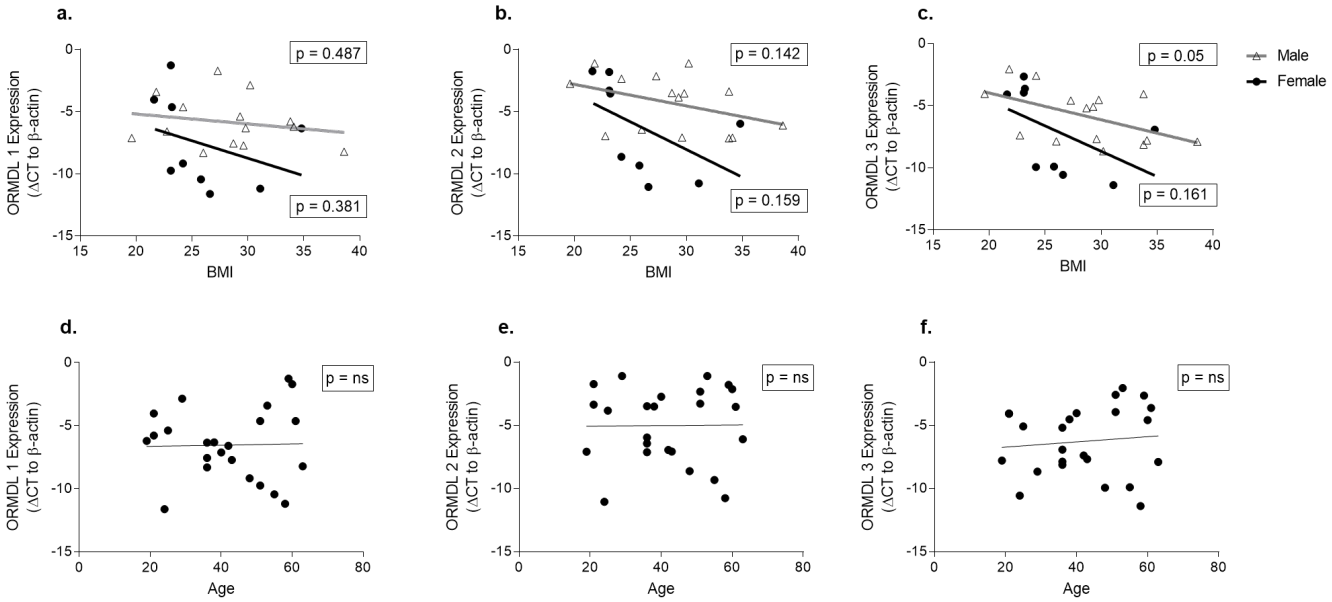

Figure S1 (Related to Figure 1). Human islet ORMDL expression is correlated with BMI and not with donor age.

(A-C) Scatter plots for (A) ORMDL1, (B) ORMDL2, (C) ORMDL3 expression vs. BMI for all donors.

(D-F) Scatter plots for (D) ORMDL1, (E) ORMDL2, (F) ORMDL3 expression vs. age for all donors. Ns: not statistically significant.

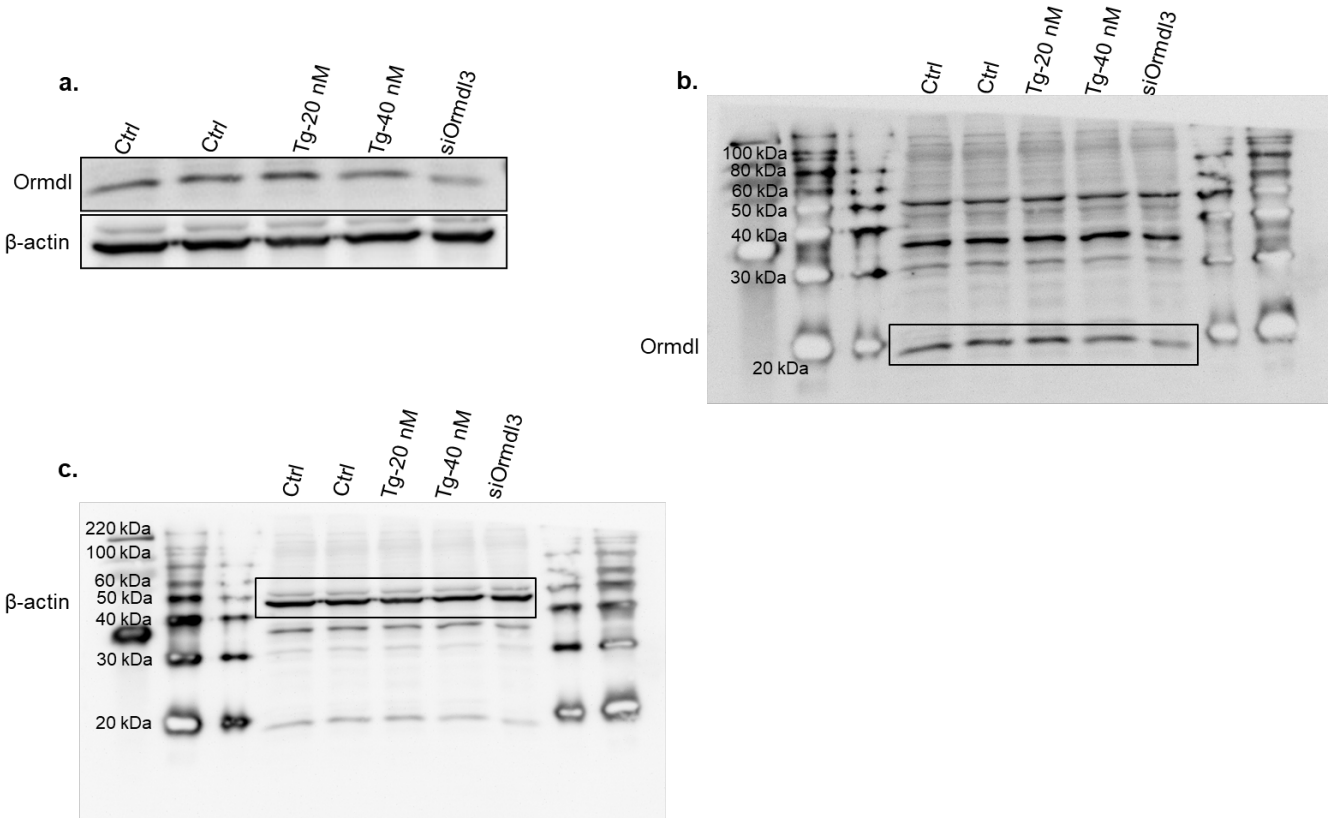

Figure S2 (Related to Figure 2). Ormdl antibody specificity and expression in lean and obese mouse islets.

(A-C). The specificity of a pan-Ormdl antibody was validated using the rat insulinoma cell line, INS-1 832/3, after transfection with siRNA against Ormdl3 (siOrmdl3) or scrambled control, as well as under stressed (thapsigargin treatment: Tg) or non-stressed conditions.

Transparent Methods

Mice

9-week-old male C57BL/6J and (B6.Cg-*Lep*^{ob}/J) mice were purchased from the Jackson Laboratory and were housed under standard conditions, under a 12:12-hr light/dark cycle, with unrestricted access to food and drinking water in an animal housing facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care. This study was carried out in accordance with the recommendations of the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The protocol (#M005064-R01-A03 by F.E. for mice) was approved by the University of Wisconsin-Madison Institutional Animal Care and Use Committee.

In vivo leptin administration

Male mice purchased from Jackson Laboratories at 9 weeks of age, acclimated for 1 week and then randomized into either vehicle or leptin treatment groups. Prior and post treatment 6-hour fasting blood glucose was measured using a Breeze2 glucometer (Bayer). At 10 weeks of age, recombinant murine leptin (PeproTech, Rocky Hill, NJ, USA) was reconstituted according to manufacturer's instructions and i.p. injected once daily to mice in the leptin group at a concentration of 4.5 µg/g body weight for 4 days, while mice in the vehicle group received filter-sterilized water. The body weight of vehicle and leptin-treated ob/ob mice as well as was control age, sex matched C57BL/6J mice measured daily.

Ex vivo leptin treatment

Primary islets were isolated from 10-week-old (*ob/ob*) male mice. After an overnight culture, 50 islets/animal were transferred into sterile non-adherent 60 mm petri dishes, in duplicates for each condition, containing 5 mL RPMI 1640 medium supplemented with 0.1% bovine serum albumin (BSA) in the presence of 100 nM murine leptin (PeproTech, Rocky Hill, NJ, USA) or an equal volume of vehicle control (0.1% BSA in water). Islets were incubated for 16 h at 37°C prior to RNA extraction and qPCR.

Cell culture

INS-1 832/3 cells were cultured in RPMI 1640 supplemented with penicillin, streptomycin, 2 mM glutamine, 10mM HEPES, 1mM sodium pyruvate, 50 µM β-ME, and 10% FBS. The cells were maintained at 37ºC in 5% CO_2 atmosphere and treated with 10 nM - 1 µM thapsigargin (Sigma-Aldrich). For gene-specific, siRNA-mediated knockdowns, 1×10^6 cells/well were used to perform reverse transfections. Transient transfections were carried out with 100 nM siRNA oligonucleotide pools (Sigma-Aldrich) using HiPerFect transfection reagent (Qiagen) per manufacturer's recommendations. Cells were harvested 24-48 hours after the start of transfections depending on the subsequent experiment. The knockdown experiments were repeated more than three times.

RNA extraction and qPCR analysis

Total RNA was extracted from *ob/ob* mouse islets, MIN6 and INS-1 832/3 cells using TRIzol reagent (Invitrogen) according to manufacturer's instructions. cDNAs were synthesized from extracted RNA by using Superscript III First Strand RT-PCR kit (Invitrogen). Real-time quantitative PCR amplifications were performed on CFX96 Touch Real-time PCR detection system (Bio-Rad). β-actin, Hprt, 18s, and Gapdh genes were used as internal controls for the quantity of the cDNAs in real time PCR assays. Primer specific for mouse: m*Ormdl1:* F: ACA GTG AGG TAA ACC CCA ATA CT, R: GCA AAA ACA CAT ACA TCC CCA GA; m*Ormdl2:* F: CAC AGC GAA GTA AAC CCC AAC, R: AGG GTC CAG ACA ACA GGA ATG: m*Ormdl3:* F: CCA ACC TTA TCC ACA ACC TGG, R: GAC CCC GTA GTC CAT CTG C. m*18s*: F: AGT CCC TGC CCT TTG TAC ACA, R: CGA TCC GAG GGC CTC ACT A. m*β-actin*: F: TCT TGG GTA TGG AAT CCT GTG GCA, R: TCT CCT TCT GCA TCC TGT CAG CAA. m*Gapdh*: F: TGT GTC CGT CGT GGA TCT GA, R: CCT GCT TCA CCA CCT TCT TGA T. Primers specific for rat: r*Ormdl1* F: CCC AAT ACT CGT GTA ATG AAT AGC, R: GGG ATG TG AGA AAT ACA ATG TG; r*Ormdl2:* F: GAT GGA CTA CGG ACT ACA GTT TAC, R: AGT GAG GCA GTG TTG ATG AG; r*Ormdl3:* F: TTG ACC ATC ACG CCC ATT, R: AGC ACA CTC ATC AAG GAC AC; r*sXbp1*: F: CTG AGT CCG AAT CAG GTG CAG, R: ATC CAT GGG AAG ATG TTC TGG; r*Grp78*: F: TGG GTA CAT TTG ATC TGA CTG GA, R: CTC AAA GGT GAC TTC AAT CTG GG; r*Chop*: F: CCA GCA GAG GTC ACA AGC AC, R: CGC ACT GAC CAC TCT GTT TC; r*Atf6*: F: TCG CCT TTT AGT CCG GTT CTT, R: GGC TCC ATA TGT CTG ACT CC. r*Gapdh*: F: AGT TCA ACG GCA CAG TCA AG, R: TAC TCA GCA CCA GCA TCA CC.

Human islet RNA was extracted using the Qiagen RNeasy Kit (Qiagen; #74106) according to manufacturer's instructions. cDNA was generated with random hexamers (High-Capacity cDNA Reverse Transcription Kit, Applied Biosystems; #4368813). qPCR was performed using SYBR green (Roche; #04913914001). Primer specific for humans: h*ORMDL1:* F: TGA CCA GGG TAA AGC AAG GC, R: CCG AAC ACC ATG TAG TTG TGG; h*ORMDL2:* F: GTG GCA CAC AGC GAA GTA AAC, R: TGC AGC AAT CCT ACC AAG ATG; h*ORMDL3*: F: GAG GCT GCT AAC CCA CTG G, R: GGT GAG GAA GTA CAG CAC GAT. All human islet cycle thresholds were normalized to β-actin.

Donor human islets

Human islets were obtained from the Integrated Islet Distribution Program (IIDP) according to an approved IRB exemption protocol stating this work is not human subjects research (UW 2012–0865). Islets were cultured in RPMI 1640 with 8 mM glucose for 24 hours before being pelleted for RNA.

Islet isolation

Islets were isolated from *ob/ob* mice using the standard collagenase/protease digestion method. Briefly, the pancreatic duct was cannulated and distended with ice-cold collagenase/protease solution using 0.5 mg/mL Collagenase P (Sigma-Aldrich, USA) in 1x Hank's balanced salt solution and 0.02% BSA. The pancreas was digested for a total of 20 minutes, with vigorous shaking every 2 minutes after 10 minutes have passed. The protease reaction was stopped using RPMI 1640 with 10% FBS. Islets were separated from the exocrine tissue using Histopaque-1077 (Sigma-Aldrich, USA) and centrifuged at 1800 g for 20 minutes. Hand-picked islets were cultured overnight at 37°C in RPMI 1640 media containing 10% FBS and 1% antibiotic/antimycotic (Thermo Fisher Scientific) before use in experiments (Truchan et al., 2015).

Western blot

Cells or islets were lysed in RIPA buffer (50 mM Tris, pH 7.4, 150 mM, NaCl, 5 mM EDTA, pH 8.0, 30 mM NaF, 1 mM Na3VO4, 40 mM β-glycerophosphate, 0.1 mM PMSF, protease inhibitors, 10% glycerol and 1% Nonidet-P40). The concentration of the isolated proteins was determined using BCA Protein Assay Reagent (Pierce, Rockford, IL). 30-45 μg of the protein was separated on a 5-12% Tris-acetate gel and electrophoretically transferred to PVDF membranes (Millipore, Billerica, MA). Membranes were then incubated with the primary antibodies against sXbp1 (Santa Cruz Biotechnology #sc-7160), Chop (Santa Cruz Biotechnology #sc-575), Grp78 (Cell Signaling Technology #3183) cleaved-Caspase-3 (Cell Signaling Technology, #9661), cleaved-Parp (Cell Signaling Technology, #9545), Ormdl (TPF, gift of Dr. Petr Draber, Academy of Sciences of the Czech Republic), β-actin (Cell Signaling Technology) and the appropriate secondary antibodies.

Statistical analysis

For all experiments the number of biological or technical replicates (*n*), error bars, and statistical analyses have been explained in the figure legends. For each experiment where statistics were computed, we used at least $n = 3$ or more biological or technical replicates. Sample sizes were not pre-determined by power analysis, but sufficiency of number of mice were estimated based on pilot experiments and previously published work (Engin et al., 2014). Data analysis was performed using GraphPad Prism v.8 (GraphPad Software, San Diego, CA). Following Shapiro-Wilks normality testing, data were analyzed by Student's *t*-test, unless otherwise stated. $p < 0.05$ was considered statistically significant. Data are represented as mean ± SEM.

Supplemental References

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