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

HIGHLIGHTS
- Islets of overweight/obese human donors display markedly reduced ORMDL3 expression.
- Ormdl3 expression was significantly upregulated in the islets of ob/ob mice.
- Leptin treatment markedly reduced Orml3 expression in the islets of ob/ob mice.
- Fumonisin B1 restores increased apoptotic marker levels induced by Orml3 silencing.
Differential Expression of Ormdl Genes in the Islets of Mice and Humans with Obesity

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SUMMARY

The orosomucoid-like (Ormdl) proteins play a critical role in sphingolipid homeostasis, inflammation, and ER stress, all of which are associated with obesity and β cell dysfunction. However, their roles in β cells and obesity remain unknown. Here, we show that islets from overweight/obese human donors displayed marginally reduced ORMDL1-2 expression, whereas ORMDL3 expression was significantly downregulated compared with islets from lean donors. In contrast, Ormdl3 was substantially upregulated in the islets of leptin-deficient obese (ob/ob) mice compared with lean mice. Treatment of ob/ob mice and their islets with leptin markedly reduced islet Ormdl3 expression. Ormdl3 knockdown in a β cell line induced expression of pro-apoptotic markers, which was rescued by ceramide synthase inhibitor fumonisin B1. Our results reveal differential expression of Ormdl3 in the islets of a mouse model and humans with obesity, highlight the potential effect of leptin in this differential regulation, and suggest a role for Ormdl3 in β cell apoptosis.

INTRODUCTION

Insulin resistance, often co-incident with obesity, dampens the brake on lipolysis, elevating plasma free fatty acid levels. Free fatty acids taken up from the plasma are the precursors for various species of intracellular lipids. Certain sphingolipids, most notably ceramide, accumulate within insulin-resistant tissues of animals (Holland and Summers, 2008; Summers, 2006) and humans (Adams et al., 2004; Straczekowski et al., 2007), including the pancreatic β cells (DeFronzo, 2004). There, they inhibit insulin action and activate processes including apoptosis, inflammation, and stress responses—a condition known as lipotoxicity (Ertunc and Hotamisligil, 2010; Cinar et al., 2014; Longato et al., 2012). Yet, despite recent progress in the field, the molecular mechanisms of sphingolipid-mediated disease pathology and the pathways generating these pathogenic lipids remain poorly understood.

The members of the orosomucoids (Orm) gene family encode transmembrane proteins localized in the endoplasmic reticulum (ER). In the budding yeast S. cerevisiae, twoOrm proteins, Orm1 and Orm2 (Han et al., 2010), have been identified as negative regulators of SPT (Breslow et al., 2010; Han et al., 2019). Orm proteins form a complex with SPT and inhibits its activity (Breslow et al., 2010). This association with SPT is regulated by Orm protein phosphorylation: an important factor for sphingolipid homeostasis (Breslow et al., 2010). Mammals, on the other hand, have three Orm-like proteins (Orm1–3) (Hjelmqvist et al., 2002). In vitro studies suggest that mammalian OrmD3 alters ER-mediated calcium (Ca²⁺) homeostasis, facilitates the unfolded protein response (UPR), induces cellular stress responses, and plays a possible role in inflammation (Cano-Rosales et al., 2010; Carreras-Sureda et al., 2013; Hsu and Turvey, 2013; Miller et al., 2012). In human genome-wide association studies (GWAS), OrmD3 is strongly associated with inflammatory diseases, including asthma, Crohn’s disease, and type 1 diabetes (T1D) (Barrett et al., 2010).
In this study, we analyzed the expression of Ormdl genes in a genetic mouse model of obesity and type 2 diabetes prior to the onset of hyperglycemia and in human pancreatic islets isolated from lean and overweight/obese non-diabetic donors. Our results, for the first time, revealed that, although ORMDL3 expression in pancreatic islets was negatively correlated with BMI in humans, leptin-deficient obese mice displayed significant upregulation of Ormdl3 expression in their islets. Administration of leptin to leptin-deficient obese mice (ob/ob) and treatment of ob/ob islets ex vivo with leptin markedly reduced Ormdl3 expression, highlighting that leptin can potentially regulate Ormdl3 expression and providing an explanation for differential expression of this gene in ob/ob mouse model and human islets in the context of obesity. Finally, we demonstrated that knockdown of Ormdl3 causes substantial upregulation of pro-apoptotic markers in a β cell line, which could be rescued by pharmacological inhibition of ceramide synthase.

RESULT

ORMDL3 Expression Is Significantly Downregulated in the Islets of Overweight/Obese Female Donors

To identify pancreatic islet ORMDL expression in the context of obesity, we used pancreatic islets isolated from lean and overweight/obese human organ donors. We grouped donors as lean (BMI<25) and overweight/obese (BMI>25) (Table 1). All ORMDL genes showed a trend toward diminished mRNA expression in islets isolated from overweight/obese humans as compared with lean (as quantified by cycle threshold compared with β-actin), with the cycles necessary to amplify ORMDL3 PCR product being significantly reduced (approximately 3.5 cycles, or 11-fold) (Figures 1A–1C). We next examined the relationship between islet ORMDL expression and donor sex. Interestingly, the cycle threshold necessary to amplify ORMDL2 and ORMDL3 expression was significantly reduced (by approximately 5–5.5 cycles) in islets from overweight/obese female donors only, corresponding with a 32- to 45-fold decrease in mRNA expression with obesity (Figures 1D–1F). ORMDL1 expression level was non-significantly decreased in islets from female donors as a factor of overweight/obesity. Although no significant changes in the expression of any ORMDL family member were observed in islets isolated from male donors as a factor of overweight/obesity, the mean ORMDL3 cycle threshold in islets from overweight/obese male donors was reduced as compared with lean (Figures 1D–1F). Correlation analyses between the ORMDL genes with BMI further show the greater decrease in ORMDL expression in female donors with increasing BMI as compared with male donors (Figures S1A–S1C). As noted above, there was also a substantial trend toward a decrease in ORMDL3 expression in islets from male donors as a function of BMI (p = 0.05) (Figure S1C). To rule out a potential confounder in our human islet analyses, we examined the correlation between ORMDL expression and donor age but did not detect any significant correlation (Figures S1D–S1F).

Ormdl3 Expression Is Significantly Upregulated in the Islets of Leptin-Deficient Obese (ob/ob) Mice

In rodent models of obesity and type 2 diabetes, increased islet ceramide and triglyceride production precede β cell dysfunction and demise (Lee et al., 1994; Unger, 2002). Since Ormdl genes were identified as negative regulators of sphingolipid biosynthesis (Davis et al., 2019; Siow et al., 2015), we asked whether the expression of these genes was altered in the pancreatic islets of leptin-deficient obese (ob/ob) mice, a model of severe insulin resistance and lipotoxicity. First, we analyzed the expression of Ormdl genes in the islets of lean and ob/ob male mice at 10 weeks of age, when ob/ob mice were still normoglycemic. Quantitative PCR analysis showed that expression of Ormdl1 and Ormdl2 was not significantly increased, whereas Ormdl3 expression was substantially upregulated in islets from male ob/ob mice (Figures 2A–2C). Next, we assessed the expression of the Ormdl genes in islets harvested from 10-week-old female lean and ob/ob mice. The expression levels of Ormdl1 and Ormdl2 were nearly identical between islets isolated from female lean and ob/ob mice, whereas the expression level of Ormdl3 was significantly increased in female ob/ob mice, similar to that of male ob/ob mice (Figures 2D–2F).
Next, we investigated the expression of Ormdls at the protein level. Ormdl1, -2, and -3 share greater than 80% sequence homology. Currently, there are no commercially available antibodies that can detect specific expression of the individual Ormdl family members. In addition, three commercially available pan-Ormdl antibodies failed validation using knockdown lysates (data not shown). We obtained a TPF-Ormdl antibody from Dr. Petr Draber’s group (Bugajev et al., 2016), and although the antibody had significant non-specific cross-reactivity, transfection with an Ormdl3 siRNA resulted in a substantial decrease in the abundance of a protein band at the expected molecular weight for Ormdl (17.5 kDa), whereas it did not affect any other “non-specific” bands, confirming the validity of this antibody for further analyses (Figures S2A–S2C). Using this antibody, we demonstrated that Ormdl protein levels were also significantly upregulated in islets from male ob/ob mice, consistent with the changes in mRNA expression (Figure 2G).

Table 1. Description of Human Islet Donors
Demographic and anthropometric data for each human islet donor is provided.

| BMI Group | Donor | Age | Mean Age ± SEM | Sex | BMI | Mean BMI ± SEM | Ethnicity |
|-----------|-------|-----|----------------|-----|-----|----------------|-----------|
| <25       | 1     | 40  | 47.3 ± 4.01    | M   | 19.6| 22.62 ± 0.48  | Black     |
|           | 2     | 21  |                | F   | 21.6|                | White     |
|           | 3     | 53  |                | M   | 21.8|                | White     |
|           | 4     | 42  |                | M   | 22.8|                | Black     |
|           | 5     | 59  |                | F   | 23.1|                | White     |
|           | 6     | 51  |                | F   | 23.1|                | White     |
|           | 7     | 61  |                | F   | 23.2|                | White     |
|           | 8     | 48  |                | F   | 24.2|                | White     |
|           | 9     | 51  |                | M   | 24.2|                | Hispanic/Latino |
| >25       | 1     | 55  | 38.6 ± 3.74    | F   | 25.8| 30.63 ± 0.96  | White     |
|           | 2     | 36  |                | M   | 26.0|                | White     |
|           | 3     | 24  |                | F   | 26.6|                | Hispanic/Latino |
|           | 4     | 60  |                | M   | 27.3|                | Asian Indian |
|           | 5     | 36  |                | M   | 28.7|                | White     |
|           | 6     | 25  |                | M   | 29.3|                | Hispanic/Latino |
|           | 7     | 43  |                | M   | 29.6|                | White     |
|           | 8     | 38  |                | M   | 29.8|                | Black     |
|           | 9     | 29  |                | M   | 30.2|                | Asian Indian |
|           | 10    | 58  |                | F   | 31.1|                | White     |
|           | 11    | 36  |                | M   | 33.8|                | White     |
|           | 12    | 21  |                | M   | 33.8|                | White     |
|           | 13    | 19  |                | M   | 34.1|                | White     |
|           | 14    | 36  |                | F   | 34.8|                | Hispanic/Latino |
|           | 15    | 63  |                | M   | 38.6|                | White     |

Leptin Administration Markedly Reduces Ormdl3 Expression in ob/ob Islets
Our data revealed that the expression of Ormdl3 had the opposite correlation to overweight/obesity in human islets compared with the mouse model. One possible explanation for the disparate results could be the difference in serum leptin levels in these models, such that obesity in humans is associated with increased circulating leptin (Al Maskari and Alnaqdy, 2006; Lonnqvist et al., 1997; Maffei et al., 1995),
whereas \(ob/ob\) mice are leptin deficient (Moon and Friedman, 1997). To test whether leptin can regulate \(\text{Orm}d3\) expression, we treated 10-week-old male normoglycemic \(\text{ob/ob}\) mice with recombinant leptin for 4 days. Our qPCR results revealed that the expression level of housekeeping gene \(\beta\)-actin was altered with leptin treatment; thus, we supplemented our analysis with two other housekeeping genes, \(\text{Gapdh}\) and \(18s\), and employed the geometric mean of these three housekeeping genes for the following qPCR analyses (Vandesompele et al., 2002). Interestingly, the expression levels of \(\text{Ormd}1\) and \(\text{Ormd}2\) did not change upon leptin treatment, whereas the expression level of \(\text{Ormd}3\) was significantly reduced in islets from \(\text{ob/ob}\) mice treated with leptin (Figures 3A–3C). The treatment of C57BL/6J lean mice with leptin did not alter \(\text{Ormd}3\) expression in islets (Figure 3D). Mean blood glucose levels in lean and \(\text{ob/ob}\) mice did not significantly change upon leptin treatment, but an approximate 10% reduction in body weight was observed in \(\text{ob/ob}\) mice, as previously reported (Harris et al., 1998; Pelleymounter et al., 1995) (Figures 3E and 3F). To demonstrate that the decrease in \(\text{Ormd}3\) expression is leptin-dependent and not a result of the change in body weight, we isolated islets from 10-week-old \(\text{ob/ob}\) mice and treated them with leptin ex vivo. Consistent with our in vivo results, expression of \(\text{Ormd}3\) was significantly decreased in isolated islets upon leptin treatment (Figure 3G). Taken together, these results suggest that leptin can play a key role in \(\text{Ormd}3\) transcriptional regulation in pancreatic islets.

Knockdown of \(\text{Ormd}3\) Leads to Significant Upregulation of Apoptotic Markers in a \(\beta\) Cell Line

To investigate the physiological function of \(\text{Ormd}\) genes in \(\beta\) cells, we knocked down all three members of the \(\text{Ormd}\) family in the INS-1-derived 832/3 rat insulinoma cell line using siRNAs specific to each gene product. A knockdown efficiency of 80%–85% was confirmed by qPCR for each of the \(\text{Ormd}\) genes (Figures 4A–4C). We assessed the expression levels of apoptotic markers in cells 48 h after knockdown of \(\text{Ormd}\)s by western blotting. The levels of pro-apoptotic markers cleaved Caspase-3 and cleaved Parp were markedly increased in \(\text{Ormd}2\)- and \(\text{Ormd}3\)-deficient cells, whereas these markers were only marginally increased in \(\text{Ormd}1\)-deficient cells (Figures 4D–4F), suggesting that \(\text{Ormd}2\) and \(\text{Ormd}3\) can play a role in regulation of apoptosis in \(\beta\) cells under physiological conditions.

\(\text{Ormd}3\) Silencing Does Not Alter the Expression of the UPR Markers in \(\beta\) Cells

\(\text{Ormd}3\) can regulate ER calcium homeostasis via inhibition of ER calcium pump \(\text{Serc}2b\) (Cantero-Recasens et al., 2010) and modulate the UPR (Cantero-Recasens et al., 2010; McGovern et al., 2010). Moreover, knockdown of \(\text{ORMDL3}\) in HEK293T cells was shown to induce a higher UPR following chemical ER stressors,
indicating that ORMDL3 expression levels can regulate UPR and that ORMDL3 may play a role to ensure ER and cellular homeostasis (McGovern et al., 2010). Thus, we investigated whether altering Ormdl3 expression levels can affect ER stress and/or the UPR in a β cell line. Interestingly, we did not observe any significant changes in the mRNA (Figures 5A–5D) or protein levels (Figure 5E) of the UPR markers sXbp1, Grp78, Chop, or Atf6 in INS-1 832/3 cells transfected with siOrmdl3 alone or in the presence of ER stressor thapsigargin, suggesting that Ormdl3 deficiency does not trigger the UPR or ER stress-mediated apoptosis in INS-1 832/3 cells.

Induction of Pro-apoptotic Pathways by Ormdl3 Knockdown Can Be Rescued by a Ceramide Synthase Inhibitor

Ormdl proteins suppress sphingolipid biosynthesis and specific classes of sphingolipids, namely, ceramides, are known to be important mediators of β cell dysfunction and apoptosis (Boslem et al., 2012; Veret et al., 2014). Thus, we hypothesized that Ormdl3 deficiency might increase ceramide production and subsequently lead to β cell apoptosis. If this is the case, blocking ceramide synthesis downstream of sphingolipid synthesis by using a pharmacological inhibitor of ceramide synthase, fumonisin B1, should reduce the expression of apoptotic markers. To test this hypothesis, we treated Ormdl3-deficient INS-1 832/3 cells with fumonisin B1 and measured the protein levels of apoptotic markers by western blotting. Consistent with our hypothesis, expression levels of apoptotic marker cleaved Caspase-3 was markedly reduced upon inhibition of ceramide synthesis in INS-1 832/3 cells (Figure 5F). Taken together, these data suggest that Ormdl3 can play a role in the regulation of apoptosis in β cells likely by affecting cellular ceramide homeostasis.

**DISCUSSION**

A growing body of evidence has suggested the genetic association of ORML3 gene polymorphisms with a diverse set of inflammatory disorders, including bronchial asthma, inflammatory bowel disease, ankylosing
spondylitis, T1D, atherosclerosis, and obesity. We have recently shown that aberrant β cell ER stress is linked to T1D pathogenesis and that there is abnormal β cell UPR activity in type 1 and type 2 diabetes animal models and human patients, suggesting that conserved cellular mechanisms can play a critical role in the pathology of both types of diabetes (Engin, 2016; Engin et al., 2013, 2014). Hence, owing to the involvement of Ormdls with inflammatory diseases, regulation of sphingolipid biosynthesis, and ER stress, we hypothesized that Ormdls could play an important role in β cell homeostasis and investigated the regulation of these genes in pancreatic islets in the context of obesity. One of the most intriguing findings of our study was that islet ORMDL3 expression was significantly influenced by obesity in both mouse and human samples, albeit in opposing directions. ORMDL3 mRNA expression was significantly reduced in islets isolated from overweight/obese human female organ donors, whereas Ormdl3 expression was actually increased in islets from both female and male ob/ob mice. We reasoned that these contrasting results might be due to leptin as obese humans have significantly increased levels of circulating leptin (Al Maskari and Alnaqdy, 2006; Lonnqvist et al., 1997; Maffei et al., 1995), whereas ob/ob mice are deficient in this adipokine (Moon and Friedman, 1997). Indeed, administration of leptin to male ob/ob mice for only 4 days significantly reduced Ormdl3 expression in islets, indicating that leptin may have a regulatory role in Ormdl3 expression. We further supported this finding with an ex vivo experiment, in which leptin treatment of islets from ob/ob mice resulted in markedly diminished Ormdl3 expression. Interestingly, leptin action in the central nervous system represses SPT expression in white adipose tissue by 30% (Bonzon-Kulichenko et al., 2009) and decreases mRNA expression of enzymes involved in de novo ceramide synthesis (SPT-1, LASS2, LASS4) and ceramide production from sphingomyelin (SMPD-1/2) (Bonzon-Kulichenko et al., 2009). Leptin receptor overexpression in the islets of obese Zucker diabetic fatty (ZDF) rats with mutant leptin receptors leads to significantly reduced SPT mRNA levels and fat content (Shimabukuro et al., 1998; Unger and Roth, 2015). However, whether such regulation also exists in human islets is not yet known. Since ob/ob mice have elevated circulating free fatty acids and increased ceramide in their islets (Sloan et al., 2011), it is possible that Ormdl3 levels in ob/ob mice in the absence of leptin is upregulated to exert compensatory inhibitory effects on sphingolipid synthesis, whereas in obese, hyperleptinemic human subjects, downregulation of ORMDL3 may lead to increased ceramide synthesis and lipotoxicity in islets. Of note, although leptin resistance in the hypothalamus is well established in obesity and diabetes, such resistance has not been definitively demonstrated in pancreatic β cells. Leptin levels are also higher in women than in men using any given measure of obesity (Kennedy et al., 1997), a finding that may provide insight into more pronounced downregulation of
ORMD2 and ORMDL3 expression in the islets of female donors compared with those of male islets. Indeed, lack of leptin in male and female ob/ob mice might have abolished this variation, leading to no apparent differences in the expression of Ormdl3 in the islets of male and female mice.

A role for Ormdl3 in calcium homeostasis and the UPR has been reported in various cell types (Cantero-Reccasens et al., 2010; Carreras-Sureda et al., 2013; Miller et al., 2012), although no such effect of Ormdl3 on the UPR was also reported (Hsu and Turvey, 2013). We demonstrated that silencing Ormdl3 in INS-1 832/3 cells neither caused a significant increase in the expression of UPR markers nor potentiated the effects of a chemical ER stressor, suggesting that changing Ormdl3 expression levels do not affect the UPR in a rat β cell line. We then showed that, even in the absence of additional stressors, Ormdl3 deficiency led to upregulation of apoptotic markers in INS-1 832/3 cells, which could be significantly reduced by administration of a pharmacological inhibitor of ceramide synthesis. Although we did not measure the ceramide levels in these cells, a recent report indicated significantly increased levels of total sphingolipids, including ceramides, in liver and serum of Ormdl3/C0/C0 mice and a marked decrease in these lipid species in Ormdl3 transgenic mice (Debeuf et al., 2019), supporting the idea that increased ceramide levels in β cells is a response to reduced Ormdl3 levels. Of note, no metabolic phenotype has yet been described in these mouse models. Whether reduced expression of ORMDL3 contributes to β cell apoptosis in overweight/obese individuals with impaired fasting glucose remains to be elucidated (Butler et al., 2003). Interestingly, silencing Ormdl3 in a mouse β cell line, Min6 cells, did not induce apoptosis (Yang et al., 2019). The discrepancy between our results and the published work can result from utilization of mouse versus rat cell line or different oligos to perform the gene silencing in these cells. Generating tissue-specific genetic loss and gain-of-function models for Ormdl3 will be essential to understand the exact function and regulation of this gene in physiological and pathological settings.

Our findings do not clarify whether the functions of Ormdl proteins themselves were altered under the conditions of obesity and/or inflammation. In yeast, Orm proteins are regulated through phosphorylation (Roelants et al., 2011; Sun et al., 2012). Although Orm proteins are highly conserved in higher organisms, Ormdl proteins have a truncated N-terminal domain and lack the phosphorylation motif found in yeast, indicating divergent post-translational regulatory mechanisms (Paulenda and Draber, 2016). Emerging data indicate that Ormdl genes might be transcriptionally regulated, such that, when sphingolipid degradation is compromised by deletion of the lyase enzyme, low SPT activity parallels a considerable elevation in Ormdl1 and Ormdl3 transcription (Hagen-Euteneuer et al., 2012). In addition, ORMDL expression strongly influences SPT activity and de novo
ceramide synthesis in macrophages (Kiefer et al., 2015). The molecular mechanisms of regulation of Ormdl transcription and post-translational processing need further investigation.

To summarize, our data provide the first comprehensive analysis of ORMDL family gene expression in mouse and human islets in the context of obesity. We demonstrated that leptin can have a significant role in the regulation of islet expression of Ormdl3. Finally, our results indicate that loss of Ormdl3 leads to significantly increased expression of pro-apoptotic markers in a rat insulinoma cell line possibly owing to increased ceramide synthesis. The molecular mechanisms by which Ormdl proteins regulate beta cell homeostasis under physiological and pathological conditions, including obesity and diabetes, remain to be determined and are worthy of future study.

Limitations of the Study

Our study reveals for the first time the expression of the Ormdl gene family members in both mouse and humans; however, it does not address the protein levels in human samples mainly because of the limitations stemming from human samples and the antibody. We also recognize the limited number of donors as a potential limitation of our study. Although this study paves the way for more detailed mechanistic studies, mechanistic work with primary islets and genetic models with tissue-specific loss and gain-of-function models of Ormdl genes will be necessary to definitively demonstrate function and regulation of these genes in beta cell pathophysiology.

Resource Availability

Lead Contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Feyza Engin (fengin@wisc.edu).

MATERIALS AVAILABILITY

This study did not generate new unique reagents.
**Data and Code Availability**

This study did not generate or analyze any new datasets or code.

**METHODS**

All methods can be found in the accompanying Transparent Methods supplemental file.

**SUPPLEMENTAL INFORMATION**

Supplemental Information can be found online at https://doi.org/10.1016/j.isci.2020.101324.

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**AUTHOR CONTRIBUTIONS**

H.L. designed and performed experiments, analyzed data, prepared the figures, and revised the manuscript. R.J.F. contributed to gene expression analyses, analyzed data, and prepared the figures. T.A. and E.D. contributed to experiments. M.E.K. and D.B.D. analyzed the data, supervised research, and edited the revised manuscript. F.E. conceived, supervised, and supported the project, designed experiments, interpreted results, and wrote the manuscript.

**DECLARATION OF INTERESTS**

The authors declare no competing interests.

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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<sup>ob</sup>/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 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 37°C in 5% CO<sub>2</sub> atmosphere and treated with 10 nM - 1 µm thapsigargin (Sigma-Aldrich). For gene-specific, siRNA-mediated knockdowns, 1×10<sup>6</sup> 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: mOrmdl1: F: ACA GTG AGG TAA ACC CCA ATA CT, R: GCA AAA ACA CAT ACA TCC CCA GA; mOrmdl2: F: CAC AGC GAA GTA AAC CCC AAC, R: AGG GTC CAG ACA ACA GGA ATG; mOrmdl3: F: CCA ACC TTA TCC ACA ACC TGG, R: GAC CCC GTA GTC CAT CTG C. m18s: F: AGT CCC TGC CCT TTG TAC ACA, R: CGA TCC GAG GGC CTC ACT A. mβ-actin: F: TCT TGG GTA TGG AAT CCT GTG GCA, R: TCT CCT TCT GCA TCC TGC CAG CAA. mGapdh: F: TGT GTC GTC CTG GGA TGG TCT GA, R: CCT GCT TCA CCA CCT TCT TGA T. Primers specific for rat: rOrmdl1 F: CCC AAT ACT CGT GTA ATG AGC, R: GGG ATG TG AGA AAT ACA ATG TG; rOrmdl2: F: GAT GGA CTA CGG ACT ACA GTT TAC, R: AGT GAG GCA GTG TGG ATG AG; rOrmdl3: F: TTG ACC ATC ACG CCC ATT, R: AGC ACA TCT ATC AAG GAC AC; rsXbp1: F: CTG AGT CCG AAT CAG GTG CTA AGC, R: ATT CAT GGG AAG ATG TTC TGG; rGrp78: F: TGG GTA CAT GTG ATT CGT CTA GA, R: CTC AAA GGT GAC TTC AAT CTG GG; rChop: F: CCA GCA GAG GTG ACG ACA AC, R: CGC ACT GAC CAC TCT GTT TC; rAtf6: F: TCG CCT TTT AGT CCG CCT GTT, R: GCC TCC ATA TGT CTG ACT CC. rGapdh: 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: hORMDL1: F: TGA CCA GGG TAA AGC AAG GC, R: CCG AAC ACC ATG TAG TTG TGG; hORMDL2: F: GTG GCA CAC AGC GAA GTA AAC, R: TGC AGC AAT CCT ACC AAG ATG; hORMDL3: 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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