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REVIEW

Unravelling the story of protein misfolding in diabetes mellitus

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Abstract

Both environmental and genetic factors contribute to the development of diabetes mellitus and although monogenic disorders are rare, they offer unique insights into the fundamental biology underlying the disease. Mutations of the insulin gene or genes involved in the response to protein misfolding cause early onset diabetes. These have revealed an important role for endoplasmic reticulum stress in β-cell survival. This form of cellular stress occurs when secretory proteins fail to fold efficiently. Of all the professional secretory cells we possess, β-cells are the most sensitive to endoplasmic reticulum stress because of the large fluctuations in protein synthesis they face daily. Studies of endoplasmic reticulum stress signaling therefore offer the potential to identify new drug targets to treat diabetes.

Key words: Endoplasmic reticulum stress; Diabetes; Unfolded protein response; PKR-like ER kinase

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INTRODUCTION

We place a heavy burden on our pancreatic β-cells. They are expected to deliver a life-long supply of insulin at the precise levels necessary to maintain glucose homeostasis while avoiding hypoglycaemia. This requires that they synthesize and secrete insulin at times of plenty, but rapidly attenuate protein synthesis when the hormone is no longer needed. Consequently, the secretory pathway of a β-cell experiences dramatic changes in client protein flux. As a consequence, it is exquisitely sensitive to defects of protein folding because large increases in the rate at which new proteins enter the endoplasmic reticulum (ER) can overwhelm the resident chaperones. This can allow incorrectly folded proteins to accumulate inside the organelle, a situation termed "endoplasmic reticulum stress". Evidence accrued over the last decade has shown that ER stress plays an important role in the pathogenesis of diabetes, both through direct toxicity to the β-cell causing loss of β-cell mass and in peripheral tissues where it contributes to insulin resistance. We propose that current views of diabetes should be revised, so that it is seen as another member of the growing list of protein misfolding diseases.

GENETIC STUDIES IDENTIFYING ER PROTEINS IN THE DEVELOPMENT OF DIABETES MELLITUS

A critical observation was made almost forty years ago when three siblings, two brothers and a sister, were reported who had developed permanent neonatal diabetes mel-

litus in association with developmental bone defects[1]. The parents of those first cases were unrelated, but many subsequent case reports were of consanguineous families[2,3]. The condition now eponymously named Wolcott-Rallison syndrome (OMIM #226980)[4] is also known as multiple epiphiseal dysplasia with early onset diabetes mellitus (MED-IDDM) to highlight its extra-pancreatic manifestations. Indeed, these are diverse and include osteoporosis, growth retardation, hepatic and renal dysfunction and cognitive impairment. It is now known to be inherited as a classical autosomal recessive trait and has been mapped in two consanguineous families to the region 2p12[2]. Each family harbored a distinct mutation, but both proved to be in the *Perk* gene.

PERK is a ubiquitously expressed kinase localized to the membrane of the ER[5,6]. Protein sequence homology showed it to be a member of the eIF2α kinase family that links cellular stresses to the inhibition of protein translation. For example, the prototypical family member GCN2 reduces protein synthesis during periods of amino acid starvation by phosphorylating and thus inhibiting the translation initiation factor eIF2α[7]. Other members of this family inhibit protein synthesis during viral infection (PKR[8]) or iron deficiency in red blood cell progenitors (HRI[9]). In each instance, reduced protein synthesis promotes cellular health, by reducing the consumption of amino acids during starvation or preventing viral replication or matching haemoglobin synthesis to available haeme. In the case of PERK, reduced protein synthesis prevents new proteins entering the ER when they cannot be correctly folded. It transpires that PERK is essential for β-cells to withstand fluctuations in proinsulin synthesis that occur daily. Mice in which the *Perk* gene is deleted faithfully recapitulate many of the phenotypic features of Wolcott-Rallison syndrome and this has been very useful in understanding this condition[10]. Remarkably, these mice are born with normal islets of Langerhans, but rapidly lose β-cells in the neonatal period. Prior to death, these cells exhibit ER dilatation, due to distention with aggregates of misfolded proinsulin.

When circulating glucose levels are low, so is the demand for insulin and PERK is inactive. When proinsulin enters the ER it is bound reversibly by molecular chaperones, such as BiP, to promote its correct folding. In addition, chaperones shield newly synthesized proteins from inappropriate interactions with other incompletely folded proteins. When glucose levels rise, β-cells are stimulated to increase insulin synthesis. If this increase were unregulated, the rise in proinsulin synthesis might overwhelm ER chaperones causing ER stress and threatening to cause protein aggregation. Indeed, this occurs in the β-cells of Wolcott-Rallison patients and in *Perk* knockout mice leading to cell death. However, in healthy cells, PERK detects this rise in ER client protein demand by monitoring the level of free BiP in the ER lumen. When free BiP levels fall due to its binding to newly synthesized protein, this triggers PERK to phosphorylate eIF2α and protein translation levels fall. In parallel, ER stress signaling pathways cause an increase in many ER resident proteins including molecular chaperones, which enable higher levels of client proteins to be folded. This mechanism has been called the Unfolded Protein Response (UPR)[11,12]

The major client protein of the β-cell ER is proinsulin[12]. Recently, defects in insulin folding have been shown to underlie rare cases of familial permanent neonatal diabetes mellitus (OMIM #606176)[13]. Initially discovered in mice by Dr. Akio Koizumi in Akita, Japan, a spontaneous *INS2* gene mutation causes β-cell death[14]. In contrast to man, mice possess two insulin genes that are functionally redundant[15] and yet the Akita mutation (C96Y) behaves as a semi-dominant trait[14,16]. This toxic-gain-of-function is caused by a substitution of a conserved cysteine residue required for the formation of an intra-molecular disulphide bond[17,18]. The mutant insulin fails to be secreted and is instead retained in the ER where, crucially, wildtype proinsulin also becomes trapped in complexes with Akita mutant proinsulin impairing secretion of the normal protein[14,19,20]. When a three-generation family with permanent neonatal diabetes mellitus was found to have a heterozygous mutation of the insulin gene, this led to 83 similar families being screened, nine of which harbored insulin mutations including one analogous to the Akita mutation of mice[13]. Several subsequent studies have also confirmed that missense mutations of the *INS* gene in man are a rare but important cause of neonatal diabetes[21-24]. When such mutant insulins were expressed in cultured cells, they caused ER sress and impaired cell viability[22,25]. This contrasts with mutations that impair proinsulin synthesis through impaired transcription, which are inherited recessive traits and fail to cause death of the β-cell[26].

EMERGING MODEL FOR ENDOPLASMIC RETICULUM STRESS INVOLVEMENT IN DIABETES

The precise details of UPR signaling and its links to cell death have been reviewed elsewhere[11,12,27]. However, it is worth examining some aspects of ER stress and cell death again, as these may provide a novel target for therapeutic intervention in the future. For example, Dr Seiichi Oyadomari observed that deletion of the *Chop* gene could delay the onset of diabetes in Akita mice[28] and prevent β-cell death following other toxic stresses[29]. CHOP is a transcription factor that is up-regulated by a number of cellular insults, notably ER stress. It has been linked to the induction of cell death and some have suggested its function is to kill cells when the degree of ER stress is insurmountable[30-32]. However, our work has suggested that the link to cell death is more complex and most likely stems from CHOP acting to boost the secretory capacity of the cell[33]. We showed that the *Gadd34* gene is transcriptionally induced by CHOP during ER stress and that deleting *Gadd34* was at least as protective as deleting the *Chop* gene in an animal model of ER stress. GADD34 functions to dephosphorylate eIF2α following ER stress so that protein translation can recover and UPR target genes can be transla-

ted[34,35]. In this manner, it behaves as a functional antagonist of PERK. Whilst cells lacking PERK are vulnerable to death during ER stress, it appears that the antagonism of PERK by GADD34 during ER stress can also result in cell death. Excessive activity of GADD34 in some circumstances creates a situation similar to that of PERK deficiency. Could GADD34 inhibitors therefore prove useful to treat diabetes? A molecule called salubrinal has been identified that promotes eIF2α phosphorylation during ER stress[36]. Its precise mechanism of action requires further elucidation, but has been suggested to involve inhibition of eIF2α dephosphorylation, which can promote cell survival in some models of ER stress.

When mice were generated with β-cells that were partially resistant to PERK by mutating the phosphorylation site of eIF2α (eIF2α^{S51A}), the animals developed diabetes due to uncontrolled proinsulin synthesis and increased oxidative stress[37]. Feeding them antioxidants in their diet ameliorated this. Interestingly, CHOP induces the transcription of *ERO1a*, which increases protein oxidation in the ER to promote disulphide bond formation[33].While this may improve protein folding, it also imposes an oxidative stress burden on the β-cell. Consequently, preventing the induction of *ERO1a* may explain why *Chop* knockout reduces oxidative damage and improves β-cell survival in models of diabetes[38]. *Chop* deletion also increases β-cell mass and prevents glucose intolerance both in high-fat fed eIF2α^{S51A} mice and in leptin receptor deficient mice. This appears to be mediated by increased β-cell proliferation and by reduced apoptosis suggesting that CHOP antagonism might help maintain β-cell mass in patients if this could be achieved pharmacologically.

β-cells, therefore, can be subject to ER stress either from poorly regulated proinsulin synthesis in Wolcott-Rallison syndrome or directly from mutant proinsulins. It is less clear, however, why ER stress should be relevant in peripheral tissues in diabetic patients. Nevertheless, ER stress in peripheral tissues plays at least as important a role in diabetes as it does in the β-cell. Some peripheral tissues, for example adipocytes, respond to raised circulating glucose by increasing ER protein synthesis thus increasing ER client load[39]. In addition, obesity increases peripheral tissue inflammation, which can also cause ER stress[40,41]. A consequence of this appears to be impaired insulin signaling and consequently insulin resistance.

While PERK regulates protein translation during ER stress and has further effects on UPR gene transcription, a second ER stress sensor called IRE1 regulates other UPR genes. IRE1 is far older than PERK in evolutionary terms, being found even in yeast. Not only can it trigger gene transcription but it can, at least in mammals, also impair insulin receptor signaling. Activated insulin receptors signal to the cell's interior *via* the phosphorylation of target molecules including insulin receptor substrate 1 (IRS1) on tyrosine residues. This can be blocked if IRS1 is phosphorylated on serine residues by the Jun N-terminal kinase (JNK)[42] and is triggered in peripheral tissues of obese subjects through activation of JNK by IRE1[43]. The notion that peripheral ER stress can impair glucose homeostasis is supported by a number of other lines of evidence. For example, if ER function is impaired in the liver by deleting the chaperone Oxygen-Regulated Protein 150 (ORP150), mice display impaired IRS1-dependent insulin signaling and develop glucose intolerance[44]. In contrast, raising the levels of ORP150 protects obese mice from diabetes[44,45]. We may yet be able to use these observations in therapies, since two small molecular "chemical chaperones", 4-phenyl butyric acid and taurine-conjugated ursodeoxycholic acid, relieve ER stress in animal models *in vivo* and improve peripheral insulin sensitivity in obese diabetic mice[46].

CONCLUSION

Substantial clinical and experimental evidence clearly shows that ER stress is important in diabetes both affecting β-cell survival and contributing to peripheral insulin resistance. This novel paradigm has already shed light on poorly understood aspects of diabetes and is providing exciting new targets for therapeutic intervention. Novel molecules, for example salubrinal and guanabenz, are already becoming available to help study ER stress in the laboratory and these may perhaps represent the lead compounds in the development of new drugs that will enable us to tackle ER stress in diabetes and will eventually help to treat this important cause of human suffering.

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REFERENCES

1 **Wolcott CD**, Rallison ML. Infancy-onset diabetes mellitus and multiple epiphyseal dysplasia. *J Pediatr* 1972; **80**: 292-297
2 **Delépine M**, Nicolino M, Barrett T, Golamaully M, Lathrop GM, Julier C. EIF2AK3, encoding translation initiation factor 2-alpha kinase 3, is mutated in patients with Wolcott-Rallison syndrome. *Nat Genet* 2000; **25**: 406-409
3 **al-Gazali LI**, Makia S, Azzam A, Hall CM. Wolcott-Rallison syndrome. *Clin Dysmorphol* 1995; **4**: 227-233
4 **Stöss H**, Pesch HJ, Pontz B, Otten A, Spranger J. Wolcott-Rallison syndrome: diabetes mellitus and spondyloepiphyseal dysplasia. *Eur J Pediatr* 1982; **138**: 120-129
5 **Shi Y**, Vattem KM, Sood R, An J, Liang J, Stramm L, Wek RC. Identification and characterization of pancreatic eukaryotic initiation factor 2 alpha-subunit kinase, PEK, involved in translational control. *Mol Cell Biol* 1998; **18**: 7499-7509
6 **Harding HP**, Zhang Y, Ron D. Protein translation and folding are coupled by an endoplasmic-reticulum-resident kinase. *Nature* 1999; **397**: 271-274
7 **Tzamarias D**, Thireos G. Evidence that the GCN2 protein kinase regulates reinitiation by yeast ribosomes. *EMBO J* 1988; **7**: 3547-3551
8 **Carroll K**, Elroy-Stein O, Moss B, Jagus R. Recombinant vaccinia virus K3L gene product prevents activation of double-stranded RNA-dependent, initiation factor 2 alpha-specific protein kinase. *J Biol Chem* 1993; **268**: 12837-12842
9 **Fagard R**, London IM. Relationship between phosphorylation and activity of heme-regulated eukaryotic initiation factor 2 alpha kinase. *Proc Natl Acad Sci U S A* 1981; **78**: 866-870
10 **Harding HP**, Zeng H, Zhang Y, Jungries R, Chung P, Plesken

H, Sabatini DD, Ron D. Diabetes mellitus and exocrine pancreatic dysfunction in perk-/- mice reveals a role for translational control in secretory cell survival. *Mol Cell* 2001; **7**: 1153-1163
11 **Ron D**, Walter P. Signal integration in the endoplasmic reticulum unfolded protein response. *Nat Rev Mol Cell Biol* 2007; **8**: 519-529
12 **Thomas SE**, Dalton LE, Daly ML, Malzer E, Marciniak SJ. Diabetes as a disease of endoplasmic reticulum stress. *Diabetes Metab Res Rev* 2010; **26**: 611-621
13 **Støy J**, Edghill EL, Flanagan SE, Ye H, Paz VP, Pluzhnikov A, Below JE, Hayes MG, Cox NJ, Lipkind GM, Lipton RB, Greeley SA, Patch AM, Ellard S, Steiner DF, Hattersley AT, Philipson LH, Bell GI. Insulin gene mutations as a cause of permanent neonatal diabetes. *Proc Natl Acad Sci U S A* 2007; **104**: 15040-15044
14 **Wang J**, Takeuchi T, Tanaka S, Kubo SK, Kayo T, Lu D, Takata K, Koizumi A, Izumi T. A mutation in the insulin 2 gene induces diabetes with severe pancreatic beta-cell dysfunction in the Mody mouse. *J Clin Invest* 1999; **103**: 27-37
15 **Leroux L**, Desbois P, Lamotte L, Duvillié B, Cordonnier N, Jackerott M, Jami J, Bucchini D, Joshi RL. Compensatory responses in mice carrying a null mutation for Ins1 or Ins2. *Diabetes* 2001; **50 Suppl 1**: S150-S153
16 **Izumi T**, Yokota-Hashimoto H, Zhao S, Wang J, Halban PA, Takeuchi T. Dominant negative pathogenesis by mutant proinsulin in the Akita diabetic mouse. *Diabetes* 2003; **52**: 409-416
17 **Nozaki J**, Kubota H, Yoshida H, Naitoh M, Goji J, Yoshinaga T, Mori K, Koizumi A, Nagata K. The endoplasmic reticulum stress response is stimulated through the continuous activation of transcription factors ATF6 and XBP1 in Ins2+/Akita pancreatic beta cells. *Genes Cells* 2004; **9**: 261-270
18 **Liu M**, Li Y, Cavener D, Arvan P. Proinsulin disulfide maturation and misfolding in the endoplasmic reticulum. *J Biol Chem* 2005; **280**: 13209-13212
19 **Allen JR**, Nguyen LX, Sargent KE, Lipson KL, Hackett A, Urano F. High ER stress in beta-cells stimulates intracellular degradation of misfolded insulin. *Biochem Biophys Res Commun* 2004; **324**: 166-170
20 **Liu M**, Hodish I, Rhodes CJ, Arvan P. Proinsulin maturation, misfolding, and proteotoxicity. *Proc Natl Acad Sci U S A* 2007; **104**: 15841-15846
21 **Edghill EL**, Flanagan SE, Patch AM, Boustred C, Parrish A, Shields B, Shepherd MH, Hussain K, Kapoor RR, Malecki M, MacDonald MJ, Støy J, Steiner DF, Philipson LH, Bell GI, Hattersley AT, Ellard S. Insulin mutation screening in 1,044 patients with diabetes: mutations in the INS gene are a common cause of neonatal diabetes but a rare cause of diabetes diagnosed in childhood or adulthood. *Diabetes* 2008; **57**: 1034-1042
22 **Colombo C**, Porzio O, Liu M, Massa O, Vasta M, Salardi S, Beccaria L, Monciotti C, Toni S, Pedersen O, Hansen T, Federici L, Pesavento R, Cadario F, Federici G, Ghirri P, Arvan P, Iafusco D, Barbetti F. Seven mutations in the human insulin gene linked to permanent neonatal/infancy-onset diabetes mellitus. *J Clin Invest* 2008; **118**: 2148-2156
23 **Molven A**, Ringdal M, Nordbø AM, Raeder H, Støy J, Lipkind GM, Steiner DF, Philipson LH, Bergmann I, Aarskog D, Undlien DE, Joner G, Søvik O, Bell GI, Njølstad PR. Mutations in the insulin gene can cause MODY and autoantibody-negative type 1 diabetes. *Diabetes* 2008; **57**: 1131-1135
24 **Polak M**, Dechaume A, Cavé H, Nimri R, Crosnier H, Sulmont V, de Kerdanet M, Scharfmann R, Lebenthal Y, Froguel P, Vaxillaire M. Heterozygous missense mutations in the insulin gene are linked to permanent diabetes appearing in the neonatal period or in early infancy: a report from the French ND (Neonatal Diabetes) Study Group. *Diabetes* 2008; **57**: 1115-1119
25 **Rajan S**, Eames SC, Park SY, Labno C, Bell GI, Prince VE, Philipson LH. In vitro processing and secretion of mutant insulin proteins that cause permanent neonatal diabetes. *Am J Physiol Endocrinol Metab* 2010; **298**: E403-E410
26 **Garin I**, Edghill EL, Akerman I, Rubio-Cabezas O, Rica I, Locke JM, Maestro MA, Alshaikh A, Bundak R, del Castillo G, Deeb A, Deiss D, Fernandez JM, Godbole K, Hussain K, O'Connell M, Klupa T, Kolouskova S, Mohsin F, Perlman K, Sumnik Z, Rial JM, Ugarte E, Vasanthi T, Johnstone K, Flanagan SE, Martínez R, Castaño C, Patch AM, Fernández-Rebollo E, Raile K, Morgan N, Harries LW, Castaño L, Ellard S, Ferrer J, Perez de Nanclares G, Hattersley AT. Recessive mutations in the INS gene result in neonatal diabetes through reduced insulin biosynthesis. *Proc Natl Acad Sci U S A* 2010; **107**: 3105-3110
27 **Marciniak SJ**, Ron D. Endoplasmic reticulum stress signaling in disease. *Physiol Rev* 2006; **86**: 1133-1149
28 **Oyadomari S**, Koizumi A, Takeda K, Gotoh T, Akira S, Araki E, Mori M. Targeted disruption of the Chop gene delays endoplasmic reticulum stress-mediated diabetes. *J Clin Invest* 2002; **109**: 525-532
29 **Oyadomari S**, Takeda K, Takiguchi M, Gotoh T, Matsumoto M, Wada I, Akira S, Araki E, Mori M. Nitric oxide-induced apoptosis in pancreatic beta cells is mediated by the endoplasmic reticulum stress pathway. *Proc Natl Acad Sci U S A* 2001; **98**: 10845-10850
30 **Zinszner H**, Kuroda M, Wang X, Batchvarova N, Lightfoot RT, Remotti H, Stevens JL, Ron D. CHOP is implicated in programmed cell death in response to impaired function of the endoplasmic reticulum. *Genes Dev* 1998; **12**: 982-995
31 **Gotoh T**, Oyadomari S, Mori K, Mori M. Nitric oxide-induced apoptosis in RAW 264.7 macrophages is mediated by endoplasmic reticulum stress pathway involving ATF6 and CHOP. *J Biol Chem* 2002; **277**: 12343-12350
32 **Oyadomari S**, Araki E, Mori M. Endoplasmic reticulum stress-mediated apoptosis in pancreatic beta-cells. *Apoptosis* 2002; **7**: 335-345
33 **Marciniak SJ**, Yun CY, Oyadomari S, Novoa I, Zhang Y, Jungreis R, Nagata K, Harding HP, Ron D. CHOP induces death by promoting protein synthesis and oxidation in the stressed endoplasmic reticulum. *Genes Dev* 2004; **18**: 3066-3077
34 **Novoa I**, Zeng H, Harding HP, Ron D. Feedback inhibition of the unfolded protein response by GADD34-mediated dephosphorylation of eIF2alpha. *J Cell Biol* 2001; **153**: 1011-1022
35 **Novoa I**, Zhang Y, Zeng H, Jungreis R, Harding HP, Ron D. Stress-induced gene expression requires programmed recovery from translational repression. *EMBO J* 2003; **22**: 1180-1187
36 **Boyce M**, Bryant KF, Jousse C, Long K, Harding HP, Scheuner D, Kaufman RJ, Ma D, Coen DM, Ron D, Yuan J. A selective inhibitor of eIF2alpha dephosphorylation protects cells from ER stress. *Science* 2005; **307**: 935-939
37 **Back SH**, Scheuner D, Han J, Song B, Ribick M, Wang J, Gildersleeve RD, Pennathur S, Kaufman RJ. Translation attenuation through eIF2alpha phosphorylation prevents oxidative stress and maintains the differentiated state in beta cells. *Cell Metab* 2009; **10**: 13-26
38 **Song B**, Scheuner D, Ron D, Pennathur S, Kaufman RJ. Chop deletion reduces oxidative stress, improves beta cell function, and promotes cell survival in multiple mouse models of diabetes. *J Clin Invest* 2008; **118**: 3378-3389
39 **Gregor MF**, Hotamisligil GS. Thematic review series: Adipocyte Biology. Adipocyte stress: the endoplasmic reticulum and metabolic disease. *J Lipid Res* 2007; **48**: 1905-1914
40 **Xue X**, Piao JH, Nakajima A, Sakon-Komazawa S, Kojima Y, Mori K, Yagita H, Okumura K, Harding H, Nakano H. Tumor necrosis factor alpha (TNFalpha) induces the unfolded protein response (UPR) in a reactive oxygen species (ROS)-dependent fashion, and the UPR counteracts ROS accumulation by TNFalpha. *J Biol Chem* 2005; **280**: 33917-33925
41 **Cardozo AK**, Ortis F, Storling J, Feng YM, Rasschaert J, Tonnesen M, Van Eylen F, Mandrup-Poulsen T, Herchuelz A, Eizirik DL. Cytokines downregulate the sarcoendoplasmic reti-

culum pump Ca2+ ATPase 2b and deplete endoplasmic reticulum Ca2+, leading to induction of endoplasmic reticulum stress in pancreatic beta-cells. *Diabetes* 2005; **54**: 452-461

42 **Thirone AC**, Huang C, Klip A. Tissue-specific roles of IRS proteins in insulin signaling and glucose transport. *Trends Endocrinol Metab* 2006; **17**: 72-78

43 **Ozcan U**, Cao Q, Yilmaz E, Lee AH, Iwakoshi NN, Ozdelen E, Tuncman G, Görgün C, Glimcher LH, Hotamisligil GS. Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. *Science* 2004; **306**: 457-461

44 **Nakatani Y**, Kaneto H, Kawamori D, Yoshiuchi K, Hatazaki M, Matsuoka TA, Ozawa K, Ogawa S, Hori M, Yamasaki Y, Matsuhisa M. Involvement of endoplasmic reticulum stress in insulin resistance and diabetes. *J Biol Chem* 2005; **280**: 847-851

45 **Ozawa K**, Miyazaki M, Matsuhisa M, Takano K, Nakatani Y, Hatazaki M, Tamatani T, Yamagata K, Miyagawa J, Kitao Y, Hori O, Yamasaki Y, Ogawa S. The endoplasmic reticulum chaperone improves insulin resistance in type 2 diabetes. *Diabetes* 2005; **54**: 657-663

46 **Ozcan U**, Yilmaz E, Ozcan L, Furuhashi M, Vaillancourt E, Smith RO, Görgün CZ, Hotamisligil GS. Chemical chaperones reduce ER stress and restore glucose homeostasis in a mouse model of type 2 diabetes. *Science* 2006; **313**: 1137-1140

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