

MINI REVIEW P 12-20

Oxidative stress, mitochondrial dysfunction and endoplasmic reticulum stress

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Diabetes mellitus is a chronic complex metabolic condition that affects carbohydrate, lipid and protein metabolism and may impair numerous organs and functions of the organism. Hyperglycemia is only the most obvious biochemical marker of diabetes, and the main contributor to the development of diabetes complications is the cumulative effect of chronic hyperglycemia. Increasing evidence suggests that oxidative stress may be the key mediators of the deleterious effects of hyperglycemia. Mitochondria play a central role in the generation of reactive oxygen species and cell apoptosis. A number of conditions including nutrient excess that interfere with proper endoplasmic reticulum (ER) function may lead to accumulation of unfolded proteins, which then trigger apoptotic as well as adaptive downstream signaling pathways. Many studies have also provided ample evidences that mitochondrial dysfunction and ER stress are most important pathogenic causes for the development of diabetes and its complications. Regulation mechanisms of how mitochondria play in the metabolism of glucose and fatty acids, the primary fuels used by cells to produce ATP, have been the subject of tremendous interests. Nonetheless, much remains to be investigated such as tissue-specific fuel selection and its relation with the pathogenesis of diabetes and complications. Cellular homeostasis depends upon the functional relationship between mitochondria and the ER. Propagation of calcium signaling from ER to mitochondria is involved in both ATP production and cell death. On the other hand, the ER requires ATP to function properly, which may make it the best site for sensing metabolic stress. In this article, oxidative stress, mitochondrial dysfunction and ER stress, especially their real-time interaction in diabetes and complication development will be reviewed.

INTRODUCTION

Diabetes mellitus comprises a number of chronic metabolic diseases characterized by hyperglycemia due to absolute or relative insulin deficiency. Hyperglycemia is only the most obvious biochemical marker of these complex disorders that affect carbohydrate, lipid, protein, and electrolyte metabolism and may impair numerous organs and functions of the organism. Type 2 diabetes mellitus (T2DM) is a systemic metabolic disorder characterized by relative insulin deficiency due to insulin resistance (IR) associated with serious and often life-threatening complications. Type 1 diabetes mellitus (T1DM) is characterized by absolute insulin deficiency resulted from autoimmune destruction of pancreatic β cell and also associated with chronic complications.

Mitochondrial dysfunction (MD) and endoplasmic reticulum (ER) stress have been known to be most important causes for the development of diabetes and its complications (Patti and Corvera, 2010; Eizirik et al., 2008). Mitochondria are the most important source of the chemical energy (ATP) required by cells.

Mitochondria also play a central role in the generation of reactive oxygen species (ROS) and lead to cell apoptosis. The ER is a subcellular compartment involved in calcium homeostasis, lipid synthesis, and protein folding. Numerous factors that interfere with proper ER function may lead to accumulation of unfolded proteins, which trigger both adaptive and apoptotic downstream signaling pathways (unfolded protein response, UPR) (Eizirik et al., 2008). However, cellular homeostasis depends upon the functional relationship between mitochondria and the ER.

OXIDATIVE STRESS ASSOCIATED WITH HYPERGLYCEMIA LEADS TO DIABETES AND DIABETIC COMPLICATIONS

There has been an interest in the role of oxidative stress (OS), especially ROS and reactive nitrogen species and their imbalance in the pathogenesis of various human diseases (Valiko et al., 2007). The overproduction of ROS poses a damage to cell structures, including membranes, proteins, and DNA, which may lead to diverse disease states, such as diabetes and atherosclerosis (Valiko et al., 2007). There have been ample evidences to support that OS is closely related to the development of diabetes, especially T2DM or metabolic syndrome (MS), which is a cluster of cardiovascular risk factors

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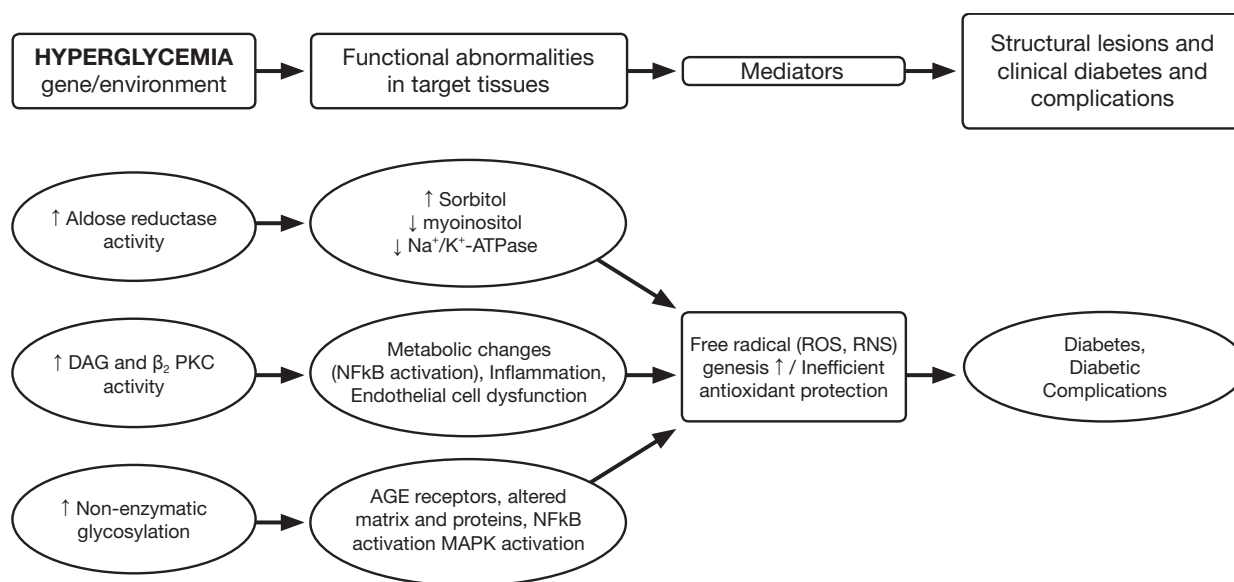


FIGURE 1 | How chronic hyperglycemia may lead to diabetes and diabetic complications.

composed of hyperglycemia, hypertension, dyslipidemia, and abdominal obesity (Hopps et al., 2010). OS has also been shown to be related to each component of MS. Importantly, abdominal obesity, visceral fat accumulation induces increase in systemic lipid peroxidation and damage through excess free fatty acids and cytokines which then trigger systemic oxidative alterations (Grattagliano et al, 2008). Secondly, patients with MS displayed significantly lower superoxide dismutase (SOD) and glutathione peroxidase activities, and T2DM patients showed positive associations with systemic OS (Roberts and Sindhu, 2009). Hypertensive subjects show elevated OS and compromised antioxidant capacities. Dyslipidemic animals and humans show increased ROS production, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity, and many OS markers and lowered SOD and endothelial nitric oxide synthase (eNOS).

Both T1DM and T2DM are characterized by the slow progression towards the generation of some specific lesions of the blood vessels affecting both small (microangiopathy) and larger (macroangiopathy) vessels. Patients with diabetes and especially with diabetic macrovascular complications always accompany more than one of these cardiovascular risk factors and expose to the condition that characterize with increased OS. Based on abundant evidences of association between OS and precedent components of MS, OS might be considered as an early event in the pathology of diabetic macrovascular complications.

The classical microvascular complications of diabetes, diabetic retinopathy (the main cause of blindness in adults), diabetic renal disease representing currently the main cause of renal substitution therapy (dialysis or renal transplantation) in developed countries, and most prevalent and troublesome

diabetic neuropathy are also associated with OS. Major epidemiologic and intervention studies showed that chronic hyperglycemia is the main contributor to diabetic tissue damage in these complications, especially in microangiopathy (UK Prospective Diabetes Study Group, 1998). The main contributor to the development of these complications is the cumulative effect of chronic hyperglycemia. Increasing evidence suggests that consequent OS and endothelial dysfunction (ED) may be the key mediators of the deleterious effects of hyperglycemia. Additional factors are represented by some accelerators such as hemodynamic (hypertension) and metabolic (IR, dyslipidemia) components.

As the primary crucial factor, the persistent hyperglycemia (duration of diabetes and level of metabolic control) is the main contributor to the microvascular complication development. An unifying mechanism was proposed by Brownlee et al. suggesting that the key element is the hyperglycemia induced overproduction of superoxide (O_2^-) anions by the mitochondrial electron transport chain (ETC) during the process of oxidative phosphorylation (OP) (Brownlee, 2005). It was postulated that hyperglycemia induces increased mitochondrial production of ROS followed by nuclear DNA strand breaks that, in turn, activate the enzyme poly ADP-ribose polymerase and may associate with and lead to a cascade of processes that finally activate 4 major pathways of diabetic complications (Figure 1).

Increased aldose reductase activity and activation of the polyol pathway

Persistent hyperglycemia increases polyol pathway activity with accumulation of sorbitol and fructose in nerves, damaging them by as yet unknown mechanisms. This is accompanied

by decreased myoinositol uptake and inhibition of the Na^+/K^+ -adenosine triphosphatase (ATPase) activity resulting in Na^+ retention, edema, myelin swelling, axoglial dysjunction and nerve degeneration (Boulton, 2007).

Activation of protein kinase C (PKC) with subsequent activation of NF- κ B pathway

Hyperglycemia is known to stimulate formation of diacylglycerol, which leads to the activation of serine/threonine kinases including protein kinase C (PKC). Then, PKC activates the transcription factor NF- κ B responsible for the modification of many genes expression (Tesfaye et al. 2005). NF- κ B is a transcriptional factor that is activated by a number of stimuli and is responsible for initiating the transcription of a number of different inflammatory and immune mediators.

Intracellular advanced glycation end-products (AGE) generation

Hyperglycemia induces a non-enzymatic glycation of proteins and AGEs in turn activate NF- κ B (Brownlee, 2005). Local generation of O_2^- also occurs by the interaction of the residues of L-lysine of the protein with α -ketoaldehydes results in oxidative modification of proteins and other biomolecules. Non-enzymatic O_2^- generation might be an element of autocatalytic intensification of pathophysiological action of carbonyl stress. The role of oxidative injury as a function of generation of ROS during the formation of AGEs which are a heterogeneous group of molecules formed from the nonenzymatic reaction of reducing sugars with free amino groups of proteins, lipids, and nucleic acids.

In summary, incidence and severity of diabetic complications associate with OS resulted from poor control of glycemia, indicating that excess glucose may be the biochemical trigger in pathogenesis. Damage of the microvessel itself as well as that of neurons resulted from the metabolic and vascular mechanisms exacerbate diabetic complications. A generic component of events occurs as the change in the phenotype of tissue cells induced by hyperglycemia. Raised extracellular glucose alters the pattern of gene expression that constitutes cell phenotype. The effects of glucose may be primary or secondary via the polyol pathway, OS, protein glycation, or other unidentified consequences of hyperglycemia. As a unifying mechanism, increased OS might be an important mediator representing the influence of hyperglycemia to the development of diabetic complications.

MITOCHONDRIAL DYSFUNCTION AS A CRUCIAL MEDIATOR OF DIABETES AND COMPLICATION DEVELOPMENT

Mitochondria are important for providing energy in the form of ATP by OP. Mitochondria are also the key regulator of glucose-stimulated insulin secretion (GSIS) in the pancreatic β cells. Numerous evidences have shown that mitochondrial function is closely related to various aspects of diabetes such as pancreatic

β -cell dysfunction, IR, obesity and vascular complications of diabetes. There is also notion that MD could be the central defect causing the abnormal glucose metabolism in the diabetic state. In the early 1990s, a specific mutation in mitochondrial DNA was identified to be causally related to the maternally inherited form of diabetes (Ballinger et al., 1992). Shulman et al. have shown that OP was decreased in IR offspring of T2DM patients (Petersen et al., 2004).

From products of the TCA cycle, fatty-acid oxidation and amino-acid oxidation, most ATPs are produced in the mitochondria via the ETC during OP. In the energy-production process, oxygen is used to oxidize molecules rich in carbon and hydrogen, with a resultant reduction of oxygen to water. This biochemical reaction gives rise to free radicals such as O_2^- and/or ROS. Approximately 90% of oxygen in the cell is consumed by the mitochondria and the ETC is the source of continuing flux of oxygen radicals. Approximately 1–5% of the oxygen consumed by mitochondria in cells is converted to ROS under normal physiological conditions. Defects in the ETC in the affected tissues of patients with mitochondrial disease or in aged individuals may contribute to increased production of O_2^- anions by mitochondria (Turrens, 2003). Therefore, mitochondria are susceptible to oxidative damage generated *in situ*. Mitochondria are the main intracellular source and also the immediate target of ROS.

To deal with the continuous production of ROS by aerobic metabolism, cells have developed antioxidative enzymes, including mitochondrial manganese (copper/zinc) superoxide dismutase, glutathione peroxidase and catalase (Battino et al., 1999). Although these enzymes in combination with other antioxidants can dispose of most of the ROS and free radicals generated under normal conditions, a fraction of ROS/free radicals may escape the defense mechanism and cause damage to critical cellular macromolecules, including nucleic acids, proteins and lipids. Therefore, by way of OS, MD induces pancreatic β -cell dysfunction, peripheral and liver IR followed by development of diabetes and diabetic complications (Figure 2).

Mitochondrial dysfunction and pancreatic β -cell dysfunction

IR patients can develop overt T2DM when pancreatic β -cells are unable to produce enough insulin to maintain normoglycemia. Pancreatic β -cells from patients with T2DM cannot sense glucose properly, which appears to be controlled by mitochondrial metabolism, and this contributes to impairment of insulin secretion. In T2DM, reduced amount of NADH or FADH_2 is generated during glucose metabolism via glycolysis and the TCA cycle. Reduced electron transfer to the ETC by NADH and FADH_2 leads to decreased production of ATP. Normally, increases in the ATP/ADP ratio in β -cells inhibit ATP-sensitive potassium channels (K_{ATP}), in turn inducing depolarization of plasma membranes. The opening of voltage-sensitive Ca^{2+} channels allows Ca^{2+} uptake by β -cells, thereby contributing to secretion of insulin. However, MD found in patients with T2DM can impair

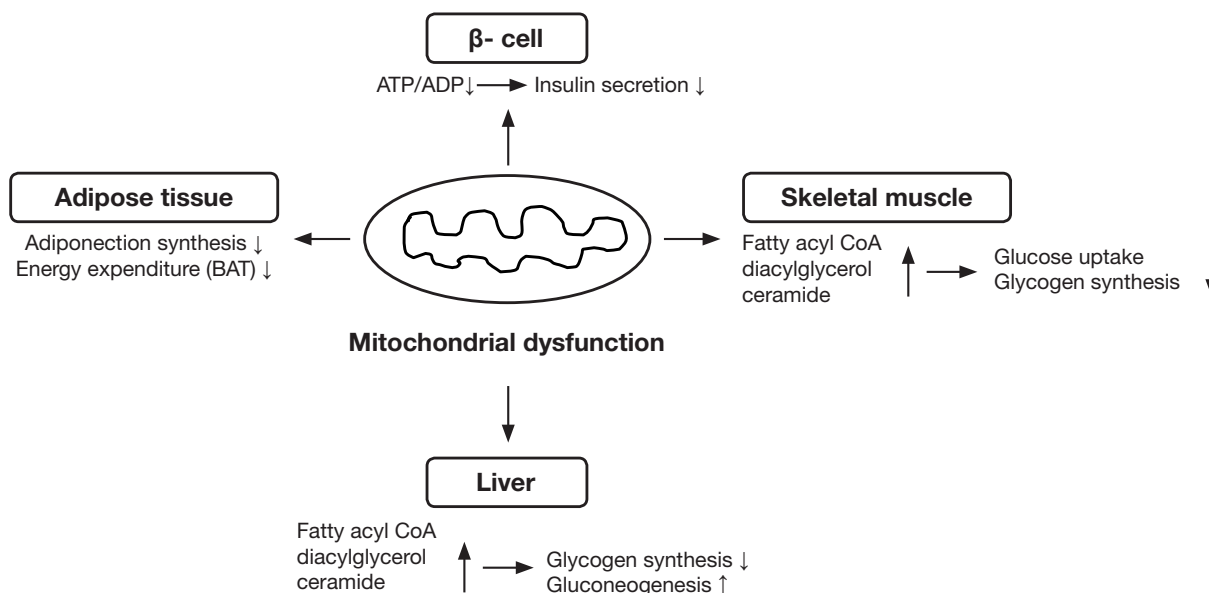


FIGURE 2 | Mitochondrial dysfunction induces pancreatic β -cell dysfunction, peripheral and liver insulin resistance followed by development of diabetes.

GSIS by reducing the ATP/ADP ratio within β -cells (Maechler and Wollheim, 2001).

Mitochondrial dysfunction and skeletal muscle insulin resistance

Defective mitochondrial fatty acid metabolism in skeletal muscle is thought to affect insulin signaling pathways, thereby leading to IR (Petersen et al., 2004; Kelley et al., 2002). Impairment of β -oxidation, either alone or in conjunction with increased delivery of free fatty acids (FFAs) from plasma, leads to elevated levels of intracellular fatty acid metabolites such as fatty acyl CoA, diacylglycerol, and ceramide. Metabolites formed under such circumstances activate PKC, leading to phosphorylation of serine sites on insulin receptor substrate-1 (IRS-1) (Tesfaye et al., 2005). Increased serine phosphorylation of IRS-1 inhibits the tyrosine kinase activity of the insulin receptor on IRS-1 and the activity of insulin-stimulated phosphatidylinositol 3-kinase (PI 3-kinase), resulting in decreased activity of insulin-stimulated protein kinase B (PKB, also known as AKT). Reduced AKT activity leads to suppression of insulin-stimulated glucose transporter 4 (GLUT4) translocation and subsequent reduction of glycogen synthesis.

Mitochondrial dysfunction and hepatic insulin resistance

The liver also plays a crucial role in the development of IR and T2DM. Defects in liver mitochondrial oxidative function can induce hepatic IR (Petersen et al., 2004; Pérez-Carreras et al., 2003). Reduced levels of mitochondrial fatty acid β -oxidation in the liver, as in skeletal muscle, lead to accumulation of intracellular fatty acid metabolites. When *de novo* hepatic lipogenesis rises or when delivery of FFAs from the plasma increases, the metabolites

adversely affect intracellular insulin signaling, leading to reduced insulin stimulation of glycogen synthesis and increased hepatic gluconeogenesis.

Mitochondrial dysfunction in adipose tissue

Adipose tissue has been known as an endocrine organ that plays a central role in fuel metabolism. Adipokines such as leptin, adiponectin, resistin, and TNF- α are released by adipose tissue, and these cytokines regulate fuel metabolism. The plasma levels of adiponectin, having insulin-sensitizing effects, are decreased in obese subjects and in T2DM patients. A recent report suggested that the levels of adiponectin in plasma and adipose tissue were decreased in obese mice, which correlated reduction of mitochondrial content and function in adipose tissue. Rosiglitazone, a peroxisome proliferator-activated receptor γ (PPAR γ) agonist, reversed decreases in plasma adiponectin levels and adiponectin expression in obese mice, and elevated mitochondrial content and function in adipose tissue (Koh et al. 2007). MD in adipose tissue leads to decreased plasma adiponectin levels in obese subjects.

Many studies on rodents have shown that the capacity of mitochondria for oxidizing fatty acids in brown adipose tissue (BAT) plays a critical role in the regulation of adaptive thermogenesis, energy balance, and body weight. Although presence of BAT was considered to be relevant only in human newborn and small mammals, recent studies using PET-CT demonstrated that adult humans still possess active BAT (van Marken Lichtenbelt et al. 2009). Thus, MD in BAT appears to be linked to impaired thermogenesis and energy expenditure, contributing to the development of obesity and IR in adult humans.

Beside the inevitable microvascular complications, in which both OS and MD always go with hyperglycemia and IR, T2DM markedly increases the risk of cardiovascular disease (macrovascular complication). ED, an early indicator of diabetic vascular disease, is common in T2DM and independently predicts cardiovascular risk. Although the precise pathogenic mechanisms for ED in T2DM remain unclear, they have been known to involve uncoupling of both eNOS activity and mitochondrial OP, as well as the activation of vascular NADPH oxidase (Hamilton and Watts, 2013). The major contributing factors include dyslipoproteinemia, OS, and inflammation. Therapeutic interventions are designed to target these pathophysiological factors that underlie ED. Therapeutic interventions including lifestyle changes, hypoglycemic agents and lipid-regulating therapies, let alone the measures to improve MD, aim to improve ED.

ROLE OF ER STRESS IN THE DEVELOPMENT OF DIABETES AND DIABETIC COMPLICATIONS

Various metabolic overloads may frequently associate with OS and stress on ER. The ER is the site of protein synthesis and modification, intracellular calcium storing, and lipid synthesis. ER maintains quality control in the face of accumulation of excess protein, misfolding, or sustained loss of calcium, all of which stress the ER leading to an adaptive response (UPR) to dampen the stress. Prolonged UPR has pathological consequences including fat accumulation, cell death, and inflammation (Eizirik et al., 2008; Ron and Walter, 2007). ER stress also plays an important role in the pathogenesis of T2DM, since such stress contributes to pancreatic β -cell dysfunction and IR (Figure 3) (Eizirik et al., 2008).

When cells and tissues are exposed to excess of glucose or FFA, at first, proteins involved in stress-regulatory pathways are usually found upregulated. One of these proteins is the 78-kDa glucose regulated protein GRP78. It is a member of the Hsp70 family of chaperones and localizes mainly in the ER. Functioning as resident chaperone regulating protein folding and preventing aggregation, GRP78 regulates UPR through the binding to the ER transmembrane proteins activating transcription factor 6 (ATF6), inositol requiring protein 1 (IRE1), and PKR-like endoplasmic reticulum kinase (PERK) (Hendershot, 2004). Accumulation of unfolded peptides titrates GRP78 away from these 3 stress sensors inducing their activation. Once activated, the UPR can be divided into 2 phases: early, pro-survival and late, pro-apoptotic UPR. It is often observed that genes up- or downstream the UPR are upregulated in several types of metabolic diseases such as T2DM suggesting chronic activation.

In fact, activated PERK mediates inhibition of protein translation via phosphorylation of eukaryotic translation initiation factor 2 α (eIF2 α), resulting in reduced protein synthesis to decrease the protein-folding load in the ER (Harding et al., 1999). PERK-mediated eIF2 α phosphorylation also contributes to the activation of a subset of translational targets including activating transcription factor 4 (ATF4). ATF4 activates transcriptionally

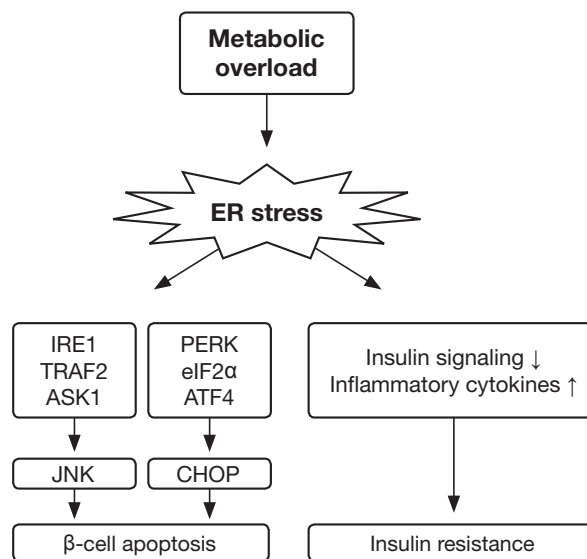


FIGURE 3 | Metabolic overload leads to ER stress, which contributes to both pancreatic β -cell dysfunction and insulin resistance followed by development of diabetes.

the proapoptotic transcription factor CCAAT/enhancer binding protein (C/EBP) homologous protein (CHOP) (Harding et al, 2000). Activation of IRE1 leads to splicing of X-box binding protein-1 (XBP1) mRNA and translation of the active form (XBP1s). XBP1s translocates to the nucleus and regulates expression of ER chaperones and proteins involved in ER-associated degradation (ERAD) (Lee et al, 2003). In addition, the cytosolic domain of IRE1 can associate with TNF receptor-associated factor 2 (TRAF2) to activate the apoptosis signal-regulating kinase 1 (ASK1) and c-Jun N-terminal kinase (JNK) pathway. In response to ER stress, ATF6, released from GRP78, translocates to the Golgi where it is cleaved by proteases into an active amino-terminal form (Shen et al., 2002). N-terminal ATF6 in turn moves to the nucleus to stimulate expression of ER chaperones and proteins involved in ERAD.

ER stress and β -cell dysfunction

ER stress also associates with both β -cell dysfunction and IR (Eizirik et al., 2008; Scheuner and Kaufman, 2008). When the demand for insulin overwhelms the folding capacity of the ER, the UPR becomes chronically activated. Several metabolic overloads have been shown to cause sustained accumulation of misfolded proteins within the ER lumen of β -cells. These include high levels of FFA and chronic hyperglycemia, as well as aggregation of islet amyloid polypeptide. Accumulation of misfolded proteins triggers chronic activation of the UPR, inducing β -cell dysfunction and apoptosis. Many components of the UPR that contribute to β -cell apoptosis have been shown in Figure 3. ER stress can induce β -cell apoptosis through prolonged activation of IRE1-TRAF2-

ASK1 cascade and JNK pathway. CHOP also plays a crucial role in the induction of ER stress-mediated β -cell apoptosis.

Besides, ER stress can also impair β cell function by a number of other mechanisms. ER stress can activate PERK to decrease insulin biosynthesis at the level of translation via eIF2 α phosphorylation. Another possibility is that activation of the UPR leads to activation of JNK, which can impair insulin gene transcription via PDX-1 nuclear exclusion and/or translation. In addition to the impairment of insulin biosynthesis, ER stress can induce mitochondrial O_2^- production, which can impair glucose oxidation and activate uncoupling protein 2 (UCP2) to decrease ATP production, and hence insulin granule exocytosis (Cunha et al., 2008).

ER stress and insulin resistance

ER stress is also involved in peripheral and hepatic IR. Metabolic overload and obesity results in chronic stimulation of ER stress, leading to continuous activation of UPR, the process of which have been suggested as a main mechanism of peripheral IR and T2DM (Özcan et al., 2004). In obese mice, levels of ER stress markers are increased in the liver and adipose tissue. Then, ER stress inhibits insulin signaling, and this leads to IR. ER stress can also activate NF- κ B signaling in the liver, thereby increasing production of proinflammatory cytokines and causing development of IR. A recent study showed that treatment of obese diabetic mice with 4-phenyl butyric acid, a chemical chaperone mimicking GRP78 and furthermore, taurine-conjugated ursodeoxycholic acid improved peripheral insulin sensitivity by alleviating ER stress (Özcan et al., 2006; Kars et al., 2010). Antioxidant therapy which was also found to improve ER stress improved insulin sensitivity in the liver and muscle of obese subjects (Kim et al., unpublished data).

The mechanism by which ER stress impairs insulin action and metabolic control is complex and can occur at varying levels to impair signal transduction and perturb specific metabolic responses. Although its molecular mechanisms for IR and dysfunction of β cells are not completely understood, there have been some hypotheses explaining the cause, such as metabolic overload associated with ER stress. IR can be induced by high carbohydrate and/ or high fat diets and people with T2DM frequently have dyslipidemia as well as hyperglycemia, which result in glucolipotoxicity involved with dysfunction or apoptosis of pancreatic β cells (Park et al., 2011; Kim et al., personal communication). Notwithstanding, relief of ER stress by any means restores GSIS as well as insulin sensitivity, which also affects the fatty acid composition and the phospholipid distribution that has important effects on membrane function. The synthesis and compositional control of lipids in the ER also needs to be tightly coordinated with lipid trafficking and secretion (Fu et al., 2012). Opportunities exist to exploit mechanisms relating to ER management of substrate flow for disease prevention or treatment. If this flow cannot be interrupted or diverted, overwhelming ER capacity initiates a vicious cycle of stress, poor metabolic control and modification of ER membrane

itself and a worsening of ER functionality.

ER stress and nonalcoholic fatty liver disease

The mechanisms of ER stress-caused enhancement of lipid synthesis are under intense investigation. First, SREBP-1c, a transcription factor in control of FFA and TG synthetic genes, and SREBP-2, a transcription factor in control of cholesterol synthetic genes, both reside in ER and were found to be up-regulated in response to ER stress. ER stress-induced JNK activation which phosphorylates IRS-1 and ER stress-induced TRB-3 which inhibit Akt signaling result in IR. Second, ER stress associated with PERK phosphorylation of eIF-2 α , was found to induce translation of C/EBP α and β leading to increased expression of PPAR γ which regulates lipid accumulation, adding another mechanism for ER stress induced steatosis. Third, XBP-1, a transcription factor mediating the ER stress response, was found to directly regulate expression of a subset of lipogenic genes independent of SREBP, adding a complexity to ER stress-induced hepatic steatosis.

It is also suggested that the hepatic lipid composition is more important than lipid quantity in the pathogenesis of non-alcoholic steatohepatitis (NASH), which frequently accompanies the development of T2DM. Some researchers examined whether antioxidant could alter intrahepatic lipid composition and distribution (Kim et al., unpublished data). When HepG2 cells were cultured with excess FFA, it increased apoptosis, and antioxidants prevented this lipotoxicity. Antioxidants restored the intracellular mitochondrial DNA copy number and reversed the morphological changes induced by lipotoxicity. Antioxidants increased the monounsaturated and polyunsaturated FA concentrations, while they decreased the total saturated FA fraction. They also prevented the movement of intracellular free cholesterol from the cell membrane to the cytoplasm. Antioxidant appears to oppose lipotoxicity by altering the intracellular lipid composition and free cholesterol distribution.

FUNCTIONAL COMMUNICATION BETWEEN MITOCHONDRIA AND THE ER

Role of ER stress in induction of mitochondrial dysfunction

Taking structural communication between mitochondria and ER into account, cellular homeostasis depends upon the functional relationship between them. Propagation of calcium signaling from ER to mitochondria is involved in both ATP production and cell death. Moreover, the ER requires ATP to function properly, which may make it vulnerable to metabolic stress. Interactions between mitochondria and the ER facilitate control of Ca^{2+} signaling and Ca^{2+} -dependent cellular processes such as apoptosis. A number of evidences have shown that ER stress induces MD, thereby leading to disruption of various physiological responses within cells (Park et al., 2011). Prolonged ER stress leads to release of Ca^{2+} from the ER lumen, which then, leads to increased Ca^{2+} uptake into the mitochondrial matrix. Elevated

Ca^{2+} uptake induces an imbalance between mitochondrial Ca^{2+} load and the buffering capacity of the matrix, which then, leads to a prolonged episode of massive mitochondrial Ca^{2+} accumulation. Sustained Ca^{2+} accumulation triggers opening of the mitochondrial permeability transition pore, and finally, results in swelling of the organelle, rupture of the outer mitochondrial membrane, and release of proapoptotic proteins into the cytosol (Deniaud et al., 2008). ROS are also thought to act as local messengers between the ER and mitochondria (Csordás and Hajnóczky, 2009). Many ROS sources and targets are localized to the ER and mitochondria. Elevated ROS levels inactivate the sarco-endoplasmic reticulum Ca^{2+} ATPase (SERCA) and activate IP_3R via oxidation. Modulation of Ca^{2+} channel activity by ROS increases the level of Ca^{2+} on the cytosolic face of the ER and also promotes Ca^{2+} uptake into the mitochondrial matrix. Therefore, ROS production in ER provides an additional mechanism by which ER stress can induce MD.

Role of mitochondrial dysfunction in induction of ER stress

It is well-known that impairment of mitochondrial function increases ER stress, which accompanies the activation of JNK and ATF3. In fact, MD activated JNK and ATF3 along with various ER stress markers in adipose tissue. Over-expression of nuclear respiratory factor-1 (NRF-1), a transcription factor that regulates the expression of nuclear-encoded mitochondrial genes, decreased the upregulated ER stress markers associated with MD (Jeon et al., 2012). These data suggest that MD induces ER stress.

ER stress induced by MD may cause hepatic IR. Inhibition of mitochondrial function disturbs insulin signaling and increases hepatic gluconeogenesis. MD elevated the level of cytosolic-free Ca^{2+} , and increased ER stress was reversed by decreasing the level of cytosolic-free Ca^{2+} . Disturbances in Ca^{2+} homeostasis in the ER are also known to trigger the ER stress response, leading to activation of p38 mitogen-activated protein kinase, as well as increasing phosphoenolpyruvate carboxykinase (PEPCK) expression. Abnormal activation of JNK by MD also increased PEPCK expression by affecting insulin signaling and forkhead box protein O1 activity (Lim et al., 2009). Together, the results suggest that MD induces ER stress in a Ca^{2+} -dependent manner, leading to disturbance of insulin signaling and an abnormal rise in gluconeogenesis within hepatocytes.

A number of other events may contribute to the linking of MD and ER stress. For example, local ATP pools in the mitochondria and the adjacent ER may be essential to supply the energy required by SERCA to import Ca^{2+} into the lumen of the ER. Inhibition of OP cause rapid local ATP depletion in mitochondria and the ER, in which SERCA is active may reduce the uptake of Ca^{2+} into the lumen of the ER. This would cause Ca^{2+} depletion within the ER, which may trigger the ER stress response (Dumollard et al., 2004). Whether this mechanism is operative in pancreatic β -cells and/or insulin-responsive tissues remains to be determined.

COMPLEX INTERACTION OF OXIDATIVE STRESS, MITOCHONDRIAL DYSFUNCTION AND ER STRESS IN T2DM

T2DM is characterized by IR and insulin secretory defect, which is inadequate to compensate for IR. The progressive failure of β cells to secrete enough insulin to compensate for IR leads to hyperglycemia, which in turn exerts deleterious effects on β cells. Chronic ER stress found in patients with T2DM is frequently associated in peripheral and hepatic IR. Moreover, one proposed mechanism of glucose-induced β cell dysfunction is also ER stress. Prolonged exposure of β cell lines or islets to glucose increased ER stress markers and over-expression of the GRP78 partially prevented glucose-induced β cell dysfunction *in vitro* and *in vivo* (Kim et al., unpublished data; Kim et al., personal communication). ROS as a crucial intervening mediator, also play a causal role in IR and glucose-induced β cell dysfunction both *in vitro* and *in vivo*. MD is also increased in islets of individuals with T2DM. Therefore, a close interrelationship between MD, OS and ER stress in β cells has been suggested. The observation that antioxidants prevent the glucose-induced increase in ER stress markers supports the notion that ER stress lies downstream of OS. A role of antioxidants in reducing ER stress to improve insulin secretion has been documented in islets. As shown previously, the ER and mitochondria interact both spatially and functionally and an increase in mitochondrial O_2^- production in the ETC during hyperglycemia may leak into the ER, which may impair protein folding and deplete ER calcium by inhibiting SERCA, leading to ER stress. Thus, it is possible that antioxidants mitigate ER stress via reducing mitochondrial O_2^- . However, there is also evidence that ER stress can induce OS.

As shown above, MD is also associated with OS. PGC-1 α , playing an important role in fatty acid oxidation and mitochondrial biogenesis, decreases intracellular ROS generation by increasing the expression of antioxidant genes, reduced cell apoptosis and ROS generation in endothelial cells by increasing ATP/ADP translocase activity of adenine nucleotide translocator (ANT) (Kim et al., 2010). ANT function is essential for PGC-1 α as it provides a continuous supply of ADP to mitochondria. Sirt 3 also functions as a downstream target gene of PGC-1 α in muscle cells and hepatocytes and mediates the PGC-1 α 's effects on cellular ROS production and mitochondrial biogenesis (Kong et al., 2010). The elucidation of the molecular mechanisms of mitochondrial regulation and its physiological functions may provide a novel target for treating diseases related with OS.

Therefore, MD and ER stress plays a causal role in IR as well as in glucose-induced β cell dysfunction *in vivo*, and that there is a reciprocal link between OS and MD induced ER stress in IR and glucose-induced β cell dysfunction *in vivo*. Therefore, it could be possible that overexpressing ER chaperones or developing small molecules mimicking ER chaperone are also of potential interest to counteract IR or to preserve β cell function in T2DM.

Glucotoxicity, lipotoxicity, and glucolipotoxicity are proposed to play a role in all forms of T2DM. Once the primary pathogenesis

of diabetes is established, probably involving both genetic and environmental forces, hyperglycemia and very commonly, hyperlipidemia ensue and thereafter exert additional damaging or toxic effects on the β -cell as well as on the insulin-responsive tissue. In addition to their contribution to the increase in IR, elevations of plasma FFA levels along with hyperglycemia affect deleterious effect on insulin secretion, insulin gene expression, and β -cell death. The central role of glucose in the mechanisms of glucolipotoxicity has been suggested, unifying the underlying mechanisms of T2DM with an emphasis on the role of OS. We all agree that the crucial role of these phenomena in the natural history of β -cell compensation, decompensation, and failure during the course of T2DM. Furthermore, MD and ER stress are considered to be the key determinants of IR as well as insulin secretory defect. Some researchers suggested mechanistic linkages among obesity, ER stress, IR, and T2DM. These include chronic 'metabolic' inflammation characterized by altered production of adipokines/cytokines and the infiltration of immune cells into tissues, lipotoxicity and ectopic fat accumulation in the liver and perhaps skeletal muscle and decreased mitochondrial function. These pathways have been studied as independent mechanisms for the induction of IR (Hotamisligil, 2010). The mechanism by which ER stress impairs insulin action and metabolic control is complex and can occur at several levels to impair signal transduction and direct or indirect perturbation of specific metabolic responses. Although its molecular mechanisms for IR and dysfunction of β cells are not completely understood, there have been some hypotheses explaining the cause, such as metabolic overload associated with ER stress.

CONCLUSION

Based on abundant data on correlations between OS and components of MS, namely atherosclerosis, hypertension, T2DM, adiposity, and IR, we could speculate that OS is an early event in the pathology of MS or a candidate for a central pathogenic role. Increase in advanced oxidized plasma protein, which are indicators of nitrosative stress, have been found in MS, and a significant decrease in antioxidant status were observed in patients with MS. T2DM, one of important component disease of MS, is characterized by IR and insulin secretory dysfunction. In fact, hyperglycemia is only the most important biochemical marker of diabetes, and the crucial contributor to the development of diabetes complications is the cumulative effect of chronic hyperglycemia. Admitting that OS may be the key mediators of the deleterious effects of hyperglycemia, mitochondria play a central role in the generation of ROS and cell apoptosis, suggesting that MD might be a crucial pathogenic cause for development of diabetes and its complication. A number of conditions including T2DM with nutrient excess that interfere with proper ER function may also lead to apoptotic downstream signaling pathways. These could accompany chronic 'metabolic' inflammation characterized by excessive production of cytokines and the infiltration of immune cells into

tissues, lipotoxicity and ectopic fat accumulation in the liver and muscle, and most importantly, decreased mitochondrial function. Owing to the structural and functional communications between mitochondria and the ER, MD and ER stress causes apoptotic cell death by increasing OS and disturbing mitochondrial biogenesis. Therefore, MD and ER stress are inseparable pathogenic causes for the development of T2DM and its complications, and they have also been known to be most important early causes for the development of diabetes and its complications. Admitting the roles of OS, MD, and ER stress are major culprits to initiate the metabolic diseases such as diabetes and its complications, we can mitigate β -cell dysfunction, peripheral IR and ED by way of targeting either stress separately. Although we do not know which comes first, abundant measures to regulate mitochondrial functions and ER stress reduction as well as antioxidant treatment are to be investigated.

ACKNOWLEDGEMENTS

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2010-0010898) and the Korea Health 21 R&D Project, Ministry of Health and Welfare, Republic of Korea (A102065).

Original Submission: February 18, 2014

Revised Version Received: March 3, 2014

Accepted: March 6, 2014

REFERENCES

- Ballinger, S.W., Shoffner, J.M., Hedaya, E.V., Trounce, I., Polak, M.A., Koontz, D.A., and Wallace, D.C. (1992). Maternally transmitted diabetes and deafness associated with a 10.4kb mitochondrial DNA deletion. *Nat Genet* **1**, 11-15.
- Battino, M., Bullon, P., Wilson, M., and Newman, H. (1999). Oxidative injury and inflammatory periodontal diseases: the challenge of anti-oxidants to free radicals and reactive oxygen species. *Crit Rev Oral Biol Med* **10**, 458-476.
- Boulton, A.J. (2007). Diabetic neuropathy: classification, measurement and treatment. *Curr Opin Endocrinol Diabetes Obes* **14**, 141-145.
- Brownlee, M. (2005). The pathobiology of diabetic complications. An unifying mechanism. *Diabetes* **54**, 1615-1625.
- Csordás, G., and Hajnóczky, G. (2009). SR/ER-mitochondrial local communication: calcium and ROS. *Biochimica et Biophysica Acta* **1787**, 1352-1362.
- Cunha, D.A., Hekerman, P., Ladrrière, L., Bazarra-Castro, A., Ortis, F., Wakeham, M.C., Moore, F., Rasschaert, J., Cardozo, A.K., Bellomo, E., Overbergh, L., Mathieu, C., Lupi, R., Hai, T., Herchuelz, A., et al. (2008). Initiation and execution of lipotoxic ER stress in pancreatic β -cells. *J Cell Sci* **121**, 2308-2318.
- Deniaud, A., Sharaf el dein, O., Maillier, E., Poncet, D., Kroemer, G., Lemaire, C., and Brenner, C. (2008). Endoplasmic reticulum stress induces calcium-dependent permeability transition, mitochondrial outer membrane permeabilization and apoptosis. *Oncogene* **27**, 285-299.
- Dumollard, R., Marangos, P., Fitzharris, G., Swann, K., Duchon, M., and Carroll, J. (2004). Sperm-triggered $[Ca^{2+}]$ oscillations and Ca^{2+} homeostasis in the mouse egg have an absolute requirement for mitochondrial ATP production. *Development* **131**, 3057-3067.
- Eizirik, D.L., Cardozo, A.K., and Cnop M. (2008). The role for endoplasmic reticulum stress in diabetes mellitus. *Endocrine Reviews* **29**, 42-61.
- Fu, S., Watkins, S.M., and Hotamisligil, G.S. (2012). The role of

- endoplasmic reticulum in hepatic lipid homeostasis and stress signaling. *Cell Metabolism* **15**, 623-634.
- Grattagliano, I., Palmieri, V.O., Portincasa, P., Moschetta, A., and Palasciano, G. (2008). Oxidative stress-induced risk factors associated with the metabolic syndrome: a unifying hypothesis. *J Nutr Biochem* **19**, 491-504.
- Hamilton, S.J., and Watts, G.F. (2013). Endothelial dysfunction in diabetes: pathogenesis, significance, and treatment. *Rev Diabet Stud* **10**, 133-56.
- Harding, H.P., Zhang, Y., and Ron, D. (1999). Protein translation and folding are coupled by an endoplasmic-reticulum-resident kinase. *Nature* **397**, 271-274.
- Harding, H.P., Novoa, I., Zhang, Y., Zeng, H., Wek, R., Schapira, M., and Ron, D. (2000). Regulated translation initiation controls stress-induced gene expression in mammalian cells. *Mol Cell* **6**, 1099-1108.
- Hendershot, L.M. (2004). The ER function BiP is a master regulator of ER function. *Mt Sinai J Med* **71**, 289-97.
- Hopps, E., Noto, D., Caimi, G., and Aversa, M.R. (2010). A novel component of the metabolic syndrome: the oxidative stress. *Nutr Metab Cardiovasc Dis* **20**, 72-77.
- Hotamisligil, G.S. (2010). Endoplasmic reticulum stress and the inflammatory basis of metabolic disease. *Cell* **19**, 900-17.
- Jeon, M.J., Leem, J., Ko, M.S., Jang, J.E., Park, H.S., Kim, H.S., Kim, M., Kim, E.H., Yoo, H.J., Lee, C.H., Park, I.S., Lee, K.U., and Koh, E.H. (2012). Mitochondrial dysfunction and activation of iNOS are responsible for the palmitate-induced decrease in adiponectin synthesis in 3T3L1 adipocytes. *Exp Mol Med* **30**, 562-70.
- Kars, M., Yang, L., Gregor, M.F., Mohammed, B.S., Pietka, T.A., Finck, B.N., Patterson, B.W., Horton, J.D., Mittendorfer, B., Hotamisligil, G.S., and Klein, S. (2010). Tauroursodeoxycholic acid may improve liver and muscle but not adipose tissue insulin sensitivity in obese men and women. *Diabetes* **59**, 1899-1905.
- Kelley, D.E., He, J., Menshikova, E.V. and Ritov, V.B. (2002). Dysfunction of mitochondria in human skeletal muscle in type 2 diabetes. *Diabetes* **51**, 2944-2950.
- Kim, E.H., Koh, E.H., Park, J.Y., and Lee, K.U. (2010). Adenine nucleotide translocator as a regulator of mitochondrial function: implication in the pathogenesis of metabolic syndrome. *Korean Diabetes J* **34**, 146-153.
- Kim, H.O., Park, L.J., Chae, H.J., and Park, Y. (submitted). The inhibition of ER stress and improvement of hepatic insulin resistance as well as β -cell dysfunction in OLETF rats. *Diabetes*.
- Kim, H.O., Park, L.J., Chae, H.J., and Park, Y. (in press). The inhibition of ER stress and improvement of alloimmunity in β -cell transplantation. *Transplantation*.
- Kim, H.O., Park, L.J., Lim, M.S., and Park, Y. (in press). Antioxidant activates AMPK and ameliorates Non-Alcoholic Fatty Liver Disease in OLETF Rats. *PLoS One*.
- Koh, E.H., Park, J.Y., Park, H.S., Jeon, M.J., Ryu, J.W., Kim, M., Kim, S.Y., Kim, M.S., Kim, S.W., Park, I.S., Youn, J.H., and Lee, K.U. (2007). Essential role of mitochondrial function in adiponectin synthesis in adipocytes. *Diabetes* **56**, 2973-2981.
- Kong, X., Wang, R., Xue, Y., Liu, X., Zhang, H., Chen, Y., Fang, F., Chang, Y. (2010). Sirtuin 3, a new target of PGC-1 α , plays an important role in the suppression of ROS and mitochondrial biogenesis. *PLoS One* **22**, e11707.
- Lee, A.H., Iwakoshi, N.N., and Glimcher, L.H. (2003). XBP-1 regulates a subset of endoplasmic reticulum resident chaperone genes in the unfolded protein response. *Mol Cell Biol* **23**, 7448-7459.
- Lim, J.H., Lee, H.J., Jung, M.H., and Song, J. (2009). Coupling mitochondrial dysfunction to endoplasmic reticulum stress response: a molecular mechanism leading to hepatic insulin resistance. *Cellular Signalling* **21**, 169-177.
- Maechler, P. and Wollheim, C.B. (2001). Mitochondrial function in normal and diabetic β -cells. *Nature* **414**, 807-812.
- Özcan, U., Cao, Q., Yilmaz, E., Lee, A.H., Iwakoshi, N.N., Ozdelen, E., Tuncman, G., Görgün, C., Glimcher, L.H., and Hotamisligil, G.S. (2004). Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. *Science* **306**, 457-461.
- Özcan, U., Yilmaz, E., Özcan, L., Furuhashi, M., Vaillancourt, E., Smith, R.O., Görgün, C.Z., and Hotamisligil, G.S. (2006). Chemical chaperones reduce ER stress and restore glucose homeostasis in a mouse model of type 2 diabetes. *Science* **313**, 1137-1140.
- Park, L.J., Min, D.S., Kim, H.O., Chung, H.Y., Lee, C.H., Park, I.S., Kim, Y.H., and Park, Y. (2011). TAT-enhanced delivery of metallothionein can partially prevent the development of diabetes. *Free Radic Biol Med* **51**, 1666-74.
- Patti, M.E., and Corvera, S. (2010). The role of mitochondria in the pathogenesis of type 2 diabetes. *Endocrine Reviews* **31**, 364-395.
- Pérez-Carreras, M., Del Hoyo, P., Martín, M.A., Rubio, J.C., Martín, A., Castellano, G., Colina, F., Arenas, J., and Solís-Herruzo, J.A. (2003). Defective hepatic mitochondrial respiratory chain in patients with nonalcoholic steatohepatitis. *Hepatology* **38**, 999-1007.
- Petersen, K.F., Dufour, S., Befroy, D., Garcia, R., and Shulman, G.I. (2004). Impaired mitochondrial activity in the insulin-resistant offspring of patients with type 2 diabetes. *N Engl J Med* **350**, 664-671.
- Roberts, C.K., and Sindhu, K.K. (2009). Oxidative stress and metabolic syndrome. *Life Sci* **84**, 705-712.
- Ron, D., and Walter, P. (2007). Signal integration in the endoplasmic reticulum unfolded protein response. *Nature Rev Mol Cell Biol* **8**, 519-529.
- Scheuner, D., and Kaufman, R.J. (2008). The unfolded protein response: a pathway that links insulin demand with β -cell failure and diabetes. *Endocrine Rev* **29**, 317-333.
- Shen, J., Chen, X., Hendershot, L., and Prywes, R. (2002). ER stress regulation of ATF6 localization by dissociation of BiP/GRP78 binding and unmasking of golgi localization signals. *Developmental Cell* **3**, 99-111.
- Testfaye, S., Chaturvedi, N., Eaton, S.E., Ward, J.D., Manes, C., Ionescu-Tirgoviste, C., Witte, D.R., Fuller, J.H. and EURODIAB Prospective Complications Study Group. (2005). Vascular risk factors and diabetic neuropathy. *N Engl J Med* **352**, 341-350.
- Turrens, J.F. (2003). Mitochondrial formation of reactive oxygen species. *J Physiol* **552**, 335-344.
- UK Prospective Diabetes Study (UKPDS) Group. (1998). Intensive blood glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Lancet* **352**, 837-853.
- Valko, M., Leibfritz, D., Moncol, J., Cronin, M.T., Mazur, M., and Telser, J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol* **39**, 44-84.
- van Marken Lichtenbelt, W.D., Vanhomerig, J.W., Smulders, N.M., Drossaerts, J.M., Kemerink, G.J., Bouvy, N.D., Schrauwen, P., and Teule, G.J. (2009). Cold-activated brown adipose tissue in healthy men. *N Engl J Med* **360**, 1500-1508.