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NEAR EAST UNIVERSITY

GRADUATE SCHOOL OF HEALTH SCIENCES

THE EFFECT OF ENDOPLASMIC RETICULUM STRESS IN TYPE 2 DIABETES MELLITUS

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MEDICAL BIOCHEMISTRY PROGRAM

GRADUATION PROJECT

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SUPERVISOR

ASSOCIATE PROFESSOR ÖZLEM DALMIZRAK

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The Directorate of Graduate School of Health Sciences,

This study has been accepted by the project committee in Medical Biochemistry Program as a Master Project.

Project committee:

Chair:	Professor Nazmi Özer Near East University
Supervisor:	Associate Professor Özlem Dalmızrak Near East University
Member:	Assistant Professor Besim Özyel Lefke European University

Approval:

According to the relevant articles of the Near East University Postgraduate Study-Education and Examination Regulations, this project has been approved by the above-mentioned members of the project committee and the decision of the Board of Graduate School of Health Sciences.

Professor Hüsnü Can Başer
Director of the Graduate School of Health Sciences

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I am grateful to Almighty God for a work well done.

I wish to express my thanks to all the faculty members of the Department of Medical Biochemistry for the continued support especially my supervisor Associate Professor Özlem Dalmızrak and Professor Nazmi Özer.

ABSTRACT

Johnson I.D. The effect of endoplasmic reticulum stress in type 2 diabetes mellitus. Near East University, Graduate School of Health Sciences, Graduation Project in Medical Biochemistry Program, Nicosia, 2018.

Endoplasmic reticulum (ER) is a significant intracellular calcium store and site where proteins migrating to the secretory pathway are synthesized, folded, modified and conveyed to their final extracellular destination. Unsettling influences in any of these capacities brings about a change in the normal folding and secretory ability of the ER and an elevated load for unfolded proteins clinched alongside its lumen characterized condition called “ER stress”. Endoplasmic reticulum activates an intricate intracellular signal transduction pathway and is principally formed on launching ER homeostasis. Failure of ER function activates cell death which is in form of apoptosis. Endoplasmic reticulum contributes to the reason for a few human pathologies in particular diabetes, obesity, neurodegeneration and malignancy.

The cell responds with to ER stress by initiating a protective mechanism known as “Unfolded Protein Response” (UPR) which comprises of;

- Rebuilding of normal cell activities by halting protein translation,
- Debasement of incorrectly folded protein and
- Initiation of signaling pathways that prompts the expansion of molecular chaperones included in the protein folding.

Over cases of extreme stress, or where the above three factors are not achieved over a particular period, initiation of apoptosis and disposal of the dead cell may be triggered eventually leading to UPR.

Key words: Endoplasmic reticulum stress, unfolded protein response, apoptosis, type 2 diabetes mellitus

ÖZET

Johnson I.D. Endoplazmik retikulum stresinin tip 2 diabetes mellitus üzerine etkisi. Yakın Doğu Üniversitesi, Sağlık Bilimleri Enstitüsü, Tıbbi Biyokimya Programı, Mezuniyet Projesi, Lefkoşa, 2018.

Endoplazmik retikulum (ER) hücre içi kalsiyum deposu ve salınacak proteinlerin sentezlenip, katlandıkları, modifiye edildikleri ve hücre dışındaki son varış yerlerine hedeflendikleri organel olarak görev yapmaktadır. Bu işlevlerden herhangi birisindeki kayıp, endoplazmik retikulumun normal katlanma ve salınım fonksiyonunu değiştirerek katlanmamış proteinlerin lümende birikmesine ve "ER stresi"nin ortaya çıkmasına neden olmaktadır. Endoplazmik retikulum hücre içinde oldukça karmaşık bir sinyal iletim yolağını aktifleştirerek ER homeostazını sağlamaya çalışmaktadır. Başarısız olduğu durumda ise apoptoz ile hücre ölümü gerçekleşmektedir. Endoplazmik retikulumun işlevleri diyabet, obezite, nörodejenerasyon ve kanser gibi birçok patolojik duruma katkıda bulunmaktadır.

Hücre ER stresine bir takım koruyucu mekanizmaları başlatarak yanıt vermektedir. "Katlanmamış Protein Cevabı" (Unfolded Protein Response-UPR) olarak bilinen bu yanıt;

- Proteinlerin translasyonunu durdurarak normal hücre aktivitelerinin yeniden oluşturulmasını,
- Yanlış katlanmış proteinlerin düzeltilmesini ve
- Proteinlerin katlanmasını sağlayan moleküler şaperonların arttırılmasını içermektedir.

Yoğun stres durumunda ya da yukarıdaki işlevler gerçekleştirilemediğinde apoptoz başlamakta ve sonuç olarak da hücrenin yıkımı gerçekleşmektedir.

Anahtar Kelimeler: Endoplazmik retikulum stresi, katlanmamış protein cevabı, apoptoz, tip 2 diabetes mellitus

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AP-1	: Activator protein-1
ASK	: Apoptosis-signal regulating kinase
ATF	: Activating transcription factor
BiP	: Ig heavy chain binding protein (also known as GRP78 or HSPA5)
C/EBP	: CCAAT/enhancer binding protein
CHOP	:C/EBP homologous protein (also known as GADD153 or DDIT3)
CPA	: Cyclopiazonic acid
eIF	: Eukaryotic translation initiation factor
ERK1	: Extracellular Signal regulated Kinase 1
FFA	: Free Fatty Acid
IL	: Interleukin (cytokine)
IRS	: Insulin receptor substrate
IRS-1	: insulin receptor signal 1
JNK	: c-Jun N-terminal kinase
MAPK	: Mitogen-Activated Protein Kinase
NF-κB	: Nuclear factor κB
NO	: Nitric oxide
NOD	: Non-obese diabetic
NOS	: Nitric Oxide System
ORF	: Open reading frame
ORP150	: Oxygen-regulated protein 150 (also known as GRP170)
PDI	: Protein disulfide isomerase
PERK	: PKR-like ER kinase
PKR	: Double-stranded RNA-activated kinase
PP	: Protein phosphatase
rER	: rough Endoplasmic Reticulum
ROS	: Reactive oxygen species

S1P	: Site-1 protease
sER	: smooth Endoplasmic Reticulum
SERCA	: Sarcoendoplasmic reticulum Ca^{2+} ATPase
SNAP	: Synaptosomal-Associated Protein
SREBP	: Sterol-response element-binding protein
STAT	: Signal transducer and activator of transcription
T1D	: Type 1 Diabetes
T2D	: Type 2 Diabetes
TRAF	: TNF receptor-associated factor
uORF	: Upstream open reading frame
UPR	: Unfolded protein response
WFS1	: Wolfram syndrome gene 1
XBP	: X-Box Binding Protein (virology)
XBP1	: X-box binding protein-1
XBP1s	: Spliced XBP1

LIST OF FIGURES

1. INTRODUCTION

Endoplasmic reticulum (ER) is an exceptionally unique organelle with a general function in lipid and protein synthesis. ER delivers the transmembrane proteins and lipids for most cell organelles and is in charge of the biosynthesis of all extracellular proteins. The endoplasmic reticulum plays a role in Ca^{2+} signaling. The resting intra-ER Ca^{2+} concentration is 3 to 4-fold higher than cytosolic Ca^{+2}, this difference is produced by the sarco(endo)plasmic reticulum Ca^{2+} ATPase (SERCA) proteins, which draw Ca^{2+} into the ER through the action of either inositol 1,4,5-trisphosphate or ryanodine receptors (Berridge, 2002). Because of its capacity to store and release Ca^{2+}, the ER regulates an extensive variety of cell functions, for example, organogenesis, transcriptional action, stress reactions and apoptosis. The key point of the unfolded protein response (UPR) is to reduce ER stress, reestablish ER homeostasis, and halt cell death. To accomplish these objectives, the UPR prompts a few facilitated reactions, including:

- entry of novel proteins into the ER reduces, consequently keeping extra protein misfolding and over-burdening of the organelle
- ER chaperones increase, hence elevating the folding rate of the ER to manage the incorrectly folded proteins
- irreversible incorrectly folded proteins from the ER increases and leading to the breakdown of these proteins in the proteasome
- apoptosis is activated if the above steps fails.

Dysfunction of the UPR plays an important role in certain diseases, especially those involving tissues dedicated to extracellular protein synthesis. Diabetes is an example of such a disease, since pancreatic beta-cells depend on efficient UPR signaling to meet the demands for constantly varying levels of insulin synthesis. Recent studies have indicated that the importance of the UPR in diabetes is not restricted to the beta-cells but also to tissues of peripheral insulin resistance such as liver and adipose tissue (Sundar Rajan et al., 2007). Better understanding of the basic mechanisms of ER stress and development of insulin resistance/type 2 diabetes is pivotal for the identification of newer molecular targets for therapeutic interventions.

2. GENERAL INFORMATION

2.1. Endoplasmic reticulum and unfolded protein response pathway

Endoplasmic reticulum (ER) constitutes about half of the membranous structure in cell. There are two main types of ER. Rough endoplasmic reticulum (rER) is found opposite to the cell nucleus with its surface covered with ribosomes. The attached ribosomes play a role in the synthesis of proteins that have a signal sequence which helps proteins to enter ER for processing while the free ribosomes located within the cell are responsible for targeting to other organelles for their synthesis and secretion. Synthesized protein has several fates. Some proteins remain where they are synthesized in endoplasmic reticulum, others are channeled to golgi complex located close to endoplasmic reticulum. Golgi complex secreted proteins are transported to the lysosomes, while others are channeled for secretion to the cell exterior. During transportation to golgi complex, proteins are transferred from ribosome into the rER lumen as shown in Figure 2.1 below where they are modified, folded and assembled. The closeness of the rER to the nucleus makes protein processing possible (Alberts et al., 2002).

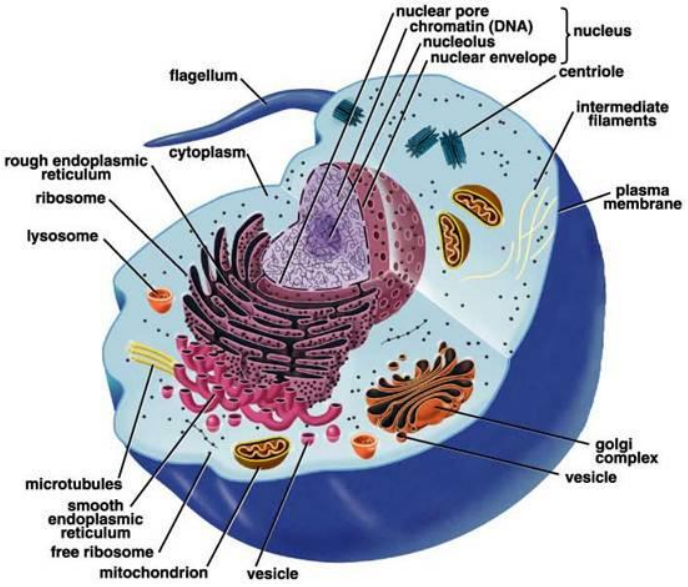

Figure 2.1. Animal cell showing smooth and rough endoplasmic reticulum (https://akinneycellproject.weebly.com)

Rough endoplasmic reticulum sends signal to the nucleus upon detecting a stress during protein folding/synthesis and hence affecting protein translation. A signaling mechanism called unfolded protein response (UPR) is activated when an unfolded or misfolded protein is found in the endoplasmic reticulum lumen. Unfolded protein response adaptive nature leads to a decrease in protein synthesis, repairs the incorrectly folded proteins or degrades them. But in cases of severe damage/misfold, the cell is triggered to undergo apoptosis (Anirikh et al., 2012).

Smooth endoplasmic reticulum is differentiated from rER by the absence of ribosomes but directly associated with the synthesis of lipids, cholesterol and phospholipids which are used in *de novo* synthesis of cell membrane. Apart from biosynthesis of steroid hormones from cholesterol, it is directly involved in the detoxification of drugs and dangerous chemicals in the liver (Alberts et al., 2002). Sarcoplasmic reticulum is a special type of smooth endoplasmic reticulum which controls the calcium ion content in the cytoplasm of muscle cell. This elevation of ER lumen is required to support the extended number of luminal constituents especially the ones that continuously synthesized to manage ER stress and appears to be adaptative to adjusting to the increasing crowdy environment which aids protein aggregation and are detrimental to the correct protein folding. Through molecule understanding, new restorative therapeutic methodologies for various human diseases caused by protein unfolding in the endoplasmic reticulum have been found (Valastyan and Lindquist, 2014).

Alarm is sent from the ER to the nucleus, since these reactions are depended to a limited extent on new gene transcription hereby demonstrating an urgent need of significant proteins and mRNAs. These signaling are moderated by three transmembrane ER proteins: inositol requiring enzyme 1 (IRE1), activating transcription factor (ATF) and protein kinase RNA-like ER kinase (PERK) (Figure 2.2). When unfolded proteins rise in the lumen of the endoplasmic reticulum, these proteins end up activated thereby making interpretation of this message into signals that balance expression of vital protein and gene (Alberts, 2002).

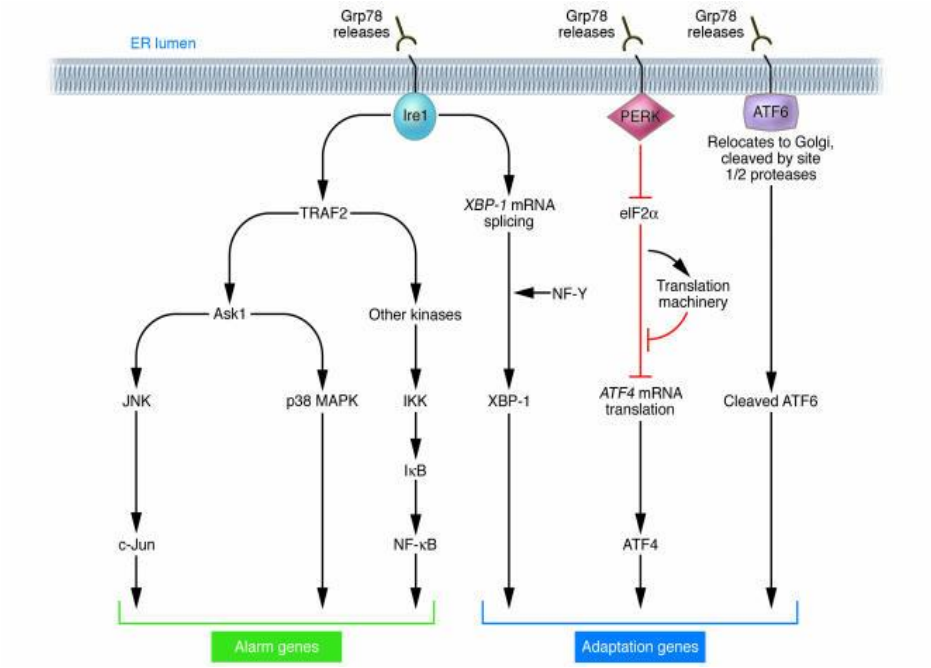

Figure 2.2. Pathway showing how grp 78 is released from endoplasmic reticulum lumen (Chunyan et al., 2005).

As the ER's protein folding needs exceed its ability to manage protein folding mechanism, cells encounter a different form of stress called "Endoplasmic Reticulum Stress" and this prompts the UPR pathway as shown in Figure 2.3. Unfolded protein response (UPR)'s outputs at first diminish endoplasmic reticulum stress through augmenting the levels off ER chaperones and protein folding activities. When ER stress cannot be managed via these adaptive outcomes in eukaryotes, the UPR initiates an inverse mechanism to rather promote destructive outcomes, including sterile inflammation and ultimately programmed cell death (Tabas and Ron, 2011).

The coordinated consequence of the UPR is lessening of general protein interpretation corresponded by up-control of ER chaperones, therefore expanding the folding rate in the ER, and the breakdown of incorrectly folded proteins. The apoptosis pathway will be activated when UPR fails to handle ER stress. Endoplasmic reticulum stress can prompt apoptosis by different pathways, including the activation of a portion of the key controllers of the UPR pathway. Consequently, IRE1α has been demonstrated to enroll the connector molecules TNF receptor-related factor 2 (TRAF2) and activate c-

Jun N-terminal kinase (JNK) with the downstream proapoptotic kinase apoptosis-signal directing kinase (ASK1) (Urano et al., 2000).

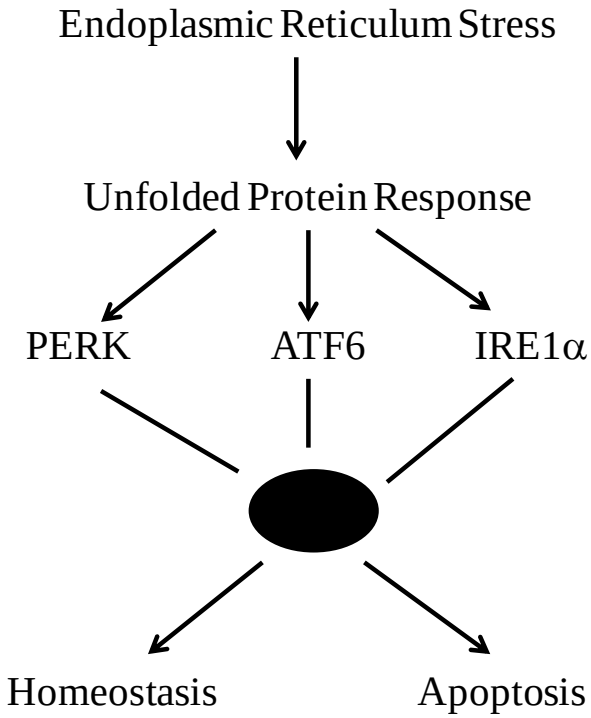

Figure 2.3. Link between endoplasmic reticulum stress and unfolded protein response (https://papalab.ucsf.edu/publications/research-0).

Neurons from ASK1−/− mice are impermeable to endoplasmic reticulum stress-mediated cell destruction, recommending that endoplasmic reticulum stress-stimulated JNK and ASK is proapoptotic (Nishitoh et al., 2002). The IRE1/TRAF2 complex can likewise prompt nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) initiation that may have an anti- or pro-apoptotic impact possible to the cell type and amount (Ortis et al,. 2006). The IRE1α/TRAF2 coupling additionally is needed for the stimulation of procaspase 12, and may add to the manifestation of ER stress activated apoptosis (van Huizen et al., 2003).

Several researchers have described the versatile benefits coming from IRE1α. Current research has shown that IRE1α has a double activity of life and death switches elective outputs relying on the strength of upstream stresses which is a crucial key in structuring therapy. IRE1α as alternate RNase outputs that are regulated by its kinase

domain to encourage dissimilar cell fates under endoplasmic reticulum stress. A study showed that excess activation of IRE1α caused the ER-localized degradation of many mRNAs in mammalian cells and ensuing exhaustion of these mRNAs prompting mammalian cells to enter apoptosis (Doyle et al., 2001). This is the principal reason of how IRE1α functions as life-death switch and it is this groundwork information that molds the essential premise of therapeutic intercession in utilization of small molecule modulators of IRE1α. Endoplasmic reticulum stress mediated apoptosis is generally linked to several critical human diseases.

2.2. Diabetes mellitus

Formally known as diabetes, it is a metabolic disorder characterized by high blood sugar concentration. Glucose builds up in the blood in the absence of insulin and cause high glucose level in the blood which has capacity to harm blood vessels in the kidney, heart, eye or nervous system causing heart disease, stroke, kidney disease, blindness and nerve damage if not treated earlier (Seino et al., 2010).

2.2.1. Type one diabetes mellitus

Type one diabetes mellitus (T1D) is also called insulin-dependent diabetes or juvenile-onset diabetes because it is commonly found in children. It is also an autoimmune condition mediated by the attack of body antibodies and destroying healthy cells that are responsible for producing β cells in the pancreas. Since the immune system mistakenly recognizes the healthy cells as foreign invaders, the body is unable to produce insulin. Though researchers do not know the reason for this autoimmune attack but current research is ongoing to give biochemical explanation to this puzzle. Hence researchers have concluded that this autoimmune attack may be due to genetic predisposition, environmental factors like exposure to virus or faulty β cells in the pancreas (Seino et al., 2010).

2.2.2. Type two diabetes mellitus

Type two diabetes mellitus (T2D) is also known as non-insulin dependent diabetes. It occurs when β-cells fail to secret the required or enough insulin to stimulate glucose uptake by peripheral tissues (Kahn, 2001) which results in glucose tolerance reduction which leads to hyperglycemia (Weyer et al., 1999). It is either the amount of insulin produced is not enough or the cells cannot use it effectively and resist it (insulin resistance - zero sensitivity to insulin commonly found in adipose tissues and muscles). Researchers are trying to explain the puzzle why some become insulin resistant while others do not, though various life activities like excess weight and inactivity may contribute to it (Wilcox, 2005)

2.2.3. Gestational diabetes

This is activated by pregnancy and leads to insulin resistance to some degree. An elevated blood glucose is present in the mother and circulated to the body via the placenta. It is normally below 2% to 10% according to National Institution of Health and 10% of ladies with gestational diabetes experience T2D. It has been reported that T2D patients experience a decrease in the apoptosis of beta cells (Butler et al., 2003). This decreased rate of beta cell loss in both T1D and T2D has been linked to stress responses controlled by vital transcription element and gene pool (Cnop et al., 2005).

2.3. Endoplasmic reticulum stress and type two diabetes

Diabetes mellitus is a model of ER stress-mediated cell degeneration. Causes of endoplasmic reticulum stress in pancreatic β-cells are given in Figure 2.4. For instance, T2D happens when half of pancreatic islet β-cells (pancreatic specialized cells that known to synthesize and secrete insulin) deteriorates, prompting low insulin response in cell. A current study has suggested that pancreatic β-cells might be weakening as they attempt to show insulin resistance in relation to obesity, over nutrition, hyperglycemia, high fat, and high carbohydrate diets prompting to apoptosis. Over work of all these cells might lead them to encounter a raised ER stress chronically. A raised ER stress puts the β-cells at a higher risk of deterioration with the UPR triggering apoptosis as

shown in Figure 2.5 more β-cells undergoes intense ER stress due to the constant of metabolic activity per cell. Similar logic may apply in T1D, as β-cells are subject to immune attack (Oakes and Papa, 2015).

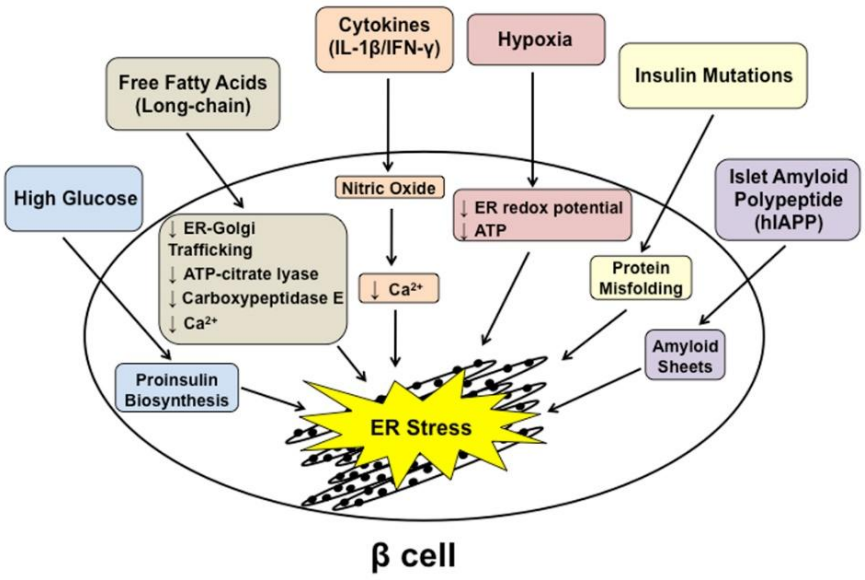

Figure 2.4. Diagram showing the major causes of endoplasmic reticulum stress (Fonseca et al., 2011).

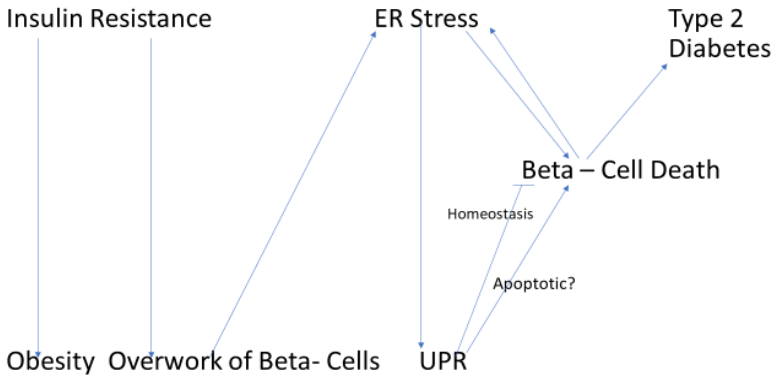

Figure 2.5. Endoplasmic reticulum stress and insulin resistance (https://papalab.ucsf.edu/publications/research-0).

2.4. Endoplasmic reticulum stress and human diabetes

Genetic change in EIF2AK3, coding for human eIF2α kinase (comparable with rat PERK), leads to monogenic diabetes in Wolcott-Rallison disorder-an uncommon health issue portrayed by neonatal or early-childhood stages insulin-independent diabetes (Delepine et al., 2000). EIF2AK3 transformations recognized currently leads to a truncated eIF2α kinase with practically zero action (Senee et al., 2004). Research studies has shown incorporate pancreatic hypoplasia and β-cell deletion (Thornton et al., 1997). This infection features the significance of PERK-intermediate endoplasmic reticulum stress reaction in the control of typical beta cell capacity and existence, in neonatal life; its significance for beta-cells in grown-ups have been addressed (Zhang et al,. 2006).

In Wolfram disorder, transformations in Wolfram disorder gene 1 (WFS1), which encodes an ER Ca^{+2} channel, prompt early diabetes related with particular beta-cell deletion, optic decay, sensorineural deafness, diabetes insipidus, and neurological signs (Inoune et al., 1998). Hereditary characteristics in the WFS1 gene has been related with T2D (Minton et al., 2002). ATF6 has additionally been specific as an uncommon T2D vulnerability gene in Pima Indians (Thameem et al., 2006). A relationship with insulin synthesis has been studied for DNA variation reaching out into the 5′ end of ATF6 gene (Das et al., 2006).

Straightforward confirmation of ER stress in T2D originates from an ongoing report by Laybutt et al. In this manner, higher levels were observed for BiP, CHOP, and p58IPK in beta-cells from pancreas segments of T2D patients was different with nondiabetic pancreas tissues (Laybutt et al., 2007), though another studies showed an increase in ER analysis in beta-cells from T2D patient when linked with nondiabetic patients (Marchetti et al., 2007). An elevation was seen in ATF3, downstream of eIF2α-ATF4 (Jiang et al., 2004), additionally appeared in insulin-positive cells in pancreatic segments of T2D individuals (Hartman et al., 2004). Huang et al., as of late revealed an elevated CHOP articulation in β-cells from overweight people, regardless of absence or presence of diabetes. In the pancreatic segments of people with diabetes, CHOP was frequently observed in nucleus rather than the cytoplasmic existence of CHOP in the

overweight people (Huang et al., 2007). It was theorized in view of these discoveries, that CHOP nuclear migration is specific and unique level for apoptosis activation, despite the fact that there is no research information in different level of ER stress to clarify this theory. CHOP may be activated by numerous of cell stress, and amyloid precursor protein appeared to initiate CHOP articulation in neurons without an UPR (Copanaki et al., 2007). Extracellular IAPP oligomers activated heat shock protein 90 in MIN-6 cells and human islets, and gentle XBP1 grafting in MIN-6 cells. Restriction of ubiquitin-proteasome signal mechanism was embroiled in the IAPP-initiated β-cell apoptosis (Casas et al., 2007). An ongoing investigation of the analysis of ER stress markers in islets differentiated from T2D organ donors demonstrated a no sign when compared with control islets after three to six days cultured at 5 mM glucose. lslets from diabetic patients activated BiP & X-box binding protein-1 bond uniquely after an elevation to 11 mM glucose, while islets were not found from nondiabetic individuals (Marchetti et al., 2007).

2.5. Endoplasmic reticulum stress and diabetes in experimental animals (mouse)

The C96Y genetic change in insulin prevents the development of a single disulfide bonds in between A and B chains (Wang et al., 1999). The incorrectly folded proinsulin elevation in ER, is sequenced to BiP, and finally broken down. The presence of typical insulins one and two allele in the heterozygous Akita mouse, makes the mice to experience diabetes because of dynamic beta cell destruction triggered by ER stress. In heterozygous, yet not homozygous, Akita mice, the homozygous interruption of CHOP postponed diabetes improvement from 8 to 10 weeks (Oyadomari et al., 2001), proposing that the cell destruction system is likely to be CHOP-independent. An ongoing report depicts the C95S change in insulin in the Munich mouse, leading to the lost of the intra-A chain disulfide bond (Herbach et al., 2007). This stimulates insulin and glucose intolerance and extreme diabetes in both heterozygous and homozygous mice, individually (Herbach et al., 2007). Deletion of the WFS1 gene leads to diabetes because of β-cell ER stress and apoptosis (Ishihara et al., 2004; Riggs et al. 2005). The WFS1 gene is an ER Ca^{2+} channel stimulated in the presence of ER stress and generates

inhibition on IRE1α, PERK, and ATF6 (Yamada et al., 2006), subsequently maintaining another feedback circle to switch off the UPR. These perceptions demonstrate that both hypo and hyper reactions to ER stress leads to β-cell destruction and diabetes.

Mice without PERK cannot phosphorylate eIF2α and inhibits insulin translation, leading to diabetes in some days after birth because of dynamic β-cell destruction (Harding et al., 2001; Zhang et al., 2002). In Wolcott-Rallison disorder, the mice display skeletal dysplasia, post-natal development resistance and exocrine pancreatic deficiency. In differentiating the reason for diabetes in PERK-deficient mice, Zhang et al. (Zhang et al., 2006) developed different tissue-and cell-specific PERK knockout mice and discovered that PERK specific significance for fetal and onset neonatal improvement of beta cell quantity and capacity. These experimental animal samples demonstrated that absence of PERK and eIF2α in β-cells is inconvenient by inhibiting their capacity to direct insulin synthesis and in this way adjust it to the present ER protein. The PERK-eIF2α pathway experiences feedback inhibition through GADD34/PP1-intermediate eIF2α dephosphorylation, and maybe additionally by the control of p58IPK, an ER-inborn co-chaperone (Yan et al. 2002; van Huizen et al., 2003; Rutkowski et al., 2007). p58IPK-deficient mice create diabetes as they achieve grown-up age because of expanded β-cell apoptosis (Ladiges et al., 2005). In spite of the fact that homozygous mice for a Ser51Ala exchange in eIF2α are destroyed after birth, the heterozygote is phenotypically typical. In the presence of food containing high fat, heterozygous Ser51Ala mouse leads to glucose intolerance (Scheuner et al., 2005). In the beta cell, the endoplasmic reticulum becomes widened, with some elevated measures of proinsulin bound to BiP, proposing postponed proinsulin expression (Scheuner et al., 2005). In islets acquired from ten to twelve weeks old diabetic db/db mice (with defective leptin receptor), endoplasmic reticulum stress markers were elevated in relative and control islets (Laybutt et al., 2007). An elevation of XBP1 splicing and BiP, GRP94, and ERp72 were expressed after eIF2α phosphorylation; ATF4, CHOP, and p58IPK were bonded (Laybutt et al., 2007). Expanded XBP1s expression may specify "pathological" β-cell ER stress (Lipson et al., 2006), strengthening the fact that ER damage affects beta cell destruction. ER stress in db/db islets in the prediabetic stage is not yet proven.

Examination of db/db mice within four weeks with exendin-4, (a glucagon-like peptide 1 receptor agonist), activated some diminished nuclear CHOP expression in beta-cells and its entire pancreatic XBP1s stages, in sequence to a lesser hyperglycemia. It was observed that exendin-4 likewise conserved beta-cell *in vitro* against the manufactured ER stressors seen in thapsigargin and tunicamycin (Yusta et al., 2006). In these *in vitro* researches, it was observed that in sequence to expanded PERK and IRE1α signalling with upgraded expression of ATF4, CHOP, and XBP1s. An experimental research proposed that the subsequent GADD34 expression (GADD34 is managed by CHOP) and PP1c activation moderated the eIF2α dephosphorylation demonstrated with exendin-4 and forskolin, along these lines lessening the ER stress reaction and permitting sustenance of translational repression (Yusta et al., 2006). Exendin-4 treatment additionally enhanced glycemia of beta-cells-particular calmodulin-overexpressing mouse, lost beta-cell by apoptosis activated by nitric oxide (NO) release and ER stress (Yu et al., 2002; Tsunekawa et al., 2007). In these animal samples, exendin-4 diminished BiP and CHOP and enlarged islet insulin content (Tsunekawa et al., 2007).

2.6. Glucotoxicity and lipotoxicity acts as activators of endoplasmic reticulum stress

An increased fatty food and obesity may add to the generation of T2D by leading to beta cell lipotoxicity insulin resistance. Palmitate has been reported to more powerful than oleate in the initiation of ER stress reaction in β-cells (Karaskov et al., 2006; Laybutt et al., 2007). Palmitate leads to the phosphorylation of PERK and eIF2α, restraint of protein release, and acceptance of ATF4 and CHOP (Karaskov et al., 2006; Cnop et al., 2007a) as shown in Figure 2.5. CHOP recognition by FFA is moderated by ATF4 binding to the C/EBP-ATF restricting site in the CHOP promoter, and in addition by c-Fos and Jun-B dimer binding to the activator protein-1 (AP-1) restricting site (Pirot et al., 2007a). Palmitate additionally activates IRE1 (as confirm by XBP1 joining) and ATF6 and modulates ER chaperones including BiP, GRP94, p58IPK, ORP150, ERp72, Dnajb9, Herp and Edem (Cnop et al., 2007a; Laybutt et al., 2007), despite the fact that BiP activation was not seen in one study (Karaskov et al., 2006). Oleate is less successful in enacting IRE1α and does not activate the PERK pathway but induces ER

chaperone association (Kharroubi et al., 2004; Cnop et al., 2007a). ER stress adds to palmitate-triggered β-cell apoptosis is seen by the mechanism that MIN-6 cells overexpressing BiP have a minor ER stress reaction and are mostly secured against palmitate-triggered apoptosis (Laybutt et al., 2007). The system by which FFA-triggered ER stress leads to β-cell apoptosis is not surely known. Since past findings recommended that wrong PERK eIF2α activation adds to β-cell destruction (Scheuner et al., 2001; Lu et al., 2004), endeavor was enhance to secure β-cells against FFA with salubrinal (Cnop et al., 2007b), a particular inhibitor of eIF2α dephosphorylation (Boyce et al., 2005). Suddenly, salubrinal-activated eIF2α phosphorylation was proapoptotic in β-cells, and it particularly increased the harmful impacts of oleate and palmitate, however not of other ER stressors, through a synergistic activation of the PERK eIF2α branch (Cnop et al., 2007b). The level of apoptosis in β-cells associated with FFA elevated by three to six folds within the level of salubrinal, while no difference was recorded with cytokines, which likewise lead to ER stress (Cnop et al., 2007b).

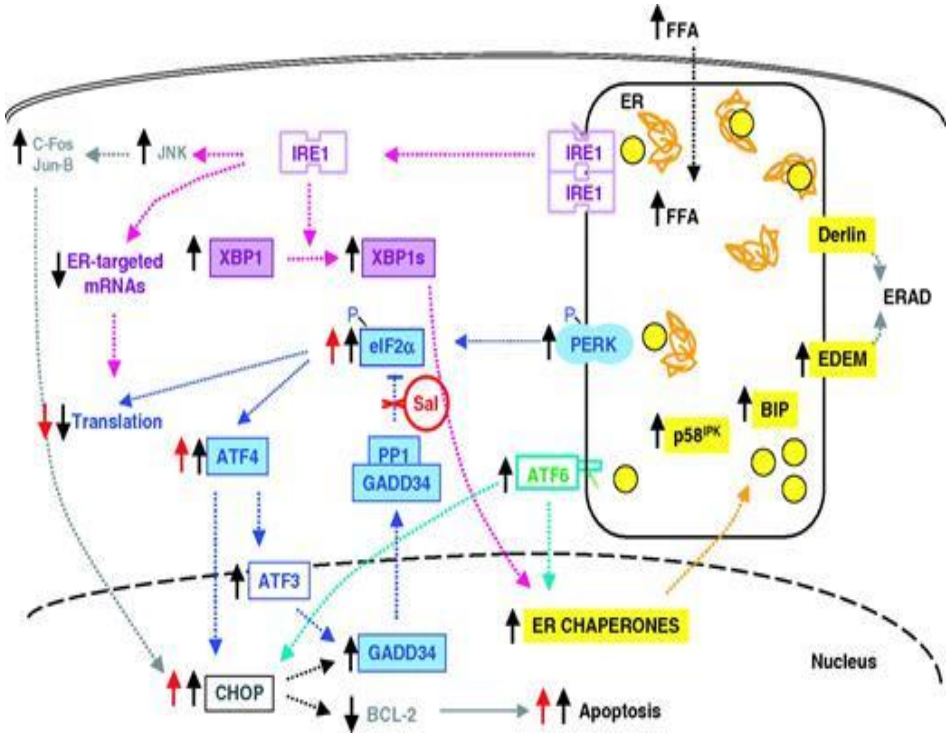

Figure 2.5. Pathway showing unfolded protein response (Eizirik et al., 2008).

An elevated glucose (30 mM) additionally initiated a moderate (around 2-overlap) initiation of the UPR in rodent islets, activating XBP1 splicing, expression of the ER chaperones BiP, GRP94 and Edem and of PERK dependent ATF3, CHOP, and GADD34 (Elouil et al., 2007). This is not reliant upon Ca^{2+} flood or insulin synthesized by the β-cells since this reaction was not influenced by diazoxide (which opens the β-cell's ATP-dependent K^{+} channels and along these lines lessens Ca^{2+} flood and insulin synthesized) or by clonidine (hinders Ca^{2+} influx) (Elouil et al., 2007). The control of CHOP expresssion by glucose in β-cells are mediated by the MAPK ERK1 and 2 (Lawrence et al., 2007). An elevated glucose having a raised effect on FFA-initiated β-cell ER stress is to resolved.

2.7. β-Cell destruction and endoplasmic reticulum stress in type two diabetes

In T1D, β-cell destruction is seen before white blood cell invasion in nonobese diabetic (NOD) mice (O Brien et al., 1997*)* and insulin-dependent diabetes mellitus rats (Jorns et al., 2005). In rat and human T1D, β-cell apoptosis harmonizes with expression of cytokines, for example, IL-1β, interferon (IFN)-γ, and TNF-α by penetrating resistant cells, and inducible NO synthase (iNOS) by both β-cells and weak cell (Eizirik et al., 2001a; Jorns et al., 2005; Uno et al., 2007), recommending that these are early intermediates of β-cell deletion. Under *in vitro* conditions, IL-1β or potentially TNF-α, with IFN-γ, activates NO generation (Eizirik et al., 1992; Eizirik et al.,2001a). Cytokine-activated death in human, rodent, and mouse β-cells, and in insulin-producing cell lines, happens for the most part by apoptosis (Mandrup-Poulsen et al., 2001; Eizirik et al., 2001a; Cnop et al., 2005), however there is a little NO-dependent necrotic segment in rat β-cells (Liu et al., 2000). Cytokine-activated β-cell apoptosis is managed by complex gene systems under the regulation of a vital interpretation factors NF-κB and Detail 1 (Cardozo et al., 2001; Cnop et al., 2005; Callewaert et al., 2007).

One of the cytokine-induced and NF-κB-controlled gene in β-cells is iNOS, prompting huge nitric-oxide arrangement (Darville et al., 1998; Eizirik et al., 2001b). The chemical NO donor SNAP slows ER Ca^{2+} in MIN-6 cells (Oyadomari et al., 2001). Since Ca^{2+} is needed for protein binding and capacity of ER chaperones, more ER Ca^{2+}

consumption will impede the nature of ER protein folding and gathering (Ni et al., 2007) and trigger CHOP expression and apoptosis (Oyadomari et al., 2001). IL-1β or more IFN-γ, through NO synthesis diminish the outflow of SERCA in essential β-cells and insulin- producing INS-1E cells, decreasing ER Ca^{2+} stores (Cardozo et al., 2005). Inhibition of SERCA by the synthetic substances thapsigargin and CPA additionally initiates endoplasmic reticulum stress and apoptosis in β-cells, and the cells are extremely delicate than fibroblasts to the proapoptotic impacts of SERCA inhibition (Zhou et al., 1998; Cardozo et al., 2005). IL-1β and IFN-γ triggers ER stress reaction, including activation of IRE1α, as demonstrated by XBP1 splicing, and of eIF2α/ATF4/CHOP/Bim, however not ATF6 (Cardozo et al., 2001; Kutlu et al., 2003; Cardozo et al., 2005; Pirot et al., 2007b). In accordance with the insufficient ATF6 activation by IL-1β and IFN-γ, the cytokines neglected to build BiP expression (Cardozo et al., 2005). Enoplasmic reticulum stress leads to cytokine-activated cell deletion is in accordance with recent research findings that insulin-delivering insulinoma cells (NIT-1) cells overexpressing BiP have a diminished CHOP expression and are in part ensured against apoptosis initiated by IL-1β and IFN-γ or cytotoxic T lymphocytes (Wang et al., 2007). In neuronal cells, NO triggers S-nitrosylation and inhibition of protein disulfide isomerase (PDI) (Uehara et al., 2006), subsequently reducing appropriate protein folding and inhibition the ER stress (Uehara, 2007). It is yet to be studied whether NO has a common impact on PDI in β-cells. Strikingly, low levels of NO, as initiated by activation of NOS by glucose, maintain against ER stress by triggering ROS (Kitiphongspattana et al., 2007), recommending that the impacts of NO on β-cell ER stress is time-dependent.

IL-1β alone prompts ER stress yet neglects to activates β-cell deletion, while IFN-γ alone causes neither but increases of effect of IL-1β- or CPA-induced cell destruction (Cnop et al., 2005; Cardozo et al., 2005; Pirot et al., 2006). By diminishing ER chaperones, and in this manner protein folding and Ca^{2+} store limit (Rasschaert et al., 2003; Pirot et al., 2006), IFN-γ diminishes β-cell conserve against ER stress and supports the proapoptotic signals, for example, CHOP and other ATF4-dependent genes. Other potential systems by which IFN-γ synergizes with IL-1β to initiate β-cell

apoptosis are increase of IL-1β-activated iNOS expression (Ni et al., 2007) and activation of expression of major histocompatibility complex (MHC) classes I and II and of different parts of the antigen presenting complex (Darville et al., 1998; Eizirik et al., 2001a); in light of the fact that the MHC complex is located in the ER, this may add to the ER excessive activity (Rasschaert et al., 2003).

Initiation of CHOP translation after β-cell presentation to palmitate or cytokines relies upon the form of ATF4 and AP-1 to the CHOP promoter, yet these two drugs triggers the arrangement of various AP-1 dimers (c-Fos and c-Jun and additionally Jun-B on account of cytokines, and c-Fos and Jun-B for palmitate) at various time focuses (Romisch et al., 1999). In accordance with this, cytokines, prompt Ser-63 phosphorylation of c-Jun. CPA, yet not cytokines or palmitate, initiates the CHOP promoter by means of ER stress reaction component (Pirot et al., 2007a). These perceptions recommend that diverse pathways of the UPR are activated in β-cells relying upon the source and power of the ER stressor. The destiny of the β-cells, deletion or survival, will rely upon the harmony between the ER stress and the UPR pathway(s) triggered, their chance course and force, and the sufficiencies of other β-cell protection components, for example, the reactive oxygen species (ROS). After the adjustment leads to apoptosis, JNK, ATF3, and CHOP are necessary intermediates of β-cell deletion (Oyadomari et al., 2001; Hartman et al., 2004).

2.8. β-Cell recuperation against endoplasmic reticulum stress

It is presumed that an elevated work in *in vivo* increases the UPR in human β-cells, as recommended by raised CHOP display in β-cells from the individuals that are overweight but not diabetic (post-mortem material) (Huang et al., 2007), surprisingly most overweight people adapt to many years of insulin resistance and not create β-cell dysfunction and diabetes. Moreover, islet cells living despite a 48-hours exposure to IL-1β *in vitro* (Eizirik et al., 1988) or presentation to the immune system attack in mice *in vivo* (Strandell et al., 1990; Eizirik et al., 1991) can resume activity after an extra six days in culture in the absence of cytokine (Eizirik et al., 1988) or the lymphocytes (Strandell et al., 1990; Eizirik et al.,1991), and β-cells presented for up to twelve hours

to a severe CPA-prompted ER stress do not achieve "the final turning point" for cell deletion (Pirot et al., 2007a). A microarray examination in INS-1E cells presented to CPA for up to twelve hours, with an extra cells treated for six hours and after that permitted to survive in the absence of CPA for three hours (Pirot et al., 2007a), showed that the two cells most influenced by CPA were those identified with cell reactions to ER stress, that experienced up-control, and those identified with separated β-cell capacities, that experienced a down-direction. After a three hours survival period, most cells came back to control levels, concerning example the proapoptotic expression models of ATF3 and CHOP, though expression of the ER chaperones BiP and GRP94 increased. This example of gene expression is most likely because of the more extended half life span of chaperones, for example, BiP, and proapoptotic cells, for example, CHOP (Rutkowski et al., 2006), and may clarify why β-cells can persist twelve hours of extreme ER stress without achieving the final turning point for cell deletion. ER stress stimulates a fast breakdown of mRNAs focused for interpretation at the ER in Drosophila cells (Hollien and Weissman, 2006). This degradation is mediated by IRE1α and supplements other UPR systems by diminishing generation of less important proteins in ER. In the breakdown of insulin mRNA, the major extensive ER-focused on mRNA in β-cells, eases practical need on ER. Both the up-regulation of ER chaperones and down-regulation of proapoptotic gene, may add to β-cell survival when the origin of ER stress is deleted.

2.9. Endoplasmic reticulum stress as a presumed connection among insulin resistance and weight gain

Endoplasmic reticulum stress is being attributed as one of the components connecting overweight with insulin resistance which may in this manner be a typical pathway for the two causes of T2D, specifically insulin resistance and β-cell destruction. From the beginning of 2004, studies were published on ER stress and insulin signaling, generally in rat liver, this course is being examined in a progression to researches (Hotamisligil, 2005).

2.10. Inhibiting action of insulin in the liver by endoplasmic reticulum stress

Ozcan et al., examined the treatment of mice liver cell with tunicamycin – an inducer of ER stress and it effectively reduced insulin stimulated tyrosine phosphorylation of IRS-1 with an elevated concentration of IRS-1. Tunicamycin treatment showed a reduced insulin stimulated Akt phosphorylation, a significant activity in the insulin receptor signaling pathway (Ozcan et al., 2004). Related result was shown when the liver cell was examined using thapsigargin. The activity of JNK was inhibited by a special synthetic inhibitor SP600125 which reversed the ER stress induced serine phosphorylation of IRS1. When liver cell was exposed to treatment with a specific inhibitory peptide sourced from the JNK binding protein, it was observed that JNK-interacting protein totally conserved insulin receptor signaling in cells treated with tunicamycin. From their findings, it was proved that ER stress encourages a JNK-dependent serine phosphorylation of IRS1 and also restrict insulin receptor signaling in cells.

No observable difference was shown between the insulin receptor signaling factor in liver and adipose tissue genotype of mice fed with regular diet. But there was a significant decrease in the followings- insulin receptor signaling in liver, IRS1/IRS2 tyrosine, Akt serine phosphorylation and insulin stimulated receptor in the group fed with elevated fat meal when compared to their control groups. Insulin receptor signaling was suppressed in adipose tissue of an elevated fat meal mice related to their control. The experimental data of Ozcan et al., showed the relationship between insulin action and ER stress *in vivo* and may not show the exact locus insulin receptor signaling pathway that is directed via this pathway (Ozcan et al, 2004).

The examination by Ozcan et al. (Ozcan et al., 2004) could not prove the mechanism of overweight stimulating endoplasmic reticulum stress signaling, which could be guessed to be lipid moderated. In this way, ER stress and insulin resistance were identified in high fat nourished XBP1+/− mice yet not in those sustained with ordinary chow. Saturated free fatty acid appeared to incite JNK activation and insulin resistance in hepatocytes both *in vitro* and when perfused into the liver *in vivo*, while hyperglycemia has a zero impact (Solinas et al., 2006).

2.11. Endoplasmic reticulum stress and obesity in the adipose tissue

Ozcan et al. (Ozcan et al., 2004), examined the sequences in various ER stressors in both genetic and high fact diet of murine obesity. Pancreatic ER kinase–an ER protein kinase, phosphorylates the alpha subunit of translation initiation factor two in the presence of ER stress. Phosphorylated ER kinase (PERK) and alpha subunit of translation initiation factor two (EIF2ά) using specific phospho-antibodies are vital factors that shows the presence of ER stress. And increased PERK and EIF2ά phosphorylation was experienced in the liver of the overweight mice while related to their control. JNK (c-Jun N-terminal kinase) was elevated as well by ER stress in respect of its increased phosphorylation has experienced in obese group. GPR78 (78KD glucose regulated immunoglobin protein) – a chaperone in ER, experienced an increase in the presence of obese mice. GRP78 messenger RNA content where increased in obese mice in comparison to the control group. In relation of GRP 78 to glucose, Ozcan et al., carried out a test to determine whether its upregulation was because of the high glucose level but they found out that GRP78 showed zero elevation in a mouse sample of hyperglycemia showing that control in obesity is not associated with glycemia alone. GRP78 activity, PERK phosphorylation and JNK activity in the liver were all increased when compared to the control with no presence of ER stress, in the muscle tissue of the obese mice. Their general results proved that overweight triggers endoplasmic reticulum stress primarily in the adipose tissue and liver (Neeraj et al., 2018).

3. CONCLUSION AND RECOMMENDATION

The UPR has been known for about two decades: The primary signs that ER stress may trigger diabetes was published about 6–7 years back, and there has been an improved development in the field from that point forward. In any case, the system generating β-cell degradation in diabetes is unpredictable (Cnop et al., 2005), and ER stress is presumably just a single of a few variables adding to β-cell destruction in diabetes. ER stress likewise appears to assume a part in an elevated fat and overweight initiated insulin resistance in liver of rat. Its work in fat tissue is not recorded yet, and, from recent accessible information, endoplasmic reticulum stress does not appear to assume a role in muscle insulin resistance. New exploratory studies will further affirm or discredit the theory that ER stress adds to the pathogenesis of diabetes and various diseases.

The followings are some of the zones / recommendations where future researches may add to explain further a more detailed work for the part of ER stress in diabetes:

- Whether there is presence of role of the unfolded protein response (UPR) and endoplasmic reticulum (ER) stress metabolism with an essential function for the control of insulin biosynthesis?
- What are the biochemical mechanisms by which free fatty acids as well as high glucose level trigger an ER stress reaction?
- What are the downstream intermediates of endoplasmic reticulum stress triggered β-cell deterioration? Are these intermediates distinctive for free fatty acid and cytokines, or is there a pathway for beta cell deletion?
- Does ER stress assume a function for insulin resistance in human type 2 diabetes? What are the included tissues if answer is in the affirmative?
- What are the action mechanisms of IFN-γ in repressing ER chaperones? Is there the existence of normal and new binding sites in the promoter site of these genes for IFN-γ-induced transcription elements?
- Apart from apoptosis as the main type of ER stress triggered by beta-cell deteriorates, does autophagy play a part?

- In the adipocytes morphologies, is there the existence of UPR roles as currently recommended for the β-cells?
- Can it be visible to guard against ER stress by β-cell and not influence their primary work of discharging insulin and detecting glucose?

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