Relationship of Adipose Tissue ER Stress in Obesity with Insulin Resistance
Research Article

Dr. Shahzad Khan¹, Dr. Chang Hua Wang², Dr. Muhammad Qadar³
¹Wuhan University School of Medicine, China
²Wuhan University School of Medicine, China
³Wuhan University School of Medicine, China

*Correspondence: Wuhan University School of Medicine, Wuhan City, 430071, China
e-mail: ycsung@postech.ac.kr, shahzadkhan571@gmail.com¹, chwang0525@whu.edu.cn², mqader85@yahoo.com ³
Phone: +8615387027401

Keywords: Adipose, Insulin resistance, ER stress, Adipokines, FFA

Received: 29 October 2013; Revised: 29 November 2013
Accepted: 9 December 2013; electronically published: 11 December 2013

Summary
Obesity, a worldwide epidemic and now has been recognized, unanimously as the leading cause of Insulin Resistance (IR). Its complications include cardiovascular and cerebrovascular events which finally results in decreased life expectancy and increased morbidity. Obesity initiates a number of metabolic derangements in various tissues including hepatic, muscle, pancreatic and adipose tissues all of which participate in increased insulin resistance (IR). Nowadays role of adipose tissue in escalating obesity associated IR is the focal topic of investigations Adipose tissue secretes important proteins which includes adipokines/ (adipocyte specific cytokines or adipocytokines) such as adiponectin, leptin, resistin, interleukin 6 (IL-6), Tumor necrosis factor TNF-α etc. An imbalance in these adipokines not only initiates but further complicates Insulin resistance. Additionally adipocyte’s endoplasmic reticulum stress (ER stress) and macrophages in adipose tissue has been found responsible as a key players behind many if not all these events. ER stress in adipocyte results in Unfolded Protein Response (UPR) which can initiate a viscous circle generating inflammatory metabolites finally leading to Insulin resistance. Furthermore these events in turn cause deranged fatty acid metabolism resulting in increased free fatty acids level (FFA) ectopic lipid deposition which promote ER stress and inflammation. Here these interlinked events are briefly discussed which ultimately culminates in IR and also an ample of light has also been thrown on the role of adipose tissue.

I. Introduction:

Insulin Resistance, how it works?
Insulin Resistance (IR) is determined by impaired sensitivity to insulin of its main target organs, i.e. adipose tissue, liver, and muscle. Insulin regulates glucose uptake and liver, and muscle. Insulin is key regulator of glucose uptake and circulating Free Fatty Acid (FFA) concentrations. In adipose tissue, insulin decreases lipolysis thereby reducing FFA efflux from adipocytes; in skeletal muscle it predominantly induces glucose uptake by stimulating the translocation of the glucose transporter GLUT4 to the plasma membrane while in liver, it inhibits gluconeogenesis by reducing key enzyme activities. Therefore Insulin resistance leads to
increased circulating FFA concentrations leading to ectopic fat deposition that hinder
insulin mediated glucose uptake in skeletal muscle as a result increased glucose generation in liver [1]. Finally, a combination of insulin resistance and abnormalities in insulin secretion can lead to Type 2 diabetes mellitus (T2DM). Insulin resistance is a complex and multisystem metabolic disorder which involves multiple events in various organs, all are directly or indirectly linked which each other. Adipose tissue, pancreatic beta cells and hepatic cells are of paramount importance in this process. ER stress in Adipocyte, initiation of UPR, generation of inflammatory metabolites, increased FFAs levels with altered fatty acid uptake, impaired and abnormal lipogenesis producing different ectopic lipid metabolites, accumulation of specific lipid metabolites in skeletal muscle and liver are some of the important role-players. [1]. In this article we mainly focus on the role of adipose tissue since in obesity increased adiposity is the key factor behind the scene.

II. Role of adipose tissue in obesity

Obesity which few years before was considered as a predisposing factor for many diseases is now considered itself as a pathophysiologic disease which requires prompt management including behavioral therapy and medications as declared in the annual conference of American medical association annual meeting 2013[2]. Obesity is a multi-organ disease which directly or indirectly increases the risk of cardiovascular diseases, cerebrovascular stroke, IR, T2DM, and even certain types of cancers. It must be pointed out over here that in this pathophysiological cascade multiple tissues play their role but there is one tissue which has been recognized or rather has been claimed for containing a molecular network which connects obesity with all of its consequences and the tissue is Adipose Tissue. Adipose tissue in which adipocytes itself can be up to 50% of the total number of cells (other are preadipocytes, macrophages and vascular cells etc.) can secrete different protein signals and factors under conditions of ER stress. Hotamisligil’s first time showed that UPR markers are overexpressed in the adipose tissue of obese rodents [3]. Investigations have shown the augmentation of ER Stress genes and eIF2a (Eukaryotic Translation Initiation Factor 2A) phosphorylation when Adult cell-derived adipocytes (ADHAS) were subjected to ER Stress [4]. Inflammation of adipose tissue which is observed in obesity and diabetes is associated with the infiltration of macrophages in to adipose tissue. These macrophages are attracted by dying adipocytes which also secretes TNF-α and IL-6 and other chemokine such as Monocyte chemo-attractant protein (MCP)-1 etc. Likewise other cells that are specialized for a high secretory capacity such as mature B lymphocytes, liver cells and pancreatic b-cells adipocyte can also expand and adopt their ER capabilities in stressful conditions like diabetes and obesity [5]. The central role of adipose tissue ER stress in ongoing pathologic conditions has been discussed in detail in many recent studies as well [6] [7, 8]. In obesity for instance, adipose tissue is poorly oxygenated [9, 10], leading to Adipose Tissue Hypoxia (ATH) which interferes with disulphide bonding in the lumen of ER thus leading to ER stress and increased ER Stress markers like CHOP (C/EBP homologous protein) and GRP78 (glucose-regulated protein 78 ) in adipose tissue [11] [12] [3, 12, 13]. Hosogai also showed increased levels of GRP78 and CHOP in hypoxic 3T3-L1 adipocytes [12]. The resulting ER stress in hypoxic adipocytes can cause apoptosis as shown in a study by Sharma et.al. where ER stress induced apoptosis by using ATF4 (Activating transcription factor 4) , GADD43 (growth arrest- and DNA damage-43) and Cyclic AMP-dependent transcription factor ATF3 as markers of apoptotic pathway [7]. ATH also
stimulate the inflammatory response of macrophages [14, 15] and induce apoptosis through cell cycle arrest at G0/G1 phase, via alpha serine/threonine-protein kinase (AKT) (also known as Protein Kinase B) and c-Jun N-terminal kinases (JNK) pathways[71]. Yet another mechanism depicted by Yin et.al [16] described that hypoxia induces cell death by promoting FFA release and inhibiting glucose uptake in adipocytes via inhibition of insulin signaling pathway.

III. Adipocyte ER stress as key factor in insulin resistance
ER stress was first observed by Ozcan and colleagues in adipose tissue of obese mice in 2004 and was proposed as a risk factor for insulin resistance [3]. Since then many researches have shown that stress inducing conditions like obesity is clearly associated with ER stress and apoptosis of beta-cells, hepatocytes and adipocytes which ultimately results in metabolic derangements, especially with insulin resistance. For instance treatment of insulin-resistant humans with Tauroursodeoxycholic acid (TUDCA), a conjugated bile acid derivative that inhibits ER stress induced apoptosis, results in increased insulin sensitivity[17]. Other studies have shown that chaperon such as 4-phenyl butyric acid (PBA) and trimethylamine N-oxide di hydrate (TMAO) which inhibit ER stress through improved ER protein folding capacity, result in increased insulin sensitivity in obese diabetic mice [18]. All these data indicate ER stress in adipose cells, liver cells and beta-cells can initiate IR partly if not solely and can further exaggerates the IR especially in the setting of obesity. However ER stress interference with insulin signaling is multifactorial which is discussed in detail in next sections.

IV. Impact of IRE1-JNK-IRS-1 signalling pathway in insulin resistance
Since ER Stress cause UPR it is possible that ER stress in adipocytes could disrupt insulin signaling through the activation of inositol-requiring enzyme 1 (IRE1) which is one of the three transmembrane proteins which take part in UPR (other include PKR-like ER kinase (PERK), and activating transcription factor 6 (ATF6) . ER stress can cause insulin receptor signaling through increase in serine (Ser) phosphorylation and decrease of tyrosine (Tyr) phosphorylation of insulin receptor substrate-1(IRS-1) which cause inactivation of signal transduction thus leading to insulin resistance[19, 20] . IκB kinase (IKK), JNK, mammalian target of rapamycin (mTOR) and protein kinase alpha (PKC- θ) are example of serine kinases which have been identified to possess this activity and many studies have shown that insulin resistant states (e.g. obesity, T2DM etc.) are associated with activation of JNK and/or IKK [21-25]. JNK is important as activated JNK phosphorylates IRS-1 at Ser307 and can be activated by both IRE1 and PKR. It is interesting that PKR can also inhibit insulin signaling by directly phosphorylating IRS1 on serine [26]. A recent study has found 2 fold up regulation of phosphorylated JNK-1 in adipose tissue from obese volunteers [27]. It has been reported that the inflammatory cytokines secreted from adipocytes and macrophages during inflammation also stimulate JNK pathways [28]. Moreover, inflammatory cytokines can hamper insulin signaling by interfering with IRS-1–insulin receptor interaction and promoting IRS-1 degradation [29]. All these information provide sufficient proofs that obesity can cause IR via JNK.

V. Adipokines importance in Insulin resistance:
Adipokines are cytokines secreted by adipose tissue and several studies conducted on rodents and humans have declared that insulin resistant states are not only associated with increased lipolysis and adipose ER stress but
also deranged adipokines production [3] [6-8, 30]. For example in obesity there is a decrease in leptin and adiponectin secretion and an increase of IL-6 secretion [30]. This altered expression of adipokines can also cause ER stress in adipose tissue. Some studies show that obese states are associated with impaired adiponectin folding and multimerization due to decreased expression of disulfide-bond A oxidoreductase-like (Dsba-L) in the ER which results in ER stress [31, 32] . On the other hand in ER stress CHOP is up regulated that impair resistin transcription in adipocytes causing its altered secretion [33]. Adipokine named adiponectin is especially important as many studies have shown its decreased expression favors insulin resistance in obesity [34] [35] [36]. The ER stress cause decrease levels of adiponectin in human adipocytes which in turn is believed to have a role in insulin sensitivity and other metabolic syndromes [37, 38]. Investigations using tunicamycin and thapsigargin as ER stress inducers found that ER stress decreases adiponectin mRNA and increase tumor necrosis factor-alpha (TNFα) mRNA in ADHAS (adult-derived human adipocyte stem) cells [4]. Target of adiponectin signaling is AMP-activated protein kinase (AMPK), which is a negative regulator of mTOR and high level of mTOR in turn cause serine phosphorylation of IRS1. Therefore when adiponectin levels are lower, inhibition of IRS1 will cause IR which is achieved by mTOR activation pathway.

Resistin a type of adipokines secreted by preadipocytes of human adipose tissue [39] also has shown links with insulin resistance. Studies have found that ER stress reduces resistin mRNA in 3T3-L1 adipocytes in a time and dose-dependent manner [33]. The role of resistin in the causation of IR which requires further clarification as it showed positive correlation with the IR in some studies [40], while other studies showed no correlation [41] [42] [43].

VI. Adipose Tissue macrophages as an important source of obesity-associated inflammation
Adipose tissue of obese can produces variety of inflammatory cytokines such as TNF α, Interleukin-1 (IL-1), Interleukin-6 (IL-6), and monocyte chemotactic protein-1 (MCP-1) [44]. Although adipocytes, preadipocytes, vascular endothelial cells-lymphocytes all take part in their secretions recent studies have clearly shown increased number of macrophages in adipose tissue of obese indicating there strong relationship with these cytokines [45] [46]. One reason behind increased macrophages recruitment is the increased cell death due to ER stress in obese states which then invites macrophages aimed to engulf dead cell debris [47]. This event is verified by the studies which showed increased expression of chemotactic proteins including MCP-1 and elevated gene expression of CC chemokine receptors such as CCR1, CCR2 and CCR3[48].

VII. Role of inflammatory cytokines
Recent studies have shown that obesity is associated with inflammation of adipose tissue playing a key role in the development of insulin resistance. This is evident by the increased levels of inflammatory markers such as TNF-α, IL-6, MCP-1, and IL-8, altered levels of adipokines including adiponectin and leptin and increased C reactive protein and osteopontin [1, 49]. Obesity especially abdominal obesity (e.g. increased waist circumference) is related with a low grade chronic inflammation which is evident by the increased levels of inflammatory cytokines which finally have a crucial role in the causation of insulin resistance and other consequences of obesity[50-52]. In one study it was shown that increased abdominal fat (increased waist hip ratio) is associated with increased C-reactive
protein (CRP), complement factor C3, IL-6 and retinol binding protein 4 concentrations [53][54, 55]. It is interesting that all three ER stress related transmembrane proteins contribute in this process. Importantly IRE1, activates JNK and NF-кB (nuclear factor kappa-light-chain-enhancer of activated B cells) through TNF receptor-associated factor 2 (TRAF2) and Apoptosis signal-regulating kinase 1 (ASK1) (also known as mitogen-activated protein kinase kinase 5 MAP3K5). PERK (pancreatic endoplasmic reticulum kinase) through eIF2a phosphorylation, inhibits IkB through eIF2a. PKR (PRKR-like endoplasmic reticulum kinase or protein kinase R) phosphorylates eIF2a and activates both JNK and IKK while ATF6 also activates NF-κB. Finally, ER stress activates CREBH (cAMP responsive element-binding protein, hepatocyte specific), which together with NF-κB augment transcription of genes taking part in inflammation [56] [24]. The role of IkB and IKK in the ER induced inflammation has been studies in ADHAS cell derived adipocytes recently. The IkB families of protein control the activation of nuclear factor-κB (NF-κB) whose entry in nucleus can initiate inflammation. Thus IkB controls NF-κB by sequestering NF-κB in the cytoplasm. IkB in turn can be degraded by IkB Kinase (IKK) which then finally results in nuclear entry of NF-κB dimers leading to inflammation. Therefore decrease levels of IkB (due to increased IKK activity) provides a pathways by which ER stress induce inflammation [52]. On the other hand interleukin-6 (IL-6) and TNFα which are increased in obesity and IR can induce ER stress which in turn increases IR in a loop manner [57]. In adipose tissues, TNF -α inhibits lipogenesis and adiponectin expression via inhibition of peroxisome proliferator-activated receptor-g (PPAR-g)-mediated mechanisms [58] [59] [60]. It is suggested that ER stress initiates TNF-α, which then not only take part in the inflammatory process as well as PPARγ mediated effects on adipocyte [61]. Therefore experiments involving PPAR activators like TZDs (thiazolidinediones) also verify that transcriptional activity of PPARγ is required for the maintenance of insulin sensitivity and lipid metabolism [62]. A recent study using RT-PCR has also documented TNF -α, in the adipose tissue of lean and obese subjects [27]. Neutralizing TNF-α in rats or using knockout mice deficient in TNF-α (Tnfr1) or the TNF-α receptor 1 gene (Tnfr1) also showed protection from insulin resistance induced by both diet and genetic obesity [44] [63]. TNF-α also activates several IR related pathways including IKK- beta in cultured murine adipocytes [64, 65]. TNF-α is thought to affect insulin sensitivity by modifying the expression of the genes for insulin receptor, IRS1, GLUT4, adiponectin, and PPARγ [66] [60]. Interestingly TNF-α through activation of NADPH oxidase also induce Reactive Oxygen Species (ROS) generation, [67, 68]. Another inflammatory cytokine IL-1β also plays important role in inducing ER stress in adipocytes in obesity. It works by increasing nitric oxide synthase (iNOS) which generates Nitric oxide (NO). NO in turn inhibits the ER calcium pump resulting in depletion of calcium stores in ER thus eventually inducing ER stress [69] [70]. A recent study has investigated the relationship of obesity induced ER stress in adipose tissue [71] using 12/15-lipoxygenase enzyme (12/15-LO) a unique inflammatory pathway regulating the ER stress response in various tissues, including adipocytes, pancreatic islets, and liver. The study showed that addition of 12/15-LO lipid products 12(S)-HETE (hydroperoxyeicosatetraenoic acid ) and 12(S)-HPETE to differentiated 3T3-L1 adipocytes induced expression and activation of ER stress markers, including BiP (binding immunoglobulin protein), XBP1 (binding immunoglobulin protein), XBP1, p-ERK (RNA-dependent protein kinase-like ER-regulated kinase), and
p-IRE (inositol-requiring enzyme)1α. The study also found that 12/15-LO action involves up regulation of interleukin-12 (IL-12) expression. This important discovery may provide a new therapeutic strategy in alleviating inflammation, β-cell dysfunction, and insulin resistance associated with ER stress, thereby reducing metabolic complications associated with visceral adiposity through blockade of 12/15-LO activation or downstream IL-12 signaling[71].

VIII. Role of FFA
The role of adipose tissue hypoxia and inflammatory mediators in adipose tissue ER stress has been discussed above. Several new studies are now focused to demonstrate that elevated levels of free fatty acids (FFAs) play important role in induction of ER stress in various cells including adipocytes [56, 72]. This is further supported by the fact that most of the obese people have elevated levels of plasma FFA [73, 74]. While inflammation also known to impair insulin action through increasing FFA and decreasing adiponectin in the blood therefore it seems that both the inflammatory cytokine and FFAs are able to target IRSs proteins thus leading to insulin resistance [75] [76]. One method of IR by high FFA is through down regulation of PPARγ protein and its mRNA which ultimately enhance IR [77]. In adipose tissue ER stress promote FFA efflux from adipocytes and high level of circulating FFA could be a cellular basis of lipotoxicity, dyslipidemia, and insulin resistance [78] [3, 7, 79, 80]. On the other hand accumulating evidences suggest that saturated long-chain FFAs like palmitate mainly and unsaturated long chain FFAs to a lesser extent induce ER stress and mediate β-cell apoptosis which eventually leads to IR and T2DM [81, 82, 83] [82-86] [85]. A recent study has described a unique pathway of the lipolysis in response to ER stress in adipocytes which is surprisingly not related with the hormone sensitive lipolysis but is related to elevated cAMP production and protein kinase A (PKA) activity. This study revealed that chemically induced ER stress in adipocyte activates cAMP/PKA (in acute phase) and ERK1/2 (extracellular signal-regulated kinase 1/2) (in chronic phase) signals and regulates lipolysis in ER-stressed adipocytes [87]. Interestingly some studies further suggests that JNK [88] and PKC [89] also modulate lipolysis and both ERK1/2 and JNK are activated during ER stress.

IX. Role of Reactive oxygen species (ROS)
There are evidences that ROS also play important role in adipocyte’s ER stress consequences which then directly or indirectly contribute in IR [11]. Increased ROS generation has been recorded due to high levels of FFAs in adipose tissue of obese mice. TNF-α has also shown increased ROS generation through activation of NADPH oxidase oxidase [67, 68] [90]. This ROS due to its oxidizing effects on nascent protein and action on calcium channels which result less availability of calcium and eventually leads to increased misfolded and unfolded proteins and further increase in ER stress [25, 91]. This FFA-mediated ROS generation model gives another way which potentially can induce ER stress in adipose tissue and leads to other manifestations of ER stress including IR.

X. Conclusion and future aspects
Above discussion demonstrate that there is a close relationship between ER stress in adipocytes and insulin resistance which is complex and multifactorial. Still above quoted evidences and findings are enough to support the theory that inhibition of this ER stress could become the basis of type 2 diabetes treatment in future and may lead to discovery of new drugs. Work has already been started in this ground. Currently at least two ER stress
inhibiting chaperons 4-phenylbutyric acid (PBA) and taurine-conjugatedursodeoxycholic acid (TUDCA) have been approved by FDA [18, 92]. Still more exploration is required to apply this complex relationship on humans in the treatment of metabolic and nutritional disorders like obesity and insulin resistance.

Figure 1: Obesity and its consequences leading to IR

Acknowledgments:
The authors are thankful to Mr. NAH (Canada) for his valuables help in proofreading and grammar check.

References:


CD8 T cells during chronic viral infection. 


