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OXIDATIVE STRESS, INSULIN SIGNALING AND DIABETES

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Abstract

Oxidative stress has been implicated as a contributor to both the onset and the progression of diabetes and its associated complications. Some of the consequences of an oxidative environment are the development of insulin resistance, β -cell dysfunction, impaired glucose tolerance, and mitochondrial dysfunction, which can lead ultimately to the diabetic disease state. Experimental and clinical data suggest an inverse association between insulin sensitivity and ROS levels. Oxidative stress can arise from a number of different sources, whether disease state or lifestyle change, including episodes of ketosis, sleep restriction, and excessive nutrient intake. Oxidative stress activates a series of stress pathways involving a family of serine/threonine kinases which in turn have a negative effect on insulin signaling. More experimental evidence is needed to pinpoint the mechanisms contributing to insulin resistance in both type 1 diabetics and non-diabetic individuals. Oxidative stress can be reduced by controlling hyperglycemia and calorie intake. Overall, this review outlines various mechanisms that lead to the development of oxidative stress. Intervention and therapy that alters or disrupts these mechanisms may serve to reduce the risk of insulin resistance and the development of diabetes.

Keywords

Oxidative stress; ketosis; obesity; diabetes

I. Introduction

Diabetes is a complex metabolic disorder characterized by defects in the body's ability to control glucose and insulin homeostasis. Diabetes has become an epidemic and remains a major public health issue. In 2007, it was estimated that 23.6 million American people (7.8% of the US population) had diabetes [1], and that diabetes would affect 210 million people worldwide by 2010 [2]. These numbers are expected to increase by 50% over the next 20 years posing a tremendous economic burden on individuals and health care systems worldwide [2]. The total annual economic cost of diabetes in the US in 2007 was estimated to be \$174 billion [1]. With the rising cost and escalating incidence of diabetes, it is increasingly important to understand the mechanisms that lead to the disease. Diabetes is divided into two main types, type 1 and type 2. Type 1 diabetes occurs when the body stops making or makes only a tiny amount of insulin, whereas type 2 diabetes occurs when the body does not make enough or has trouble using the insulin. Type 1 diabetes has been linked

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mostly to genetics and the production of auto-antibodies that destroy pancreatic β -cells [3]. Type 2 diabetes results primarily from insulin resistance and has been linked to factors such as obesity and age. Type 2 diabetes accounts for more than 90% of individuals diagnosed with diabetes [4].

Oxidative stress is thought to be a major risk factor in the onset and progression of diabetes. Many of the common risk factors, such as obesity, increased age, and unhealthy eating habits, all contribute to an oxidative environment that may alter insulin sensitivity either by increasing insulin resistance or impairing glucose tolerance. The mechanisms by which this occurs are often multi-factorial and quite complex, involving many cell signaling pathways. A common result of both types of diabetes is hyperglycemia, which in turn contributes to the progression and maintenance of an overall oxidative environment. Macro- and microvascular complications are the leading cause of morbidity and mortality in diabetic patients, but the complications are tissue specific and result from similar mechanisms [5], with many being linked to oxidative stress. There is a large body of clinical evidence correlating diabetic complications with hyperglycemic levels and length of exposure to hyperglycemia [6]. This review will discuss the current understanding of insulin signaling and the role of oxidative stress in the insulin signaling process. It will also focus on the many risk factors that alter insulin sensitivity through mechanisms linked to oxidative stress and potentially lead to insulin resistance and diabetes.

II. Insulin and normal insulin signaling

Insulin is a key hormone with an important role in the growth and development of tissues and the control of glucose homeostasis [7]. Insulin is secreted by pancreatic β -cells as an inactive single chain precursor, preproinsulin, with a signal sequence that directs its passage into secretory vesicles. Proteolytic removal of this signal sequence results in the formation of proinsulin. In response to an increase in blood glucose or amino acid concentration, proinsulin is secreted and converted into active insulin by special proteases. The active insulin molecule is a small protein that consists of A and B chains held together by two disulfide bonds [8]. The primary role of insulin is to control glucose homeostasis by stimulating glucose transport into muscle and adipose cells, while reducing hepatic glucose production via gluconeogenesis and glycogenolysis. Insulin regulates lipid metabolism by increasing lipid synthesis in liver and fat cells while inhibiting lipolysis. Insulin is also necessary for the uptake of amino acids and protein synthesis [9]. The pleiotrophic actions of insulin are all crucial for maintenance of normal cell homeostasis and allow cellular proliferation and differentiation.

Normal insulin signaling occurs through activation of a specific insulin receptor, which belongs to a subfamily of receptor tyrosine kinases [10]. The insulin molecule binds to the α subunit of the receptor, releasing the inhibition of tyrosine auto-phosphorylation by the β subunit [11, 12]. The receptor is auto-phosphorylated at distinct tyrosine residues. In contrast to most tyrosine kinase receptors, the activated insulin receptor directly phosphorylates insulin receptor substrates (IRS-1-4) on multiple tyrosine residues. There are currently four members of the IRS family known to be involved in insulin signaling, with IRS-1/2 being the most important for glucose transport [12, 13]. The subcellular distribution of these proteins between the cytoplasm and low density membrane compartments of the cell has been shown to play a vital role in transmitting the proper insulin response [13, 14]. Tyrosine phosphorylated IRS proteins then act as a binding site for signaling molecules containing SH-2 (Src-homology-2) domains such as phosphatidylinositol 3'-kinase (PI3K), GRB-2/mSos, and SHP-2. These molecules bind the phosphorylated tyrosine residues of IRS proteins, forming a signaling complex to mediate downstream signaling. PI3K is the main signal mediator of the metabolic and mitogenic actions of insulin. It is composed of a

p85 regulatory subunit, which binds to IRS proteins, and a p110 catalytic subunit. Following association of p85 with IRS-1/2, the p110 subunit has increased catalytic activity. This allows phosphorylation of its substrate, PtdIns(4,5)P₂, on the 3' position of the inositol ring to generate PtdIns (3,4,5)P₃ [11]. The second messenger, PtdIns (3,4,5)P₃, recruits the serine kinases PDK-1, PKB/Akt, and PKC to the plasma membrane via their PH domains. The activation of these kinases results in several of insulin's responses, such as GLUT4 translocation to the membrane, glycogen synthesis by phosphorylation of GSK-3, and lipogenesis by up-regulating synthesis of the fatty acid synthase gene.

In addition to insulin signaling via PI3K, insulin can activate the mitogen-activated protein (MAP) kinase, ERK, which leads to gene expression for various cellular proliferation or differentiation components. After phosphorylation of IRS-1/2, the adaptor proteins Grb-2 and SOS are recruited and work together with a stimulated tyrosine phosphatase, SHP-2, to activate membrane bound Ras. Activated Ras leads to a kinase cascade, allowing ERK to translocate to the nucleus for gene expression [12].

Insulin's main action of glucose uptake also requires activation of another signaling pathway involving tyrosine phosphorylation of the Cbl proto-oncogene. Cbl is associated with the adaptor protein CAP, which contains three SH3 domains and a sorbin homology (SoHo) domain. The SoHo domain of the phosphorylated Cbl-CAP complex allows translocation to lipid rafts and association with the protein flotillin. A signaling complex is formed at the site of the lipid raft, resulting in the activation of a small G protein, TC10. TC10 is thought to act as a second signal in recruitment of the GLUT4 protein to the membrane [7, 12].

III. Reactive oxygen species and redox state

Reactive oxygen species (ROS) and the cellular redox state are increasingly thought to be responsible for affecting different biological signaling pathways. ROS are formed from the reduction of molecular oxygen or by oxidation of water to yield products such as super oxide anion (O₂⁻), hydrogen peroxide (H₂O₂), and hydroxal radical (OH·). In a biological system, the mitochondria and NAD(PH) oxidase are the major sources of ROS production [15]. In moderate amounts, ROS is involved in a number of physiological processes that produce desired cellular responses. However, large quantities of ROS in a biological system can lead to cellular damage of lipids, membranes, proteins, and DNA. Nitric oxide (NO·) is another contributor to ROS concentrations and the formation of reactive nitrogen intermediates (RNIs). NO· is generated by specific nitric oxide synthases (NOSs) that also contribute to a large number of physiological processes. NO· can react with superoxide to form a potent oxidizing agent, peroxynitrite (ONOO⁻), which contributes to cellular damage and oxidative stress [15]. Oxidative stress results from overproduction of ROS and/or decreased system efficiency of scavengers such as vitamin C, vitamin E, and glutathione [16]

The direction of many cellular processes, such as phosphorylation and dephosphorylation and regulation of the cell cycle [17-21], can be determined by the redox state. Increases in ROS can lead to an imbalance of the cellular oxidation state, disrupting the redox balance. The intracellular ROS concentration can be estimated using the redox potential, E. A cell contains many biological redox couples, such as NADP⁺/NADPH, GSSG/2GSH, Cys(SH)₂/CySS and TrxSS/Trx(SH)₂, which allow the cell to maintain redox homeostasis. NADPH has the lowest reduction potential and thus serves as the driving force for other redox couples [17]. GSSH/GSH is the main redox buffer of the cell and is found throughout all cellular compartments, which enables determination of the cellular redox potential using the following Nernst equation

$$E = C + \kappa \log \left(\frac{[\text{GSSG}]}{[\text{GSH}]^2} \right),$$

where C and κ are constants [18]. The addition of oxidants to a cell system results in an increased [GSSG]/[GSH] ratio, thereby increasing the value of E above a specific threshold, which is representative of an oxidative state. Studies have shown that the redox state common in diabetics results in an abnormally high E [16, 18, 22], which leads to disease progression and complications.

Oxidative stress conditions have been shown to be caused by conditions such as hyperglycemia, UV radiation, and increased intake of free fatty acids (FFAs) [23]. It is now known that oxidative stress conditions can result in the activation of various stress pathways such as NF- κ B, JNK/SAPK, and p38 MAPK. The apparent crosstalk between oxidative stress induced pathways and normal insulin signaling creates the possibility for multiple disruptions in the ability of insulin to maintain its normal functions.

IV. Hyperglycemia, oxidative stress and diabetes

In the current literature there are numerous studies indicating that diabetic subjects tend to have more oxidative internal environments than those of healthy normal subjects [11, 24, 25]. From these studies it is clear that diabetic subjects show an increase in ROS generation and oxidative stress markers, with an accompanying decrease in antioxidant levels. Hyperglycemia can cause an increase in oxidative stress markers such as membrane lipid peroxidation. The degree of lipid peroxidation in erythrocytes was directly proportional to the glucose concentrations in vitro [24] and the blood glucose concentrations, as assessed by the glycosylated hemoglobin, in diabetic patients [26]. The increase in the lipid peroxidation was preventable after the control of glycemia with insulin in streptozotocin treated diabetic rats [25]. Thus, hyperglycemia, one factor shared by both type 1 and type 2 diabetics, is a major contributor to oxidative stress. Hyperglycemia induced oxidative stress has been hypothesized to contribute to oxidative stress either by the direct generation of ROS or by altering the redox balance. This is thought to occur via several well studied mechanisms, including increased polyol pathway flux, increased intracellular formation of advanced glycation end-products, activation of protein kinase C, or overproduction of superoxide by the mitochondrial electron transport chain [5, 27].

The polyol pathway leads to reduction of glucose to sorbitol via aldose reductase in an NADPH dependent manner. Sorbitol is then oxidized to fructose by the enzyme sorbitol dehydrogenase, with NAD⁺ reduced to NADH. The main function of aldose reductase is to reduce toxic aldehydes formed by ROS or other substrates to inactive alcohols. Under normal conditions, aldose reductase has a low affinity for glucose, with a very small percentage of total glucose converted to sorbitol via this pathway. Under hyperglycemic conditions, there is an increase in the enzymatic activity and production of sorbitol, resulting in an overall decrease in NADPH. NADPH is an essential cofactor for the production of GSH, a critical intracellular antioxidant [5, 27, 28]. It has also been proposed that the increase in sorbitol and its conversion to fructose increases the NADH:NAD⁺ ratio, which can lead to PKC activation and inhibition of the enzyme glyceraldehyde-3-phosphate dehydrogenase (GADPH) [27, 28]. Increased glucose flux through the polyol pathway does not produce ROS directly, but contributes greatly to an overall redox imbalance in the cell that leads to oxidative stress.

The next mechanism by which hyperglycemia contributes to the oxidative stress environment of a diabetic is through the increased production of advanced glycated end

products (AGEs) [4, 5, 27]. AGEs are formed through the covalent binding of aldehyde or ketone groups of reducing sugars to free amino groups of proteins, creating a Schiff's base. A Schiff's base then spontaneously rearranges itself into an amadori product, which is a more stable ketoamine. Amadori products can then be directly converted to AGEs or undergo auto-oxidation to form reactive carbonyl intermediates. Glucose alone can also undergo auto-oxidation to form reactive carbonyl intermediates, of which glyoxal and methyl-glyoxal are the two main intermediates. These reactive carbonyl intermediates then complete a complex series of chemical rearrangements to yield irreversible AGE structures [29, 30]. AGEs can signal through the cell surface receptor called "RAGE," which is a receptor for other non-AGE pro-inflammatory related molecules as well. RAGE is highly conserved across species and expressed in a wide variety of tissues. It is upregulated at sites of diseases such as atherosclerosis and Alzheimer's [29]. One of the main consequences of RAGE-ligand interaction is the production of intracellular ROS via the activation of an NADPH oxidase system. The ROS produced in turn activates the Ras-MAP kinase pathway, leading to activation of NF- κ B [27, 29]. Activation of NF- κ B results in the transcriptional activation of many gene products, one of which is RAGE, as well as many others associated with diseases such as atherosclerosis [29].

Hyperglycemia can contribute to the direct and indirect production of ROS via the activation of the DAG-PKC pathway [5, 27]. The protein kinase C family consists of a number of different PKC isoforms, most of which are activated by the lipid second messenger DAG. Under hyperglycemic conditions, there is an increase in the glycolytic intermediate, dihydroxyacetone phosphate. Increased levels of this intermediate are then reduced to glycerol-3-phosphate, consequently increasing the *de novo* synthesis of DAG. Hyperglycemia can also activate PKC indirectly through ligation of AGE receptors and by the influx of the polyol pathway [5]. Nevertheless, activation of various PKC isoforms can result in a range of alterations in cell signaling. It has been reported that under hyperglycemic conditions, PKC- α is a potent activator of NADPH oxidase, which could be inhibited by α -tocopherol [31]. It has also been shown that PKC- α and PKC- δ can activate NADPH oxidase and in turn responsible for inducing TLR-2 and TLR-4 expression under high glucose conditions [32]. These alterations can contribute to an oxidative stress environment either by direct production of ROS or indirectly by activating other pathways. PKC activation has been shown to depress nitric oxide production, which is a potent vasodilator, by inhibiting endothelial nitric oxide synthase (eNOS). In contrast, it increases vasoconstriction by activating endothelin-1, resulting in abnormal blood flow. Activation of PKC can also induce expression of the permeability enhancing factor VEGF, contributing to blood flow and vessel permeability changes. PKC also contributes to matrix protein accumulation by inducing expression of TGF- β 1, fibronectin and type IV collagen. This activation is thought to be a result of PKC induced nitric oxide inhibition. Activated PKC contributes directly to the oxidative stress environment by activating NF- κ B and various membrane associated NADPH oxidases, resulting in excessive ROS production [27]. Hyperglycemia contributes to an oxidative stress environment by activating PKC, which alters a number of different pathways involved in stress responses.

A prominent mechanism for ROS production is overproduction of superoxide by the mitochondrial electron transport chain (ETC) [27]. Under normal conditions, glucose oxidation begins in the cytoplasm, where glucose undergoes glycolysis. During glycolysis, NADH and pyruvate are generated. NADH donates electrons to the mitochondrial electron transport chain by two different shuttle systems, while pyruvate donates reducing equivalents by entering the TCA cycle and producing NADH and FADH. Both NADH and FADH provide the electrons that fuel ETC and ATP production. NADH derived from glucose oxidation and from the TCA cycle donates electrons to complex I of the ETC and FADH₂ donates its electrons to complex II. Complex I and II then transfer the electrons to

ubiquinone. Ubiquinone passes its electrons to complex III, cytochrome C, complex IV, and finally to molecular oxygen. As the electrons are transferred through the ETC the energy is used to shuttle protons across the membrane. This creates a voltage across the inner and outer membrane of the mitochondria and drives ATP synthesis. In hyperglycemic conditions, the number of substrates entering the TCA cycle is greatly increased and consequently the number of reducing equivalents donating electrons to the ETC is also increased. Once the ETC reaches a threshold voltage across the membrane the electrons begin to back up at complex III. These electrons are then donated to molecular oxygen, which in turn results in an increase in mitochondrial superoxide production [27].

Cells and tissues contain antioxidant defense mechanisms, which aid in preventing the buildup of ROS and maintain the redox balance of the cell or tissue. Diabetes is associated with reduced levels of antioxidants such as GSH, vitamin C, and vitamin E [33-35]. Glycation of antioxidative enzymes during hyperglycemia can impair cellular defense mechanisms, leading to the development of oxidative stress and the progression and complications of diabetes. Studies have reported that glycation of Cu-Zn-superoxide dismutase [36] and esterase [37] can inhibit their enzymatic activity. A more recent study has shown that glycation of thioredoxin inhibits its antioxidant and organ protective actions [38]. Protein glycation not only reduces the actions of the antioxidant system, but also affects normal functions of other proteins, resulting in altered cellular functions in diabetes. Glycation of proteins such as platelet derived growth factor (PDGF) [39] and collagen have been reported to contribute to complications by promoting vascular stiffness and altering vascular structure and function [40]. Thus, a reduction in antioxidative enzymes and inhibition of enzymatic activity due to glycation in diabetes significantly contributes to the overall oxidative environment seen in diabetics.

V. The influence of oxidative stress on insulin signaling

ROS and RNIs have been shown to affect the insulin signaling cascade; however, the disruption seems to be dose and time dependent. Millimolar ROS concentrations have been shown to play a physiological role in insulin signaling via an NAD(P)H oxidase-dependent mechanism. Upon insulin stimulation there is a burst in H_2O_2 production, creating a short-term and low dose exposure to ROS. This enhances the insulin cascade by inhibiting tyrosine phosphatase activity, leading to an increase in the basal tyrosine phosphorylation level of both the insulin receptor and its substrates [41].

The most common outcome of disrupted insulin signaling is insulin resistance. Insulin resistance occurs when normal levels of insulin are inadequate to produce a normal insulin response from fat, liver, or muscle cells. Multiple cellular studies have shown that under oxidative stress conditions, insulin signaling is impaired, resulting in insulin resistance of the cell [42]. This is frequently investigated by measuring glucose uptake, glycogen, and protein synthesis in a cell after exposing it to H_2O_2 . The exact link between oxidative stress and impaired insulin signaling is not fully understood, but several well accepted mechanisms have been proposed. These include ROS impaired insulin signaling caused by inducing IRS serine/threonine phosphorylation, disturbing cellular redistribution of insulin signaling components, decreasing GLUT4 gene transcription, or altering mitochondrial activity [11, 43].

Chronic oxidative stress is now well known to induce a number of stress sensitive signaling pathways, such as NF- κ B, JNK/SAPK, and p38 MAPK. NF- κ B is a transcription factor that plays a major role in mediating immune and inflammatory responses. The NF- κ B pathway is activated by phosphorylation of the inhibitory subunit, I κ B, by an active serine kinase, IKK. The serine kinase IKK has been shown to exert a negative effect on insulin signaling.

Along with IKK, a number of mitogen-activated protein kinases (MAPK) are also activated when exposed to an oxidative environment. The MAPKs are composed of a family of related serine/threonine protein kinases, such as JNK, ERK, and p38 MAPK. These kinases can be activated in response to cellular stimuli such as stress, inflammatory cytokines, and G protein coupled receptor agonists. The proposed mechanism of insulin signal interference by activated serine/threonine kinases is attributed to the increased serine/threonine phosphorylation of key components in the insulin signaling pathway, such as IR and IRS. Serine/threonine phosphorylation of IRS or the IR impairs the protein's ability to recruit and activate downstream SH-2 containing signaling molecules and disrupts the ability of the IRS protein to interact with the insulin receptor [11, 23, 44, 45]. Other serine/threonine kinases involved in insulin signaling can also be directly activated by ROS. Among these are PKC, PKB, mTOR, and GSK3, all of which can act synergistically to desensitize the insulin signal by phosphorylating IR or IRSs on select serine/threonine residues [11, 46] (Figure 1).

The distribution of insulin signaling components inside a cell plays a very important role in insulin signaling. In the past, studies that looked at total cell lysate indicated that the basal levels of PI3K and other insulin signaling components were not diminished upon exposure to oxidative stress. Thus different subcellular compartments of the cell were examined and oxidative stress is now thought to contribute to impairment of the translocation of the insulin signaling components between the different subcellular compartments. It has been shown that IRS-1 and IRS-2 are mainly located in the low density microsome (LDM) fraction of the cell and that, upon activation, PI3K is recruited from the cytosol to the LDM [13, 14]. In BSO treated rats and 3T3-L1 adipocytes, it was demonstrated that oxidative stress disrupted the translocation of PI3K from the cytosol to the LDM fraction of the cell, leading to insulin resistance. In both the whole cell lysate and the cytosolic fraction of the cell, PI3K protein levels were not affected in the adipose or skeletal muscle of BSO treated rats. However, PI3K levels in the LDM fraction were significantly decreased [47]. By disrupting the phosphorylation of IRS proteins, the recruitment of PI3K from the cytosol is impaired; when PI3K does not get activated, insulin resistance results.

The facilitated diffusion of glucose into the cell is mediated by a family of glucose transporters. GLUT4 is a glucose transporter expressed in tissue sensitive to insulin for glucose transport such as adipose tissue, skeletal muscle, and cardiac muscle [48]. GLUT1 is ubiquitously expressed and mostly responsible for basal glucose uptake, but it is not insulin responsive and is generally expressed in low levels in adipose tissue [48, 49]. Gene transcription in response to oxidative stress affects insulin signaling by altering the availability of GLUT4. It has been shown that in L6 myotubes and 3T3-L1 adipocytes exposed to H₂O₂, there is an increase in GLUT1 mRNA and protein content, which is credited to an increase in DNA binding for the transcription factor AP1. Along with the increase in GLUT1, a decrease in the mRNA and protein levels of GLUT4 are observed after exposure to H₂O₂. In 3T3-L1 adipocytes, the decrease in GLUT4 was attributed to a decrease in binding of DNA binding proteins to the insulin response element (IRE) in the GLUT4 promoter [11]. By decreasing GLUT4 gene expression in a cell, one would expect to observe a decrease in the glucose uptake, resulting in insulin resistance.

VI. Mitochondrial dysfunction and insulin signaling

Mitochondria are the main power supply for our cells and play a central role in cell life and death [50, 51]. They provide energy for almost all cellular processes, from muscle contraction to maintenance of ionic gradients for vesicle fusion, and the cycling necessary for secretion of hormones and neurotransmitters. The mitochondria also play an important role in how β -cells release insulin in response to glucose levels and the sensing of oxygen tension in the carotid body and pulmonary vasculature [50, 52]. Increasing amounts of

evidence have demonstrated that mitochondrial dysfunction is associated with disease states such as insulin resistance and T2D [50-56]. Genetic factors, oxidative stress, mitochondrial biogenesis, and aging are all factors that have been shown to affect mitochondrial function and lead to insulin resistance [53]. About 98% of inhaled oxygen is consumed by the mitochondria [50], of which about 0.2% to 2.0% results in ROS production [53], one of the primary sources of mitochondrial injury. Some of the elements vulnerable to damage via ROS within the mitochondria include lipids, proteins, oxidative phosphorylation enzymes, and mtDNA, with the major consequence being mitochondrial dysfunction. It has been shown that mitochondrial dysfunction results from oxidative stress in skeletal muscle [53, 57] and in other tissues including liver, fat, heart, blood vessels, and pancreas [53].

It has been known for years that mitochondrial dysfunction in genetic diseases leads to insulin resistance and is an underlying cause in the development of diabetes. For example, MELAS (myopathy, encephalopathy, lactic acidosis and stroke-like episodes) syndrome is caused by a maternally inherited mtDNA mutation; the disease is associated with diabetes due to insufficient insulin secretion by the pancreatic β -cells [58]. Since the mitochondrial genome encodes many of the proteins involved in oxidative phosphorylation, mutations caused by stress conditions may be one of the underlying mechanisms of insulin resistance caused by mitochondrial dysfunction.

It has also been reported that obese or T2D subjects and their offspring contain fewer and smaller-sized mitochondria upon skeletal muscle biopsy [53, 59]. Oxidative capacity has been shown to correlate with the number and density of mitochondria, and to be related to the reduction in expression of mitochondrial proteins involved in mitochondrial biogenesis and ATP production [53]. Furthermore, aging is also a factor in decreased mitochondrial biogenesis and ATP production. Respiration is decreased in isolated mitochondria from elderly subjects who have reduced mitochondrial number and function [60]. The decreased number of mitochondria, density, and mitochondrial gene expression may all play important roles in contributing to the development of insulin resistance and diabetes.

Mitochondria can also contribute to the influx of fatty acids and activation of stress related kinases. Studies have shown that fatty acid induced insulin resistance can be caused by direct inhibition of insulin-stimulated glucose transport activity [61]. A decrease in mitochondrial fatty acid oxidation, which is caused by mitochondrial dysfunction or reduced mitochondrial biogenesis and density, results in increased levels of fatty acyl CoA and diacylglycerol (DAG). These in turn activate stress related Ser/Thr kinase activity and inhibit glucose transport by mechanisms discussed earlier [52]. In relation to stress activated kinases, oxidative stress also contributes to impaired insulin signaling by increased uncoupling protein-2 (UCP2) activity. Uncoupling proteins are mitochondrial transporters of the inner membrane that, when activated, cause protons to leak across the inner membrane, generating heat without contributing to ATP production [62, 63]. UCP2 is thought to negatively regulate glucose stimulated insulin secretion by reducing the amount of ATP produced. This idea is supported by studies that have demonstrated stimulation of UCP2 in vitro and in vivo by hyperglycemia and lipid fuels and in animal models of type 2 diabetes [52]. Furthermore, it has been demonstrated that β -cell functions improved in a type 2 diabetes animal model in which UCP2^{-/-} mice demonstrated enhanced insulin secretory capacity after a high-fat diet [64]. Since ATP production is key to providing energy for almost all cellular processes, it is likely that decreased ATP production will affect insulin signaling in many different cell types.

Oxidative stress seems to play a major role in mitochondrial dysfunction, which can further exacerbate stress signals and reduce ATP production. The pathways leading to insulin resistance may be synergistic and the mitochondrial dysfunction can create a feedback loop,

adding to the overall oxidative stress environment (Figure 2). Further studies are needed to determine the exact effect of oxidative stress on the mitochondria and the link between the mitochondria and insulin resistance.

VII. Ketosis and oxidative stress and insulin signaling

Ketosis is a state characterized by elevated serum levels of ketone bodies. In addition to hyperglycemia, type 1 diabetics frequently experience ketosis due to insulin deficiency. This condition is more common and severe in patients with type 1 versus type 2 diabetes, but it may exacerbate insulin resistance in type 2 diabetes. Several markers of vascular inflammation have been shown to be influenced by the presence of ketosis. It has been reported that acetoacetate, but not β -hydroxybutyrate, increases lipid peroxidation and growth inhibition in cultured human endothelial cells [65], as well as lowering glutathione levels in human erythrocytes [66]. It has also been reported that acetoacetate increases TNF- α and IL-6 secretion in cultured monocytes and in hyperketonemic diabetic patients [67, 68]. Other reports have shown that chronic exposure to β -hydroxybutyrate can impair insulin action in cardiomyocytes [69]. These studies also showed an increase in ROS production and inhibition of the AMPK/p38 MAPK signaling pathway in cardiomyocytes treated with β -hydroxybutyrate. These results indicate that hyperketonemia may alter glucose uptake during metabolic stress conditions and could be a contributing factor to diabetic cardiomyopathy [69-71]. They also show that high levels of ketone bodies can increase cellular oxidative stress, which may contribute to the development of the insulin resistance seen in both types of diabetes.

II. Insulin sensitivity and nutrient availability

Both obesity and excessive intake of nutrients have long been risk factors for a variety of adverse health outcomes, such as high blood pressure, insulin resistance, oxidative stress, and type 2 diabetes. Furthermore, studies have shown that calorie overload in rodents results in rapidly induced skeletal muscle and liver insulin resistance, while calorie restriction enhances skeletal muscle, liver, and insulin sensitivity [72]. Several key modulators are thought to act as sensors to excessive intake of nutrients, including the regulatory subunits of PI3K, the protein deacetylase sirtuin 1 (SIRT1), and mTOR, a serine/threonine protein kinase [72], all of which also play key roles in modulating insulin action. Excess regulatory subunits of PI3K can have a negative effect on insulin signaling by binding to IRS-1 and inhibiting normal insulin signals. It has been reported that in insulin resistant subjects, there is an excess amount of regulatory subunits of PI3K [73], and that calorie restriction increases the ratio of PI3K catalytic-to-regulatory subunits in rat skeletal muscle [74]. mTOR is also a nutrient sensing pathway and overactivation in rodent and human systems is associated with insulin resistance [72]. SIRT1 plays a key role in sensing calorie restriction and resulting in positive insulin sensitivity. Calorie restriction increases expression of SIRT1 through eNOS expression. Activation of SIRT1 results in activation of PGC-1 α , one of the components of mitochondrial biogenesis [53]. Thus it is safe to say that calorie restriction may be a positive mechanism by which to increase mitochondrial biogenesis and insulin sensitivity. Two approaches have been proposed to attenuate the effect of excessive nutrients leading to insulin resistance, one being weight loss to enhance insulin sensitivity and the other being alterations in the macronutrient content of diets to avoid stimulating compensatory insulin mechanisms [75]. These forms of therapeutic intervention may be a good way to improve insulin sensitivity and delay or stop the onset of insulin resistance.

IX. PTEN and insulin sensitivity

PTEN is a phosphoinositide phosphatase that regulates the PI3K/Akt signaling pathway. It was originally identified as a tumor suppressor gene and later determined to act as a negative

regulator of the insulin signaling pathway [76-78]. Overexpression of PTEN in 3T3-L1 adipocytes results in inhibition of insulin-induced PtdIns(3,4)P₂ and PtdIns(3,4,5)P₃ production, Akt/PKB activation, GLUT4 translocation to the cell membrane and glucose uptake [79, 80]. In contrast, attenuation of PTEN expression by siRNA in 3T3-L1 adipocytes enhanced insulin-stimulated Akt and glycogen synthase kinase 3 α phosphorylation [77]. These results have also been confirmed in animal studies where PTEN antisense oligonucleotides normalized blood glucose concentrations in db/db and ob/ob mice. These studies also showed that inhibition of PTEN expression dramatically reduced insulin concentrations in ob/ob mice and improved the performance of db/db mice during insulin tolerance tests [81]. This suggests that PTEN may make individuals more susceptible to the development of type 2 diabetes by modulating insulin sensitivity. Only one report has examined the PTEN gene and identified three mutations of the gene in type 2 diabetes patients, suggesting that the PTEN gene is associated with insulin resistance and type 2 diabetes [82]. However, the question still remains whether upregulation of PTEN expression or activity could be responsible, at least in part, for the loss of insulin sensitivity in specific tissues and a cause of insulin resistance and diabetes. No studies have reported the activity or expression level of PTEN in normal versus insulin resistant or diabetic subjects; however, results from a rodent model reported increased PTEN gene expression in soleus muscle from obese Fa/Fa Zucker rats [83]. Overall, this suggests that PTEN plays a major role in regulating glucose metabolism via the Akt/PKB signaling pathway and that it may serve as a potential target when developing the therapeutic remedies aimed at enhancement of insulin sensitivity.

X. Sleep restriction and insulin sensitivity

Sleep plays a vital role in the normal homeostasis of glucose metabolism and insulin sensitivity and sleep loss is now considered a novel risk factor for insulin resistance and type 2 diabetes. Sleep loss, whether voluntary or disease related, affects millions of individuals in our modern society. Over the past few decades, the average sleep duration of Americans has decreased by 1.5 to 2 hours [84]. Interestingly, the trends in increased obesity and diabetes seem to mirror the time period for the increase in sleep loss. This suggests that sleep loss may contribute to the development of insulin resistance and type 2 diabetes. The mechanisms for this seem to be multifactorial and subject to multiple feedback and feedforward mechanisms that increase the risk of developing diabetes.

Increased levels of pro-inflammatory markers and oxidative stress are known precursors to insulin resistance and diabetes. Several studies have reported that sleep loss results in acute inflammation marked by small increases in pro-inflammatory cytokines or other inflammation markers [85-87]. Data from both human and animal studies suggest that IL-6 and TNF- α may induce insulin resistance, and elevated levels of these cytokines have often been reported in metabolic syndrome [88]. A couple of studies have shown that modest daily restriction of sleep is associated with increased secretion of the both IL-6 and TNF- α [86, 87]. C-reactive protein (CRP) is a major marker of the “acute-phase response” and a known indicator of inflammation. Both acute total and short-term partial sleep deprivation resulted in elevated CRP concentrations [85]. Inflammation and oxidative stress have been linked by many different pathways. Very little is known regarding whether oxidative stress is a cause or effect of sleep deprivation, although it has been reported that glutathione and catalase activity is decreased in sleep-deprived animals [89]. This indicates that sleep loss is linked to low-grade inflammation and oxidative stress, which is a prominent mechanism and risk factor for the development of insulin resistance and type 2 diabetes.

Experimental evidence has shown that sleep duration affects blood glucose levels and that sleep loss can be detrimental to carbohydrate metabolism and endocrine function [90, 91]. It

has been shown that, under periods of sleep deprivation, sleep extension and normal sleep, the sleep deprived individuals had significantly impaired glucose tolerance, and reductions in their acute insulin response to glucose and in glucose effectiveness when compared to fully rested individuals [90]. In another study, slow wave sleep suppression was shown to decrease insulin sensitivity, reduce glucose tolerance and increase the risk of type 2 diabetes [91]. A recent study conducted in type 1 diabetics concluded that partial sleep deprivation during a single night induced peripheral insulin resistance in these patients [92].

Disturbances in the counter-regulatory hormones growth hormone (GH) and cortisol may be linked to negative effects on glucose regulation due to sleep restriction. Studies have shown that sleep restriction is associated with and extended the duration of elevated nighttime GH concentrations [93] as well as with an increase in evening cortisol levels [90]. These results demonstrate how sleep deprivation could adversely affect glucose regulation by decreasing glucose uptake and reducing insulin sensitivity on the following morning [94]. Increased levels of cortisol may also affect other pathways that influence overall food intake and obesity. A study done in sheep examined the effects of a chronic increase in plasma cortisol concentrations on energy balance and endocrine function and concluded that elevated cortisol concentrations can affect food intake, adiposity, and reproductive function [95]. Sleep loss also has an impact on the hormones involved in appetite regulation. Two of the important appetite regulating hormones, leptin and ghrelin, are both altered by sleep deprivation in such a way as to influence food intake that is not in response to caloric need. Leptin is an appetite-inhibiting hormone, while ghrelin is an appetite-stimulating hormone. Several studies have shown that partial and chronic sleep loss is associated with a significant decrease in levels of leptin and an increase in levels of ghrelin [96-98]. Reduced time spent sleeping also allows more time to eat, which can in turn contribute to a person's overall food intake and lead to obesity. The mechanisms underlying the role of sleep loss in insulin resistance are multi-factorial but can be linked back to the effects of oxidative stress (Figure 3). Further studies are needed to determine the direct effect of hormonal changes, such as changes in cortisol levels during sleep deprivation, to better understand the underlying cause of insulin resistance and development of obesity and diabetes.

XI. Therapy

Antioxidant therapy has been of great interest as a way to combat oxidative stress in diabetic patients over the past decade. Although a very logical approach, it may take more than simple dosing with an antioxidant. Classical antioxidants such as vitamins E and C do not always appear to be helpful among all diabetic patients [99]. Recent reports now show that patients with type 2 diabetes mellitus and the haptoglobin (Hp) 2-2 genotype benefit from vitamin E supplementation [100, 101]. The main function of the Hp gene product is to bind free hemoglobin and facilitate its removal from circulation, ultimately inhibiting hemoglobin-induced oxidative damage to tissues [102]. Diabetic patients and Hp 2 transgenic mice with the (Hp) 2-2 genotype show increased oxidative stress markers and are at increased risk for cardiovascular disease [101, 103]. Therefore identifying patients with this genotype is more likely to benefit them by providing antioxidant treatment specific to their disease genotype. This work presents a different approach to treating diabetic patients with antioxidants by suggesting the use of more tailored treatment regimens. Although some antioxidants have been unsuccessful in preventing CVD and reducing diabetic risk, new insights into mechanisms leading to oxidative stress conditions may provide new antioxidant discoveries.

XII. Conclusion

Oxidative stress appears to be an underlying cause of many diseases, in particular diabetes. Oxidative stress has been implicated as a contributor to both the onset and the progression of

diabetes. Some of the consequences of an oxidative environment are development of insulin resistance, β -cell dysfunction, impaired glucose tolerance, and mitochondrial dysfunction, which can lead ultimately to the diabetic disease state. Experimental and clinical data suggest an inverse association between insulin sensitivity and ROS levels. Chronic exposure to oxidative stress activates a series of stress pathways involving a family of serine/threonine kinases, which in turn has a negative affect on insulin signaling. Oxidative stress can arise from a number of different sources, including disease states or lifestyle changes. Although it is clear that oxidative stress can arise from episodes of ketosis, sleep restriction, and excessive nutrient intake, more experimental evidence is needed to pinpoint the mechanisms contributing to insulin resistance in both type 1 diabetics and non-diabetic individuals. By avoiding hyperglycemia and monitoring calorie intake, generation of ROS can be reduced and the redox state of a diabetic can remain under control; however, this is not always an easy process and tailored treatment options may also be of some benefit. Overall, these data contribute to increased knowledge of various processes involving oxidative stress where intervention and therapy may serve to reduce the risk of insulin resistance and the development of diabetes.

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Abbreviations

AGE	advanced glycated end products
CAP	c-Cbl-associated protein
DAG	diacylglycerol
eNOS	endothelial nitric oxide synthase
eNOS	endothial nitric oxide synthase
ERK	extracellular signal regulated kinase
ETC	electron transport chain
GH	growth hormone
GLUT4	glucose transporter type 4
GRB-2	growth factor receptor-bound protein 2
GSH	glutathione
GSK-3	glycogen synthesis kinase 3
IKK	I κ B kinase
IL-6	interleukin 6
IR	insulin receptor
IRE	insulin response element
IRS	insulin receptor substrate
JNK	jun n-terminal kinase
LDM	low density microsome

MAPK	mitogen activated protein kinase
MELAS	myopathy, encephalopathy, lactic acidosis and stroke-like episodes
mtDNA	mitochondrial DNA
NF-κB	nuclear factor κ B
NOS	nitric oxide synthases
PDGF	platelet derived growth factor
PDK-1	phosphoinositide dependant kinase 1
PGC-1α	peroxisome proliferator activated receptor α
PH	pleckstrin homology domain
PI3K	phosphatidylinositol 3'-kinase
PKB	protein kinase B
PKC	protein kinase C
PTEN	phosphatase and tension homolog
RAGE	receptor for advanced glycated end product
RNI	reactive nitrogen intermediates
ROS	reactive oxygen species
SAPK	stress activated protein kinase
SH-2	Src-homology-2
SHP-2	Src homology 2-containing tyrosine phosphatase
SIRT1	sirtuin 1
SoHo	sorbin homology domain
T1D	type 1 diabetes
T2D	type 2 diabetes
TCA	tricarboxylic acid
TNF-α	tumor necrosis factor- α
UCP2	uncoupling protein 2
VEGF	vascular endothelial growth factor

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Figure 1.
Schematic of the effect of chronic oxidative stress on the insulin signaling pathway.

Figure 2.
Schematic of the putative pathways linking mitochondrial dysfunction and diabetes.

Figure 3.
Schematic of proposed pathways leading from sleep loss to diabetes.