Chapter 2

Review of Literature
2. Review of literature

2.1. Insulin signaling and insulin action

Insulin structure and synthesis

Insulin is the most potent anabolic hormone known and is essential for appropriate tissue development, growth and maintenance of whole-body glucose homeostasis. Insulin hormone is secreted by the β-cells of the pancreatic islets of Langerhans in response to increased circulating levels of glucose and amino acids after a meal. Insulin is a polypeptide hormone containing two chains of amino acids linked by disulfide bridges. In humans, the A chain contains 21 amino acids and B chain contains 30 amino acids. Minor differences occur in the amino acid composition of the insulin molecule from species to species. These differences are not sufficient to affect the biologic activity of particular insulin in heterologous species, but are sufficient to make the insulin antigenic. The gene for insulin is located on the short arm of chromosome 11 in humans. Like most of the other polypeptide hormones, insulin is synthesized as a large preprohormone (Fig. 2.1). Preproinsulin has a 23 amino acid signal peptide which is removed as it enters the endoplasmic reticulum. The remainder of the molecule is then folded, and the disulfide bonds are formed to make proinsulin. The two peptide segments, A and B are connected by the connecting peptide (C-peptide) which facilitates the folding. The C-peptide dissociates from the granules before insulin secretion. In humans the half-life of insulin in the circulation is only 5 minutes. In the target cells, both the insulin and insulin receptor are internalized and then degraded by proteases present in the endosomes.
Although insulin affects diverse processes such as cellular growth, differentiation, apoptosis, lipid and protein metabolism, the primary action of insulin is in the regulation of glucose homeostasis. Insulin regulates glucose homeostasis by reducing the hepatic glucose output (via decreasing gluconeogenesis and glycogenolysis) and increasing the rate of glucose uptake, primarily into striated muscle and adipose tissue. In muscle and fat cells, the transport of circulating glucose depends on the insulin-stimulated translocation of the glucose transporter GLUT4 isoform to the cell surface (Shulman, 2000). Insulin also increases lipid synthesis in liver and fat cells, and attenuates the fatty acid release from triglycerides in fat and muscle. The signaling mechanisms involved in the various biologic responses to insulin remain somewhat indefinable, but recent progress has shed light on a few pathways that are critical for its regulation of glucose and lipid metabolism.
The insulin receptor

Insulin action is initiated through the binding to and activation of its cell-surface receptor, which consists of two α-subunits and two β-subunits (Fig. 2.2). These subunits are linked by disulfide bonds into an α2β2 heterotetrameric complex. Insulin binds to the extracellular α subunits, transmitting a signal across the plasma membrane that activates the intracellular tyrosine kinase domains of the β subunits. The receptor then undergoes a series of intramolecular transphosphorylation reactions in which one β subunit phosphorylates its adjacent partner on specific tyrosine residues. Some evidence suggests that different tyrosine residues account for distinct functions. For example, phosphorylation of COOH-terminal tyrosines mediates the mitogenic actions of insulin. The phosphorylated tyrosines in the juxtamembrane domain may participate in substrate binding, whereas those found within the kinase domain regulate the catalytic activity of the insulin receptor β subunit.
Once activated, the insulin receptor phosphorylates a number of important proximal substrates on tyrosine, including members of the insulin receptor substrate family (IRS - 1/2/3/4), the Shc adapter protein isoforms, SIRP family members, Gab-1, Cbl, and APS. Tyrosine phosphorylation of the IRS proteins creates recognition sites for additional effector molecules containing Src homology 2 (SH2) domains. These include the small adapter proteins Grb2 and Nck, the SHP2 protein tyrosine phosphatase and, most importantly, the regulatory subunit of the phosphatidylinositol 3-kinase (PI3-kinase). Critical physiologic functions for both IRS-1 and IRS-2 have been recently established. The homozygous IRS1 knockout mice develop a mild state of insulin resistance (Araki et al., 1994; Tamemoto et al., 1994) but do not become diabetic, presumably owing to β-cell compensation. On the other hand, homozygous disruption of
the IRS2 gene results in impaired insulin secretion, in addition to peripheral insulin resistance and diabetes (Withers et al., 1998). Skeletal muscle IRS2 does not appear to be necessary for insulin or exercise-stimulated glucose transport. The insulin resistance observed in the IRS2 knockout animals most likely reflects secondary events occurring as a consequence of alterations in β-cell function or survival (Higaki et al., 1999). This finding is consistent with recent studies on β cell – specific insulin receptor knockout mice. These animals develop both peripheral insulin resistance and diabetes, presumably due to alterations in the normal pattern of insulin secretion (Kulkarni, 1999).

**Downstream signaling events**

At present, only one downstream signaling molecule has been identified as unequivocally essential for insulin-stimulated GLUT4 translocation, the PI3-kinase. Multiple studies using various pharmacologic inhibitors, microinjection of blocking antibodies and expression of dominant interfering and constitutively active mutants are all consistent with a necessary role for PI3-kinase activity in insulin-stimulated glucose uptake and GLUT4 translocation (Czech et al., 1999). Several studies have suggested that the interaction of IRS with PI3-kinase is necessary for the appropriate activation and/or targeting of the enzyme to a critical intracellular site, perhaps including its association with GLUT4 vesicles. The targets of PI3-kinase action are likewise controversial. Two classes of serine/threonine kinases are known to act downstream of PI3-kinase, namely the serine/threonine kinase Akt, also known as protein kinase B (PKB), and the atypical protein kinase C isoforms α and δ (PKCα/δ). Stable expression of a constitutively active, membrane-bound form of Akt in 3T3L1 adipocytes results in increased glucose transport and persistent localization of GLUT4 to the plasma membrane (Kohn et al., 1996 & 1998). Conversely, expression of a dominant interfering Akt mutant inhibits insulin-
stimulated GLUT4 translocation (Cong et al., 1997; Wang et al., 1999). Similarly, PKCz is also activated by the formation phosphoinositides, which accumulate in insulin-treated cells; PKCz is therefore also sensitive to pharmacologic PI3-kinase inhibitors, such as wortmannin (Bandyopadhyay et al., 1997). Expressions of PKCz or PKCl are also reported to induce GLUT4 translocation, whereas expression of a dominant-interfering PKCl inhibited GLUT4 translocation (Kitamura et al., 1998; Kotani et al., 1998). Thus, although PI3-kinase activation is essential, the protein kinase targets that mediate the effects of this pathway remain uncertain.

Several investigators have examined the role of Akt and PI 3-kinase in the regulation of peripheral insulin sensitivity. There appears to be a relative decrease in insulin-stimulated association of IRS proteins with PI 3-kinase and activation of Akt in insulin-resistant skeletal muscle (Cusi K et al., 2000; Krook et al., 1998). Surprisingly however, patients with reduced insulin-stimulated PI3-kinase maintain normal activation of Akt (Kim et al., 1999). Even though these studies involved a small number of patients, the data suggest that PI3-kinase is in substantial excess, with only a relatively small activation necessary for the full expression of downstream signaling. These data further imply that defects in the pathway leading from IRS tyrosine phosphorylation to Akt activation may not be responsible for insulin resistance in patients with type II diabetes.

Although PI3-kinase activity is necessary for insulin-stimulated glucose uptake, additional signals are also required for the stimulation of GLUT4 translocation. Thus, activation of PI3-kinase by stimulation with IL-4 or by engagement of certain integrins does not induce GLUT4 translocation (Guilherme et al., 1998; Isakoff et al., 1995). Furthermore, two natural insulin receptor mutations that were fully capable of activating PI3-kinase nevertheless failed to induce GLUT4 translocation and glucose uptake (Krook
et al., 1997). The most compelling evidence for a required additional PI3-kinase-independent pathway makes use of a cell-permeable analog of PI(3,4,5)P3 (Tag et al., 1998). In these experiments, addition of the PI(3,4,5)P3 analog had no effect on GLUT4 translocation. As expected, treatment of cells with wortmannin prevented insulin-stimulated translocation of GLUT4. However, treatment of adipocytes with wortmannin, insulin plus the PI(3,4,5)P3 analog, resulted in enhanced glucose uptake. These data suggest that although the PI3-kinase pathway is necessary, there is at least one additional pathway that is independent of PI 3-kinase activation.

Recent studies have shown that insulin can also rapidly induce the tyrosine phosphorylation of the Cbl proto-onco protein, but only in insulin-responsive cells (Ribon et al., 1997). This phosphorylation requires the presence of the adapter protein CAP, which associates with a proline rich domain in Cbl through its COOH-terminal SH3 domain. CAP appears to be important in insulin signaling, as it is markedly induced during adipocyte differentiation and is transcriptionally regulated by the thiazolidinedione family of insulin-sensitizing PPARγ agonists (Ribon et al., 1997). In support of this hypothesis, recently it was observed that expression of a dominant-interfering CAP mutant (CAPDSH3) completely inhibited insulin-stimulated glucose uptake and GLUT4 translocation. This occurred through a marked reduction in the localization of tyrosine-phosphorylated Cbl in the plasma membrane. Together, these data suggest that the insulin-dependent tyrosine phosphorylation and/or compartmentalization of CAP/Cbl complex may provide a necessary second signal that functions in parallel with the activation of the PI3-kinase-dependent signaling pathway.
GLUT4 vesicle trafficking, docking, and fusion

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**Figure 2.4.** Insulin stimulated GLUT4 recruitment to cell membrane.

The mechanisms by which upstream signaling pathways converge on the intracellular GLUT4-containing vesicles to translocate this protein to the cell surface remain obscure. In the basal state, GLUT4 continuously recycles between the cell-surface membrane and various intracellular compartments. After insulin stimulation, there is marked increase in the rate of GLUT4 vesicle exocytosis, with a small decrease in the rate of internalization. The insulin-stimulated exocytosis of GLUT4 resembles the regulated exocytosis of synaptic vesicles. In particular, GLUT4 vesicles contain the v-SNARE (Soluble NSF attaching factor receptor) proteins VAMP2 and VAMP3, which physically interact with their t-SNARE counterparts (syntaxin 4 and SNAP23) in the plasma membrane during GLUT4 vesicle translocation (Fig. 2.4). Several lines of evidence have suggested that insulin specifically stimulates the translocation of the GLUT4 from VAMP2-containing compartments (Pessin et al., 1999). Although these SNARE interactions are essential, none of these core proteins appear to be direct targets.
of insulin action. Similarly, although several important SNARE accessory proteins, such as Munc18c, Synip, and NSF, also appear required for the control of GLUT4 docking and fusion events, the molecular mechanism by which insulin regulates their function are yet to be elucidated.

**Insulin and MAPK pathway**

Apart from PI3K pathway, insulin also activates the mitogen activated protein kinase (MAPK) pathway, involved in the mitogenic activity of insulin. The MAP kinase pathway is activated by the binding of Grb2 to Tyr-phosphorylated She or IRS via its SH2 domain. Grb2 is pre-bound to mammalian Son of Sevenless (mSOS), a nucleotide exchange protein that catalyzes the exchange of GDP for GTP on Ras (a small GTPase protein); this results in activation of Ras. The phosphorylated form of Ras binds the inner leaflet of the plasma membrane, and on activation, it binds the NH2-terminal region of Raf, recruiting Raf to the plasma membrane. Ras-Raf interaction displaces the 14-3-3 proteins that are bound to Raf and allows the phosphorylation of Raf by a number of (Ser/Thr) kinases, thus disinhibiting Raf kinase (Kulkarni., 1999). Raf-1 activates a dual-specificity kinase, MEK1, by phosphorylating two regulatory Ser residues. In turn, MEK1 activates extracellular signal-regulated kinase (ERK)-1 and ERK2 by phosphorylating regulatory Tyr and Thr residues (Czech et al., 1999). Activated ERKs mediate the growth-promoting effects of insulin by phosphorylating transcription factors such as Elk-1, leading to the induction of genes.

**Regulation of insulin signaling cascades**

An essential component of cellular signal transduction is regulation of the system. Insulin action is terminated by the action of protein tyrosine phosphatases (PTPs). These phosphatases dephosphorylate the insulin receptor and IRS-1 and terminate insulin action.
in target cells. Agents like vanadium which inhibits PTPs action are known to improve insulin sensitivity. Ser/Thr residues of IRS proteins have a dual function and serve either as positive or negative modulators of insulin signal transduction. Phosphorylation of Ser residues within the PTB domain of IRS-1 by insulin-stimulated PKB (Paz et al., 1999) protects IRS proteins from the rapid action of PTPs and enables the Ser-phosphorylated IRS proteins to maintain their Tyr-phosphorylated active conformation. These findings implicate PKB alone as a positive regulator of IRS-1 functions. In contrast, Ser/Thr kinase, which is different from PKB (Paz et al., 1999) has been implicated as the kinase(s) that phosphorylates IRS-1 and acts as the negative feedback.

2.2. Insulin resistance

Insulin resistance is a common pathologic state in which target cells fail to respond to normal levels of circulating insulin. Insulin resistance is often associated with central obesity, hypertension, polycystic ovarian syndrome, dyslipidemia, and atherosclerosis. This constellation of symptoms is often referred to as syndrome X, or insulin resistance syndrome. At the molecular level, impaired insulin signaling results from mutations or post-translation modifications of the insulin receptor itself or any of its downstream effector molecules (Taylor et al., 1998). In some cases, insulin resistance could be accounted for by a defect in insulin binding to its receptor (Roach et al., 1994); however, insulin resistance is most often attributed to a post-binding defect in insulin action. A marked reduction in the receptor kinase activity was observed in several patients with extreme resistance to insulin, but with normal insulin binding (Grigorescu et al., 1986). Similarly, severe defects in receptor kinase activity are associated with naturally occurring mutations of the insulin receptor gene (Taylor et al., 1992). However,
these events are rare and do not play an important role in the pathophysiology of typical type 2 diabetes or obesity (Krook et al., 1996).

Whereas the insulin receptor is down regulated in human obesity, there is no further decrease in its activity in liver and muscle of type 2 diabetic patients, suggesting that aggravated insulin resistance of type 2 diabetes is primarily of a post-receptor nature (Caro et al., 1987). Insulin receptor Tyr kinase activity in patients with type 2 diabetes is significantly reduced (Thies et al., 1990). The effect of kinase activity could be getting nullified because of elevation in Tyr phosphatase activity (Kusari et al., 1994) or enhanced Ser/Thr phosphorylation of the receptor that impairs its Tyr kinase activity (Dunaif et al., 1995; Haring et al., 1991). Serine/Threonine phosphorylation of the insulin receptor occurs in response to the treatment of cells with insulin (Tavare et al., 1991), or with activators of PKC or the cAMP-dependent protein kinase (Stadtmauer et al., 1986; Takayama et al., 1988). Accordingly, downstream signaling cascades should be decreased in proportion to the defect in insulin receptor Tyr kinase activity; however, when compared with the reductions in Tyr phosphorylation of the insulin receptor and IRS-1, PI3-K is more severely reduced in type 2 diabetic patients (Goodyear et al., 1995).

Glycogen synthesis is markedly reduced in the muscle of type 2 diabetic patients. Shulman et al. demonstrated that one of the defects resulting in these changes was a reduction in glucose transport (Shulman et al., 2000). Because intracellular glucose and glucose-6- phosphate levels were reduced, Shulman et al. hypothesized that glucose transport was defective. Insulin-stimulated signaling pathways studied in isolated muscle preparations from type 2 diabetic patients demonstrated normal insulin receptor Tyr phosphorylation, normal MAP kinase (ERK) phosphorylation and normal glycogen synthase activity. In contrast, insulin stimulated glucose transport was reduced (Krook et
al., 2000); this was accompanied by a decrease in Tyr phosphorylation of IRS-1 and a reduced association of IRS-1 and PI3-K. Hence, insulin resistance could be attributed to the uncoupling of the insulin receptor and IRS proteins, which could be the result of excessive Ser/Thr phosphorylation of the latter (Paz et al., 1997).

**Ser/Thr phosphorylation of IRS proteins and insulin resistance**

Agents that enhance Ser/Thr phosphorylation of IRS proteins or other downstream effectors of the insulin signaling cascade play negative-regulatory roles in insulin action. Ser/Thr phosphorylation impairs insulin-stimulated Tyr phosphorylation of IRS proteins, uncouples insulin signal transduction and has been implicated in the development of insulin resistance (Hotamisligil et al., 1996; Tanti et al., 1994). Increased serine phosphorylation of IRS-1 has been observed after treatment of cells with activators of PKC, Ser/Thr phosphatase inhibitors such as okadaic acid, platelet-derived growth factor (Staubs et al., 1998), insulin or angiotensin II and with activation of cellular stress pathways by tumor necrosis factor (TNF) (Kanety et al., 1995) and other cytokines (Cohen et al., 1999). Decreased Tyr phosphorylation of IRS proteins and a reduction in their associated PI3-K activity is observed in skeletal muscle and adipocytes both in obesity and type 2 diabetes (Kerouz et al., 1997). Similarly, insulin-stimulated Tyr phosphorylation of IRS proteins and the activation of their downstream effectors are decreased in both genetic and diet induced rodent models of obesity and insulin resistance (Folli et al., 1993).

**TNF-α and insulin resistance**

TNF-α expression is increased in abdominal fat and muscle tissues of obese individuals and in many animal models of obesity. The degree of TNF-α expression is positively correlated with the degree of obesity and the levels of plasma insulin and
decreases with the improvement of insulin sensitivity (Hotamisligil et al., 1995; Saghizadeh et al., 1996). Circulating levels of TNF-α are elevated in obese subjects and decrease with weight reduction (Dandona et al., 1998). Further support for its role in affecting insulin sensitivity resulted from studies using a soluble TNF-α receptor IgG fusion protein, which neutralized TNF-α when administered to animals with insulin resistance; this neutralization was associated with improvement in insulin action (Hotamisligil et al., 1993). Whereas similar studies in humans were unsuccessful (Ofei et al., 1996), the possibility remains that TNF-α causes insulin resistance in a paracrine fashion and that circulating levels of TNF-α represent leakage into the circulation after increased tissue expression. Whether the effects of TNF-α are direct or indirect has not yet been determined, because it has been shown that TNF-α stimulates leptin secretion from adipocytes, and free fatty acid (FFA) levels are correlated with TNF-α levels. Both leptin and FFAs play a role in insulin resistance (Cheung et al., 1998; Grunfeld et al., 1996).

TNF-α has direct effects on the insulin signaling cascade in cultured cells. TNF-α increases the Ser phosphorylation of IRS-1 and IRS-2. Serine phosphorylation of these substrates results in a reduction in both insulin-receptor Tyr autophosphorylation and Tyr kinase activity of the receptor and markedly reduces the ability of the IRS molecules to dock with the receptor and interact with downstream pathways, such as PI3K and glucose transport (Paz et al., 1999). The mechanisms by which TNF-α enhances IRS Ser phosphorylation is not known. Interestingly, activation of the peroxisome proliferator-activated receptor-γ (PPAR γ) by thiazolidinediones (TZDs) reduces the expression of TNF-α and hinders TNF-α’s inhibition of insulin action (Jiang et al., 1998).
Fatty acids and insulin resistance (lipotoxicity)

In both obesity and type 2 diabetes, plasma free fatty acid (FFA) levels are elevated. Increasingly, there is evidence to support the contention that FFAs affect insulin action at the peripheral target tissues. The exact site of inhibition of insulin action has not yet been well defined; however, FFA induced defects at the level of glucose uptake into muscle, phosphorylation of glucose by hexokinase and glycogen synthesis has been demonstrated (Boden et al., 1994; Roden et al., 1996). It has been further proposed that the mechanisms by which TNF-α and leptin cause insulin resistance and TZDs improve insulin sensitivity may be triggered indirectly via a reduction in FFAs levels (Muller et al., 1997).

Our understanding of insulin signal transduction pathways and insulin resistance has evolved rapidly and extensively over the past few years. Still, further studies are required to unravel the mechanisms controlling these intricate regulatory processes that presumably mediate, at least in part, the insulin resistance associated with obesity and hyperinsulinemia. These new emerging paradigms and target substrates should facilitate the development of compounds that will activate or inhibit various signaling elements in these cascades, thereby renewing our hope for new families of therapeutic agents to treat obesity and type 2 diabetes.

2.3. Oxidative stress and antioxidants

Free radicals can be defined as molecules or molecular fragments containing one or more unpaired electrons in atomic or molecular orbitals (Halliwell et al., 1999). This unpaired electron(s) usually gives a considerable degree of reactivity to the free radical. Radicals derived from oxygen represent the most important class of radical species generated in living systems (Miller et al., 1990). Oxygen free radicals or more generally,
reactive oxygen species (ROS) as well as reactive nitrogen species (RNS) are products of normal cellular metabolism. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are well recognised for playing a dual role as both deleterious and beneficial species since they can be either harmful or beneficial to living systems (Valko et al., 2004). Beneficial effects of ROS (Fig. 2.5) occur at low/moderate concentrations and involve physiological roles in cellular responses to noxia, in defence against infectious agents and in the function of a number of cellular signaling systems. One further beneficial example of ROS at low/moderate concentrations is the induction of a mitogenic response.

![Diagram of Metabolism/Environment and Reactive Oxygen Species (ROS)](attachment)

**Figure 2.5.** Pleiotropic roles of reactive oxygen species (ROS) known to occur in higher organisms, indicative of the fact that ROS are not always harmful to cells.

The harmful effect of free radicals causing potential biological damage is termed oxidative stress and nitrosative stress (Kovacic et al., 2001; Ridnour et al., 2006). This occurs in biological systems when there is an overproduction of ROS/RNS on one side
and a deficiency of enzymatic and non-enzymatic antioxidants on the other. In other words, oxidative stress results from the metabolic reactions that use oxygen and represents a disturbance in the equilibrium status of prooxidant/antioxidant reactions in living organisms. The excess ROS can damage cellular lipids, proteins or DNA inhibiting their normal function. Because of this, oxidative stress has been implicated in a number of human diseases as well as in the ageing process. The delicate balance between beneficial and harmful effects of free radicals is a very important aspect of living organisms and is achieved by mechanisms called "redox regulation". The process of "redox regulation" protects living organisms from oxidative stress and maintains "redox homeostasis" by controlling the redox status invivo.

![Diagram of Oxidative Stress](image)

Figure 2.6. Oxidative stress results from imbalance between the levels of antioxidants (AOX) and reactive oxygen species (ROS). Cells are normally able to balance the production of oxidants and antioxidants to maintain redox equilibrium. Oxidative stress occurs when this equilibrium is upset by excess levels of ROS or depletion of antioxidant defenses.

**Reactive oxygen species (ROS)**

Free radicals derived from oxygen represent the most important class of radical species generated in living systems (Miller et al., 1990). Molecular oxygen (dioxgen) has a unique electronic configuration and is itself a radical. The addition of one electron to dioxygen forms the superoxide anion radical (O$_2^-$) (Miller et al., 1990). Superoxide
anion is formed either through metabolic process or following oxygen activation by physical irradiation. Superoxide anion can further interact with other molecules to generate ‘secondary’ ROS, either directly or through enzyme or metal-catalysed processes (Valko et al., 2005).

The production of superoxide occurs mostly within the mitochondria of a cell (Cadenas et al., 1998). The mitochondrial electron transport chain is the main source of ATP in the mammalian cell and thus is essential for life. During energy transduction, a small number of electrons leak to oxygen prematurely, forming the oxygen free radical superoxide, which has been implicated in the pathophysiology of a variety of diseases (Kovacic et al., 2005; Valko et al., 2004). Measurements on sub-mitochondrial particles suggest an upper limit of 1–3 % of all electrons in the transport chain leaking to generate superoxide instead of contributing to the reduction of oxygen to water. Superoxide is produced from both Complexes I and III of the electron transport chain, and once in its anionic form it is too strongly charged to readily cross the inner mitochondrial membrane. Recently, it has been demonstrated that Complex I-dependent superoxide is exclusively released into the matrix and that no detectable levels escape from intact mitochondria (Muller et al., 2004). This finding fits well with the proposed site of electron leak at Complex I, namely the iron–sulphur clusters of the (matrix-protruding) hydrophilic arm. In addition, experiments on Complex III show direct extra-mitochondrial release of superoxide.

The hydroxyl radical (·OH) is the neutral form of the hydroxide ion. The hydroxyl radical has a high reactivity, making it a very dangerous radical with a very short invivo half-life of approximately $10^{-9}$ seconds (Pastor et al., 2000). Thus when produced invivo hydroxyl radical reacts close to its site of formation. The redox state of the cell is largely
linked to an iron (and copper) redox couple and is maintained within strict physiological limits. It has been suggested that iron regulation ensures that there is no free intracellular iron. However under stress conditions, an excess of superoxide anion releases 'free iron' from iron-containing molecules. The release of iron by superoxide has been demonstrated for [4Fe - 4S] cluster containing enzymes of the dehydratase-lyase family (Liochev et al., 1994). The released Fe$^{2+}$ can participate in the Fenton reaction, generating highly reactive hydroxyl radical (Fe$^{3+}$+H$_2$O$_2$→Fe$^{3+}$ + 'OH+OH$^-$). Thus under stress conditions, O$_2^-$ acts as an oxidant of [4Fe - 4S] cluster-containing enzymes and facilitates 'OH production from H$_2$O$_2$ by making Fe$^{3+}$ available for the Fenton reaction (Valko et al., 2005; Leonard et al., 2004). The superoxide radical participates in the Haber-Weiss reaction (O$_2^-$+H$_2$O$_2$→O$_2$+ 'OH+OH$^-$) which combines a Fenton reaction and the reduction of Fe$^{3+}$ by superoxide, yielding Fe$^{2+}$ and oxygen (Fe$^{3+}$ +O$_2^-$→Fe$^{2+}$ +O$_2$) (Liochev et al., 1994).

Additional reactive radicals derived from oxygen that can be formed in living systems are peroxyl radicals (ROO$^-$). The simplest peroxyl radical is HOO$^-$, which is the protonated form of superoxide (O$_2^-$) and is usually termed either hydroperoxyl radical or perhydroxyl radical. It has been demonstrated that hydroperoxyl radical initiates fatty acid peroxidation by two parallel pathways: fatty acid hydroperoxide (LOOH)-independent and LOOH-dependent (Aikens et al., 1991). The LOOH-dependent pathway of HOO$^-$ initiated fatty acid peroxidation may be relevant to mechanisms of lipid peroxidation initiation invivo.

Xanthine oxidase (XO) and xanthine dehydrogenase (XD) are interconvertible forms of the same enzyme, known as xanthine oxidoreductase (XOR) (Borges et al., 2002; Vorbach et al., 2003). In purine catabolism, XOR catalyzes the oxidative
hydroxylation of hypoxanthine to xanthine and subsequently of xanthine to uric acid. Uric acid acts as a potent antioxidant and free radical scavenger. Xanthine oxidoreductase (XOR) has, therefore, important functions as a cellular defense enzyme against oxidative stress. With both XO and XD forms but particularly with the XO form, numerous ROS and RNS are synthesized (Vorbach et al., 2003). Thus, the synthesis of both an antioxidant (uric acid) and numerous free radicals (ROS and RNS) makes XOR an important protective regulator of the cellular redox potential.

Peroxisomes are known to produce $H_2O_2$, but not $O_2^-$, under physiologic conditions (Valko et al., 2006). Peroxisomes are major sites of oxygen consumption in the cell and participate in several metabolic functions that use oxygen. Oxygen consumption in the peroxisome leads to $H_2O_2$ production, which is then used to oxidize a variety of molecules. The organelle also contains catalase, which decomposes hydrogen peroxide and presumably prevents accumulation of this toxic compound. Thus, the peroxisome maintains a delicate balance with respect to the relative concentrations or activities of these enzymes to ensure no net production of ROS. How the organelle maintains this equilibrium is unclear. When peroxisomes are damaged and their $H_2O_2$ consuming enzymes are down regulated, $H_2O_2$ releases into the cytosol which significantly contributes to oxidative stress.

If a phagocytic cell such as the neutrophil is exposed to a stimulus, it has the ability of recognising the foreign particle and undergoing a series of reactions called the respiratory burst (DeCoursey et al., 2005). Nicotine adenine dinucleotide phosphate [NAD(P)H] oxidase is best characterised in neutrophils, where its production of $O_2^-$ generates the respiratory burst necessary for bacterial destruction. The non-phagocytic
NAD(P)H oxidases produce superoxide at a fraction (1–10 %) of the levels produced in neutrophils and are thought to function in intracellular signaling pathways.

**Reactive nitrogen species (RNS)**

Nitric oxide (NO') is a small molecule that contains one unpaired electron on the antibonding $2\pi^*\gamma$ orbital and is, therefore, a radical. Nitric oxide (NO') is generated in biological tissues by specific nitric oxide synthase (NOS), which metabolise arginine to citrulline with the formation of NO' via a five electron oxidative reaction (Ghaforifar et al., 2005). Nitric oxide (NO') is an abundant reactive radical that acts as an important oxidative biological signaling molecule in a large variety of diverse physiological processes, including neurotransmission, blood pressure regulation, smooth muscle relaxation and immune regulation (Bergendi et al., 1999). Nitric oxide (NO') has a half-life of only a few seconds in an aqueous environment. Nitric oxide (NO') has greater stability in an environment with a lower oxygen concentration (half life > 15 sec). However, since it is soluble in both aqueous and lipid media, it readily diffuses through the cytoplasm and plasma membranes (Chiueh, 1999). In the extracellular milieu, NO' reacts with oxygen and water to form nitrate and nitrite anions. Cells of the immune system produce both the superoxide anion and nitric oxide during the oxidative burst triggered during inflammatory processes. Under these conditions, nitric oxide and the superoxide anion may react together to produce significant amounts of a much more oxidatively active molecule, peroxynitrite anion (ONOO^-), which is a potent oxidising agent that can cause DNA fragmentation and lipid oxidation (Carr et al., 2000).
Antioxidants

Exposure to free radicals from a variety of sources has led organisms to develop a series of defence mechanisms (Cadenas, 2000). Defence mechanisms against free radical-induced oxidative stress involve: (i) preventative mechanisms, (ii) repair mechanisms, (iii) physical defences, and (iv) antioxidant defences. Enzymatic antioxidant defences include (Table 2.1) superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT). Non-enzymatic antioxidants (Table 2.1) are represented by ascorbic acid (Vitamin C), tocopherol (Vitamin E), glutathione (GSH), lipoic acid (LA), carotenoids, flavonoids and other antioxidants. Under normal conditions, there is a balance between both the activities and the intracellular levels of these antioxidants. This balance is essential for the survival of organisms and their health.

<table>
<thead>
<tr>
<th>Non-enzymatic antioxidant molecules</th>
<th>Subcellular location</th>
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<tr>
<td>Ascorbate (vitamin C)</td>
<td>Plastid; apoplastic; cytosol; vacuole</td>
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<tr>
<td>β-Carotene</td>
<td>Plastid</td>
</tr>
<tr>
<td>Glutathione, reduced (GSH)</td>
<td>Plastid; mitochondrion; cytosol</td>
</tr>
<tr>
<td>Polyamines (e.g., putrescine, spermine)</td>
<td>Nucleus; plastid; mitochondrion; cytosol</td>
</tr>
<tr>
<td>α-Tocopherol (vitamin E)</td>
<td>Cell and plastid membranes</td>
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<td>Zeaxanthin</td>
<td>Chloroplast</td>
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<tr>
<th>Antioxidant enzymes</th>
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<tr>
<td>Ascorbate peroxidase</td>
<td>1.11.1.11</td>
<td>Plastid stroma and membranes</td>
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<td>Peroxidases (non-specific)</td>
<td>1.11.1.7</td>
<td>Cytosol; cell wall-bound</td>
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<td>Catalase</td>
<td>1.11.1.6</td>
<td>Glyoxy some; perox isome; cytosol; mitochondrion</td>
</tr>
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<td>Super oxide dismutase (SOD)</td>
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<td>Cytosol (Cu/ZnSOD); plastid (Cu/ZnSOD; FeSOD); mitochondrion (MnSOD); perox isome</td>
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<td>Dehydrosascorbate reductase</td>
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<td>Cytosol; plastid</td>
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<td>Glutathione reductase</td>
<td>1.8.4.2</td>
<td>Mitochondrion; cytosol; plastid</td>
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<td>Cytosol; microsomal</td>
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Table 2.1. Enzymatic and non-enzymatic antioxidant systems.
Antioxidant enzymes

Superoxide dismutase (SOD)

Superoxide dismutase is a very important enzyme that functions as a cellular antioxidant. It is present in cell cytoplasm (copper-zinc enzyme) and in mitochondria (manganese enzyme) in order to maintain a low concentration of superoxide anion. It catalyzes the dismutation of superoxide anion in the following manner.

\[ 2 \text{O}_2^- + 2 \text{H}^+ \rightarrow \text{O}_2 + \text{H}_2\text{O}_2 \]

The absence of this enzyme is lethal. The amount of superoxide dismutase is controlled by specific redox-sensitive genes in cells. There is also an extracellular form of superoxide dismutase in plasma, lymph, and synovial fluid that is different from the intracellular forms of the enzyme. The extracellular enzyme may function at cell surfaces.

Catalase

Catalase is a heme protein that catalyzes the reaction shown below in which hydrogen peroxide is detoxified. It is usually found in peroxisomes except in cells like erythrocytes that do not contain these organelles. In that case catalase is a cytoplasmic enzyme. Catalase provides a protective role that is similar to that of glutathione peroxidase because both are important means of removing hydrogen peroxide. Both catalase and glutathione peroxidase are important in hydrogen peroxide detoxification.

\[ 2 \text{H}_2\text{O}_2 \rightarrow \text{O}_2 + 2 \text{H}_2\text{O} \]
Glutathione peroxidase

Glutathione peroxidase is a cytoplasmic and mitochondrial enzyme that is important for detoxifying H$_2$O$_2$ in most cells. This protein is a seleno protein, i.e., it contains a selenocysteine amino acid at the active site instead of a normal cysteine. The selenium that replaces the normal sulfur in this amino acid has enhanced nucleophilic properties and ionizes more readily to release a proton. It is a much more effective catalyst in the reaction catalyzed by this enzyme.

\[ H_2O_2 + 2 \text{glutathione (GSH)} \rightarrow \text{glutathione disulfide (GSSG)} + H_2O \]

Glutathione reductase

The flavoprotein, glutathione reductase (the enzyme uses bound FAD, flavin adenine dinucleotide, in an interesting electron transfer reaction), uses the reducing power for the pentose phosphate pathway (NADPH) to keep the glutathione pool in cell in a very reduced state. Even when large amounts of hydrogen peroxide are present this enzyme is very effective at reducing the cellular glutathione pool. Cells contain at least 100 reduced glutathione molecules for every molecule of glutathione disulfide

\[ \text{Glutathione disulfide (GSSG)} + \text{NADPH} \rightarrow 2 \text{glutathione (GSH)} + \text{NADP}^+ + H^+ \]

The net result of this cycle is to use NADPH to reduce hydrogen peroxide to water, a process that requires two electrons. Other reductases can also catalyze reactions that reduce lipid peroxides, i.e., LipidOOH, instead of hydrogen peroxide. Thus the reaction is:
LipidOOH + 2 glutathione (GSH) → glutathione disulfide (GSSG) + LipidOH

There are a number of other enzymes that contribute to less well defined antioxidant functions of specific cells. These enzymes are usually quite abundant and the amounts present usually reflect the oxidative status of the cell.

**Non enzymatic antioxidants**

**Glutathione**

![Glutathione molecule](image)

**Figure 2.7. Structure of glutathione.**

The tripeptide, glutathione (GSH) is the major thiol antioxidant and redox buffer of the cell. The oxidised form of glutathione is GSSG, glutathione disulphide. Glutathione is highly abundant in the cytosol (1–11 mM), nuclei (3–15 mM) and mitochondria (5–11 mM), and is the major soluble antioxidant in these cell compartments. Because GSH is synthesized in the cytosol by the sequential action of glutamate–cysteine ligase and glutathione synthetase, its mitochondrial presence requires inner membrane transport. Two mitochondrial electroneutral antiport carrier proteins have been shown to have the capacity to transport GSH, the dicarboxylate carrier protein and the 2-oxoglutarate carrier protein. Recently, it has been shown that externally added GSH is readily taken up by mitochondria, despite the 8 mM GSH present in the
mitochondrial matrix (Shen et al., 2005). It therefore appears that GSH is taken up against a concentration gradient. Glutathione (GSH) in the nucleus maintains the redox state of critical protein sulphhydrils that are necessary for DNA repair and expression. Oxidised glutathione is accumulated inside the cells and the ratio of GSH/GSSG is a good measure of oxidative stress of an organism (Nogueira et al., 2004). Too high a concentration of GSSG may damage many enzymes oxidatively.

The main protective roles of glutathione against oxidative stress are (Masella et al., 2005): (i) glutathione is a cofactor of several detoxifying enzymes against oxidative stress, e.g. glutathione peroxidase (GPx), glutathione transferase and others; (ii) GSH participates in amino acid transport through the plasma membrane; (iii) GSH scavenges hydroxyl radical and singlet oxygen directly, detoxifying hydrogen peroxide and lipid peroxides by the catalytic action of glutathione peroxidase; (iv) glutathione is able to regenerate the most important antioxidants, Vitamins C and E, back to their active forms; glutathione can reduce the tocopherol radical of Vitamin E directly, or indirectly, via reduction of semi-dehydroascorbate to ascorbate. The capacity of glutathione to regenerate the most important antioxidants is linked with the redox state of the glutathione disulphide-glutathione couple (GSSG/2GSH) (Pastore et al., 2003).

**Vitamin E**

![Figure 2.8. Structure of Vitamin E.](image)

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The main function of vitamin E is as a chain-breaking, free radical trapping antioxidant in cell membranes and plasma lipoproteins. It reacts with the lipid peroxide radicals formed by peroxidation of polyunsaturated fatty acids before they can establish a chain reaction. The tocopheroxyl free radical product is relatively unreactive and ultimately forms nonradical compounds. Commonly, the tocopheroxyl radical is reduced back to tocopherol by reaction with vitamin C from plasma. The resultant mono dehydroascorbate free radical then undergoes enzymic or nonenzymic reaction to yield ascorbate and dehydroascorbate, neither of which is a free radical. The stability of the tocopheroxyl free radical means that it can penetrate farther into cells and, potentially, propagate a chain reaction. Therefore, vitamin E may, like other antioxidants, also have pro-oxidant actions, especially at high concentrations. This may explain why, although studies have shown an association between high blood concentrations of vitamin E and a lower incidence of atherosclerosis, the effect of high doses of vitamin E have been disappointing.

**Vitamin C**

![Figure 2.9. Structure of Vitamin C.](image)

As an antioxidant vitamin C's primary role is to neutralize free radicals. Since vitamin C is water soluble it can act both inside and outside the cell to combat the free
free radicals such as hydroxyl and super oxide radicals and quench the.
versatile vitamin C also plays an important role in the regeneration of
such as vitamin E and lipoic acid.

Lipoic acid

![Structure of lipoic acid](image)

Figure 2.10. Structure of lipoic acid.

The naturally occurring antioxidant α-lipoic acid, a cofactor in the α-keto-acid
dehydrogenase complex has been demonstrated to 1) quench radicals, 2) exhibit chelating
activity, 3) reduce lipid peroxidation, 4) increase intracellular reduced glutathione levels
and 5) regenerate vitamin C and vitamin E. Since lipoic acid is both water and lipid
soluble, it can act both at lipophilic and lipophobic environment of cell. Due to this property
lipoic acid plays a central role in the sparing/regeneration of other antioxidants.

2.4. Redox homeostasis and redox signaling

Free radicals operate at low, but measurable concentrations in the cells. Their
'steady state' concentrations are determined by the balance between their rates of
production and their rates of removal by various antioxidants. Thus each cell is
characterized by a particular concentration of electrons (redox state) stored in many
cellular constituents and the redox state of a cell and its oscillation determines cellular
functioning (Schafer et al., 2001). The term "redox state" has not only been used to describe the state of a redox pair, e.g. GSSG/2GSH, Asc*-/-AcsH- and others, but also to describe more generally the redox environment of a cell (Butler., 2000). The redox state of a cell is kept within a narrow range under normal conditions - similar to the manner in which a biological system regulates its pH. Under pathological conditions, the redox state can be altered to lower or higher values. A 30mV change in the redox state means a 10-fold change in the ratio between reductant and oxidant species (Schafer et al., 2001).

The term redox signaling is used to describe a regulatory process in which a signal is delivered through redox reactions. Redox signaling requires either an increase in ROS formation or a decrease in the activity of antioxidant system(s). The regulated increase in free radicals (ROS/RNS) leads to a temporary imbalance that represents the physiological basis for redox regulation. Thus physiological demonstration of redox regulation involves a temporary shift of the intracellular redox state toward more oxidising conditions. Signaling mechanisms that respond to changes in the redox state involve: (i) Nuclear factor-kappa B (NF-kB) pathway, (ii) protein tyrosine phosphatases, (iii) Src family kinases, (iv) C-Jun-N-terminal kinase (JNK) and p38 mitogen activated protein kinase (p38MAPK) signaling pathways, (v) insulin receptor kinase activity and others (Galter et al., 1994; Hehner et al., 2000; Kuge et al., 1994). The process of redox signaling is adopted by various organisms including bacteria to induce protective responses against oxidative stress and to restore the original state of 'redox homeostasis' after temporary exposure to ROS/RNS. Under pathological conditions, however, abnormally large concentrations of ROS/RNS may lead to permanent changes in signal transduction and gene expression, typical for disease states. Thus recent studies have
linked the role of redox sensitive NF-kB, P38MAPK and JNK pathways in the pathogenesis of insulin resistance.

**Nuclear Factor kappa-B (NF-kB) pathway**

One major intracellular target of oxidative stress is the transcription factor NF-kB (Baldwin., 2001; Mercurio et al., 1999). NF-kB can be activated by a wide array of exogenous and endogenous stimuli including hyperglycemia, elevated FFA, ROS, TNF-α, IL-1β and other proinflammatory cytokines, viral infection and UV irradiation (Barnes et al., 1997). NF-kB pathway plays a critical role in mediating immune and inflammatory responses and apoptosis. The aberrant regulation of NF-kB pathway is associated with a number of chronic diseases including diabetes and atherosclerosis.

![Figure 2.11. Activation of NF-kB pathway](image)

NF-kB pathway is activated through a common pathway, which involves the phosphorylation-induced proteasome-mediated degradation of the inhibitory subunit, IkB (Karin et al., 1999). A general overview of the sequence of events leading to NF-kB
activation is shown in Fig. 2.11. In resting cells, NF-kB is present in the cytoplasm as an inactive heterodimer, consisting of the p50 and p65 subunits complexed with an inhibitor protein subunit, IkB. After stimulation, a serine kinase cascade is activated leading to the phosphorylation of IkB (Karin et al., 2000). This event primes IkB as a substrate for ubiquitination and subsequent degradation, freeing the NF-kB heterodimer to translocate to the nucleus. NF-kB regulates the expression of a large number of genes, including growth factors [e.g., vascular endothelial growth factor (VEGF)], proinflammatory cytokines (e.g., TNF-α and IL-1β), RAGE, adhesion molecules (e.g., vascular cell adhesion molecule-1) and others. Many products of the genes regulated by NF-kB also, in turn activate NF-kB (e.g., VEGF, TNF-α and IL-1β). Enzymes that catalyze the ubiquitination and degradation of phospho-IkB are constitutively active, indicating that the principal regulatory step in the activation of NF-kB is IkB phosphorylation (Karin et al., 1999 & 2000).

The enzyme that phosphorylates IkB is IkB kinase (IKK), a heterotrimeric complex consisting of two catalytic subunits, IKKa (also called IKK1) and IKKB (also called IKK2), and a regulatory subunit, IKKγ (DiDonato et al., 1997; Mercurio et al., 1997). The IKK is activated after serine phosphorylation catalyzed by upstream serine kinases, including NF-kB inducing kinase (NIK) (Ling et al., 1998) and NF-kB activating kinase (NAK) (Tojima et al., 2000). Although both IKKa and IKKB subunits are subject to serine phosphorylation, only substitution of these sites in IKKB completely prevents the activation of total IKK activity (Delhase et al., 1999). Interestingly, IKKB is directly inhibited by aspirin and salicylate (Yin et al., 1998) along with several anti-inflammatory cyclopentenone prostaglandins (Rossi et al., 2000), making these agents important tools with which to study the NF-kB pathway. Recent discoveries and characterization of
IKKβ, NIK and NAK provide a unique opportunity to investigate and potentially identify novel molecular targets of antioxidant action, which have the demonstrated ability to block activation of the NF-κB pathway.

**JNK and p38MAPK pathways**

The C-Jun N-terminal Kinase (JNK) also referred to as SAPK (stress activated protein kinase) and p38 MAPKs (mitogen activated protein kinase) are members of the complex superfamily of MAPserine/threonine protein kinases (Fig. 2.12). This superfamily also includes the ERKs (Lewis et al., 1998). In contrast to ERKs (also referred to as MAPKs), which are typically activated by mitogens, JNK and p38MAPK are known as stress-activated kinases. This can be attributed to the fact that the activities of these enzymes are stimulated by a variety of exogenous and endogenous stress-
inducing stimuli including ROS, oxidative stress, osmotic stress, proinflammatory cytokines, heat shock, and UV irradiation.

Activated JNK bind to and phosphorylate the transcription factor cJun, which is one component of the activator protein (AP)-1 transcription factor complex (along with other members of the cFos and cJun families). Transactivation of cJun by JNK enhances the expression of genes with AP-1 recognition sites including cJun, thereby initiating a positive feedback loop. The redox regulation of AP-1 has been studied extensively and serves as a model for the redox regulation of other transcription factors including NF-κB. A closely related member of this family of transcription factors is AP-2. This transcription factor is activated by inflammatory cytokines and prostaglandins in cultured mesangial cells (Suyama et al., 2001) and its DNA-binding activity in vitro is redox sensitive (Huang et al., 1998). Activation of AP-2 is associated with decreased expression of SOD, a major antioxidant enzyme (Zhu et al., 2001).

The most familiar function attributed to the JNK pathway is its role as a mediator of apoptosis (Basu et al., 1998). Blockage of the JNK pathway by expression of dominant negative cJun increases cell survival, an effect that can also be achieved by treatment with the thiol antioxidant and redox regulator, N-acetyl cysteine (Xia et al., 1995; Park et al., 1996). JNK is activated by hyperglycemia-induced oxidative stress and is likely involved in apoptosis mediated by hyperglycemia in human endothelial cells (Ho et al., 2000). Interestingly, H₂O₂ generation, JNK activity, and subsequent apoptosis induced by hyperglycemia could be suppressed by the antioxidant vitamin C. Thus, induction of AP-1 by JNK could serve as a potential marker for pathologies associated with chronic oxidative stress.
Activation of p38 MAPK also influences a large number of cellular processes including inflammation, immunity, cell growth, apoptosis, tissue-specific responses to stress by regulating gene expression, other signaling pathways (e.g., NF-kB, insulin, cytokine, arachidonate, and others), and cytoskeletal rearrangement. In addition, p38 MAPK rapidly regulates other serine kinases (Lewis et al., 1998). Chronic activation of the p38 MAPK pathway is often associated with disease pathology, including inflammation, ischemia/reperfusion injury, infectious disease, and neuronal disease (Obata et al., 2000). In this regard, selective p38 MAPK inhibitors are in clinical development as anti-inflammatory agents (Lee et al., 2000).

p38 MAPK is activated in response to hyperglycemia and in diabetes. In vascular smooth muscle cells, treatment with insulin (100 nm) and hyperglycemia (25 mm) for 12–24 hours induced the activation of p38 MAPK. In glomeruli of rats made diabetic by streptozotocin, p38 MAPK activity was increased compared with controls, followed by increased phosphorylation of heat shock protein 25, a downstream substrate of p38 MAPK (Dunlop et al., 2000). These effects appeared to be the result of increased ROS production. Taken together, these recent data suggest that the NF-kB, JNK and p38MAPK pathways are candidate redox sensitive signaling systems and their chronic activation leads to various complications.

2.5. Oxidative stress, redox sensitive serine kinase pathways and insulin resistance

Oxidative stress is not only associated with complications of diabetes, but has been linked to insulin resistance (Paolisso et al., 1994 & 1996; Ceriello et al., 2001). In 3T3-L1 adipocytes, induction of oxidative stress with H₂O₂ inhibits insulin-stimulated glucose transport (Rudich et al., 1997&1999; Tirosh et al., 1999). Studies (Najib et al., 2001) have recently reported the direct protective effect of glutathione on insulin action
in HTC rat hepatoma cells transfected with the insulin receptor. In vivo studies in animal models of diabetes indicate that antioxidants, especially LA, improve insulin sensitivity. Several clinical trials have also demonstrated improved insulin sensitivity in insulin-resistant and/or diabetic patients treated with the antioxidants like vitamin C, LA, vitamin E, and glutathione (Caballero., 1993; Hirai et al., 2000; Hirashima et al., 2000). In patients with type 2 diabetes, both acute and chronic administration of LA improves insulin resistance as measured by both the euglycemic-hyperinsulinemic clamp and the Bergman minimal model.

Oxidative stress leads to the activation of multiple serine kinase cascades (Adler et al., 1999; Cohen et al., 1996; Kyriakis et al., 1996). There are a number of potential targets of these kinases in the insulin signaling pathway, including the insulin receptor (IR) and the insulin receptor substrate (IRS) family of proteins. Increased phosphorylation of the IR or IRS on discrete serine or threonine sites decreases the extent of their tyrosine phosphorylation and is consistent with impaired insulin action (Kellerer et al., 1998; Paz et al., 1997). Recently it has been reported that IKKβ, which activates NF-kB, is increased in insulin-resistant muscle from a variety of sources (Yuan et al., 2000). Activation of IKKβ inhibits insulin action; salicylates and ligands for PPARγ, both of which inhibit IKKβ activity restore insulin sensitivity both in vitro and in vivo (Yuan et al., 2001). Treatment with aspirin and salicylates alters the phosphorylation patterns of the IRS proteins, resulting in decreased serine phosphorylation and increased tyrosine phosphorylation (Yuan et al., 2001). After acute exposure to H₂O₂, the NF-kB and p38 MAPK pathways are markedly activated and that their activation can be blocked by pretreatment with LA. In L6 muscle cells, activation of p38 MAPK by oxidative stress (H₂O₂) is linked to H₂O₂-mediated inhibition of insulin-stimulated glucose transport.
Inhibition of insulin signaling was reversed by a specific inhibitor of p38MAPK (Blair et al., 1999). In addition, both TNF-α and anisomycin (strong activators of JNK) stimulate IRS-1-associated JNK activity, resulting in increased serine phosphorylation of IRS-1 catalyzed by JNK (Aguirre et al., 2002). Consequently, insulin-stimulated tyrosine phosphorylation of IRS-1 was substantially reduced and insulin action was impaired.

Taken together, there is strong evidence to indicate that the NF-κB, JNK and p38 MAPK are redox sensitive signaling systems that can be activated by oxidative stress invitro and invivo. Chronic activation of these pathways is associated with insulin resistance. Even though, oxidative stress and stress sensitive serine kinase activities are studied in insulin resistance, their associated role in the pathogenesis of insulin resistance has not been studied together. Moreover, the precise component of insulin signaling protein(s), which was disturbed by these redox sensitive serine kinases are not identified. Even though, the beneficial effect of antioxidants in insulin resistance is documented, the molecular mechanism by which these antioxidants improve insulin sensitivity is not known. New insights into the molecular pathogenesis of insulin resistance may help in the identification of pharmacological targets for treatment and/or prevention of insulin resistance and its complications.