Insulin-Mediated Signaling Pathways in Healthy State and Insulin Resistance State

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Global burden of type 2 diabetes mellitus is the main focus of the research studies in the world to understand fine details of normal metabolic and Mitogenic actions of insulin coupled with minute knowledge of the molecular causes of the insulin resistance and defect in the insulin signaling pathways that contribute to impaired carbohydrate metabolism in body. This review article provides thorough description of the insulin mediated signaling pathways and role of implicated effector molecules that are part of impaired signaling pathways to reach a consensus helpful in developing newer diagnostic as well as therapeutic molecules.

Keywords: Type 2 diabetes, insulin resistance, insulin actions, insulin signaling, impaired insulin signaling.

1. INTRODUCTION

Insulin is a peptide hormone secreted by the pancreatic beta cells and is encoded by INS gene among humans. Insulin is the important hormone that has anabolic impact on the metabolism of proteins, carbohydrates and lipids. Insulin hormone mediates actions through insulin receptor. It is the glycoprotein receptor expressed on the cell surface of target cells. Insulin receptor is linked to tyrosine kinase receptors family. Insulin receptor is composed of an alpha-subunit and beta-subunit. Insulin hormone is linked to alpha-subunit while tyrosine specific protein kinase activity is associated with the beta-subunit.

The insulin activated beta subunit further generates cascade of molecular events in the cytosol that could be essential in expressing metabolic actions of insulin in healthy states of body. Furthermore, impaired insulin receptor signaling could be implicated in the insulin resistance state. In the review article, we shall discuss insulin mediated signaling in healthy state, its regulation and impairment in the insulin mediated signaling in the insulin resistance state.

2. BIOCHEMISTRY OF INSULIN

Insulin is secreted by pancreatic beta cells. It is composed of 51 amino acids which are arranged into two polypeptide chains, namely chain A and chain B. The chain A is composed of 21 amino acids, while chain B is made up of 30 amino acids. Both chainsare inter-linked disulfide bonds. Insulin contains two categories of disulfide bonds namely inter-chain disulfide bond and intra-chain disulfide bond. In the former disulfide

bond, 7th cysteine residue in chain A is linked with 7th cysteine residue of chain B, another inter-chain disulfide bond is located between 20thcysteine residue ofchain A and 19thcysteine residue of chain B as in Figure 1.

Insulin hormone contains one intra-chain disulfide bond that links 6th cysteine residue to 11th cysteine residue located in chain A [1].

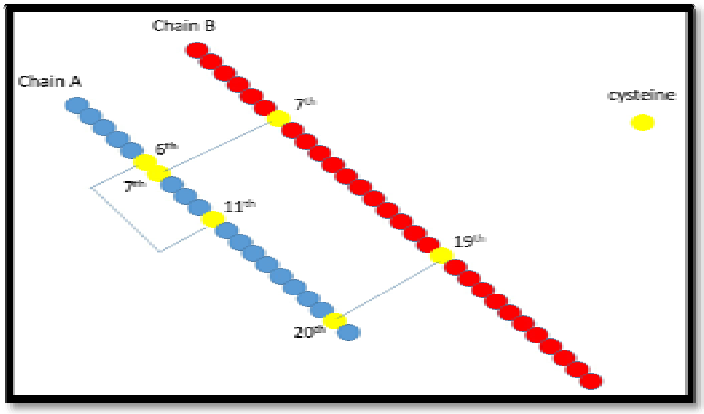

Fig. 1: Structure of Insulin.

3. BIOCHEMISTRY OF INSULIN RECEPTOR

3.1. Location of Receptor

Insulin receptor is a transmembrane glycoprotein expressed on the cell surface of target tissue. It belongs to family of tyrosine kinase receptors having high affinity to the insulin hormone and ligands. Insulin, and insulin growth factor-I (IGF-1) activate insulin receptor [2].

3.2. Splicing of Insulin Receptor Gene

Insulin receptor gene (INSR gene) encodes the insulin receptor. The chromosome 19 contains the insulin receptor gene. Structurally, INSR gene is composed of 21 introns and 22 exons end. The peptide made up of 12 amino acids is encoded by exon 11. Thisexon undergoes splicing and forms IR-A, insulin receptor A and IR-B, insulin receptor Bexhibiting variation in the affinity towards insulin [3].

3.3. Insulin Receptor Post-Translational Modification

The post-translational modification of newly synthesized insulin receptor isoforms could lead to the synthesis of α and β subunits of the receptors. The two subunits are subjected to dimerisation to form heterodimer (α2β2) that are held together by disulfide-bonds [4]. In the heterodimer, two β-subunits exhibit have transmembrane character differentiated into extracellular domain, intracellular domain and trans-membrane domain.

Moreover, two α-subunits are totally extracellular in location. The disulfide bonds hold α and β subunits of insulin receptor. The alpha-subunit contains 723 amino acids with molecular weight of nearly 130 kDa, whereas β-subunit is composed of 620 amino acids having molecular weight of 95 kDa [4].

3.4. Insulin Receptor Molecular Characterization

Three domains of insulin receptor are termed as ectodomain, transmembrane domain and juxta-membrane domain.

3.4.1. Insulin receptor Ectodomain

The ectodomain of insulin receptor is made up of 723 amino acids of each alpha subunit and another 194 amino acids contributed by each beta subunit. Two globular domains (homologous) marked as L1 and L2 contribute to the synthesis of N-terminals of the ectodomain of receptor.

The leucine rich domains (L1 and L2 domains) are both distanced by 150 amino acid residues domain designated as cysteine-rich domain. Additionally, this domain possesses 7 repeats and 2 disulfide bonds [5]. The three fibronectin-III domains marked as FnIII-1, FnIII-2, and FnIII-3 contribute to the synthesis of C-terminals of the ectodomain of receptor. Each domain (FnIII-1 and FnIII-3) contains 100 amino acids, while domain FnIII-2 is composed of 120-amino acids [6].

The domain, FnIII-1 is positioned in the alpha-subunit, whereas domain FnIII-3 is located in the beta-subunit. The FnIII-2 domain is termed as insert domain (ID). It harbors α-β cleavage site that is termed as furin cleavage site. The domain FnIII-2 is split by furin at α-β cleavage site into FnIII-2a and FnIII-2b subdomains [7].

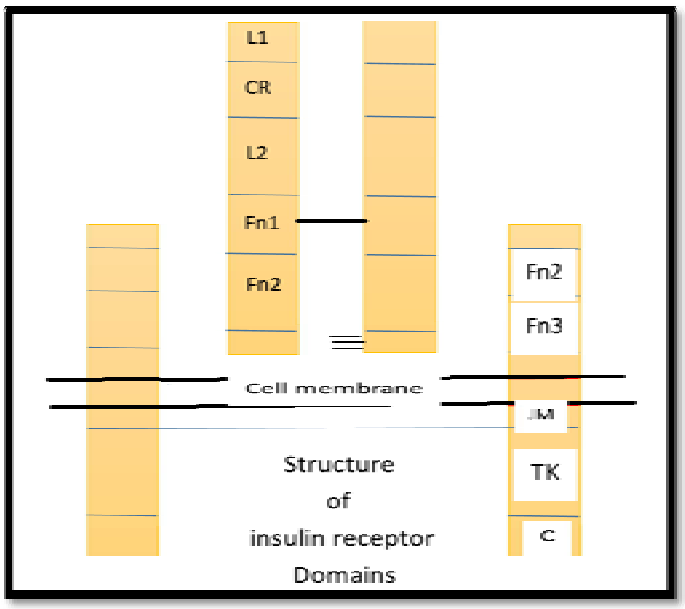

Fig. 2: Structure of insulin receptor Domains.

Two alpha-subunits of insulin receptor bind together via formation of disulfide bonds through two cysteine residues (Cyst 524) located in the domain FnIII-1. The cysteine residues namely Cys 685, 683 and 682 present in the insert domain (ID) in domain FnIII-2 under-write disulfide bonds in the two alpha subunits as in Figure 2 [8]. Single disulfide bond between the residue Cysteine 647 in the insert domain and residue Cys 872, single disulfide bond lead to bonding of alpha and beta subunits in ectodomain in receptor[8]. Ectodomain of insulin receptor represents as inverted (V) shape having its one side made up of L1-CR-L2 sequence; another side is contributed by FnIII-1, FnIII-2 and FnIII-3 domains [9].

3.4.2. Transmembrane domain and Juxta-membrane domain of Insulin receptor

The transmembrane domain is composed of 23 amino acids (930 to 952 residues), while the juxta-membrane domain is located after the trans-membrane domain and is consisted of 29 amino acid residues (953 to 982 residues). Function of juxta-membrane domain is to serve in internalization of insulin receptor and docking insulin receptor substrates 1, 2 and 3 [10].

3.4.3. Cytoplasmic domain of Insulin receptor

Domain, tyrosine kinase is located in the cytoplasmic domain of insulin receptor and is composed of residue 980 to 1255 residue along with 100 residues of beta-CT domain. The (Tyr1158, Tyr1162 and Tyr1163) tyrosine residues are docked in the domain, tyrosine kinase. The tyrosine residues Tyr1162 and Tyr1163 possess auto-phosphorylation property that could lead to activation of tyrosine kinase [11].

3.5. Insulin Binding Sites on Insulin Receptor

Thorough knowledge of the fine structure of the ectodomain of insulin receptor is helpful in grasping the insulin binding sites on receptor. The alpha-subunits of the insulin receptor possess insulin binding sites namely site 1 and site 2. The insulin binding site 1 is composed of peptide domain in the C-terminal made up of residues 704 to 715 termed as α-CT segment and L1 domain. The site 1 is the main insulin binding site in receptor [12].

Another insulin binding site called as insulin binding site 2 is composed of FnIII-1 domain (C-terminal portion) and leucine rich domain, L2 domain. The insulin binding site 1 and site 2 are referred as high and low affinity binding sites, respectively. The binding sequence of insulin is that insulin links to site 1 located in the 1^{st} alpha-subunit followed by binding of insulin to site 2 located in the 2^{nd} alpha-subunit in the insulin receptor[13].

The same insulin molecule binds with site 1 of 1^{st} monomer and site 2 of 2^{nd} monomer due to the close approximation of two insulin binding sites located on the receptor. Thereafter, 2^{nd}insulin molecule attaches with unbound site 1 and site 2 in alpha subunits leading to cross linking of insulin receptor protomers [14].

3.6. Insulin Receptor Phosphorylation

In the non-appearance of insulin, insulin receptor is kept in inactive phase by the parting of transmembrane domains by extracellular domains. The distance in transmembrane domains is maintained by link between L1 domain-FnIII2'-3' domain [15].

After binding of insulin with ectodomain of the insulin receptor, the forces between L1domain-FnIII2'-3' domains are disrupted leading to close approximation of transmembrane domains resulting into auto-phosphorylation of tyrosine residues in the tyrosine kinase domains. Conclusively, extracellular domains have inhibitory role on the tyrosine kinase property of in the insulin receptor in the absence of insulin molecule [16].The Tyr-1162 residue is positioned in the catalytic site of domain, tyrosine kinase. It has role in the auto-phosphorylation mechanism.

The OH^- group of Tyr-1162 residue is attached to the $COOH^-$ group of amino acid, Asp 1132 through formation of hydrogen bond in beta subunit. It inhibits binding of ATP molecule. Thus Tyr-1162 residue inside each beta subunit located at substrate binding site function as cis-auto-inhibitory molecule that suppresses auto-activation of kinase domain [17].

The binding of insulin with its receptor leads to substantial conformational modification in the intra-cellular domain of insulin receptor and further displacement of Tyr-1162 residue from the catalytic domain and subsequent attachment of ATP molecule in the kinase domain [18]. It is possible now that three tyrosine residues domain in 1^{st}beta-subunit is closely approximated with the catalytic domain of 2^{nd} beta subunit resulting into trans-phosphorylation of tyrosine residues. Three tyrosine residues domainsphosphorylation in each beta-subunit bring about insulin receptor in the activated state for its effect on the downstream effector molecules [19].

4. INSULIN MEDIATED SIGNAL TRANSDUCTION IN HEALTHY STATE

Insulin mediated and Insulin receptor–activated molecular signal transduction can be differentiated based upon the involvement of insulin receptor substrate in the signal transduction. The insulin receptor substrate is the best characterized protein and serves as substrate of activated insulin receptor. Thus IRS-activated signal transduction and non-IRS-activated signal transduction are the molecular signaling pathways in the insulin receptor mediated signal transduction in healthy state of body for the regulation of metabolism and tissue growth.

5. IRS-ACTIVATED SIGNAL TRANSDUCTION PATHWAYS

5.1. Insulin Receptor Substrates

Insulin receptor substrate is the protein ligand that binds with activated insulin receptor in the insulin activated signaling pathway. It's a family of proteins from insulin receptor substrate-1 (IRS-1) to IRS-6, which serves as scaffold for the interaction of downstream effector molecules [20]. The IRS is composed of a pleckstrin homology (PH) domain and 40 amino acid residues downstream, a PTB domain, both oriented at amino-terminus, which are further followed by C-terminus tail [20].

The pleckstrin homology (PH) domain is a protein made up of nearly 120 amino acid residues which is highly prevalent in protein molecules serving as effector molecules in the cytosolic signaling [21]. The insulin receptor substrate is recruited by the activated IR to the plasma membrane through the pleckstrin homology domain and phosphotyrosine binding (PTB) domain [22]. The IRS is further phosphorylated by the activated insulin receptors through the tyrosine residues which serve as binding sites for downstream effector molecules containing Src-homology 2 (SH2) domain [23].

The IRS-1 to 6 exhibit same phosphorylation motifs for the tyrosine residues, but their functions in the body are distant apart from each other. Study reported in IRS-1 knockout (KO) mice compromised insulin actions on skeletal muscles and retarded growth in the mice models [24]. The study in IRS-2 knockout mice model showed retarded growth inspecific neurons and pancreatic islets with impaired insulin signaling in the hepatocytes cells. These findings could lead to pathology of type 2 diabetes mellitus [25].

Furthermore, IRS-1 knockout mice model showed compromised differentiation in the pre-adipocytes while differentiation in the pre-adipocytes was normal in IRS-2 KO model with defective insulin-mediated glucose uptake in adipocytes[26]. The insulin receptor substrate 1 is essential in skeletal muscles for the differentiation of myoblasts, uptake and metabolism of glucose, while insulin receptor substrate 1 essential for the metabolis of lipids in skeletal muscles [27].

In rodents, insulin receptor substrate 3(IRS-3) is highly expressed in liver, adipocytes, and lung tissues, while IRS-3 gene in humans is pseudogene, resulting into absence of synthesis of protein [28]. In mice, deletion of IRS-1 and IRS-3 genes could result into impaired adipogenesis [29]. The gene IRS-4 is transcribed in the tissues in liver, brain, skeletal muscle, heart, and kidneys. Furthermore, IRS-4 knockout mice model showed retarded tissue growth and minimal glucose intolerance. Insulin receptor substrate -5

(IRS-5) is also termed as docking protein-4 (DOK4) and IRS-6 is called as DOK5. These proteins have restricted expression in tissues and serve as poor substrates for the insulin receptor [30].

The phosphorylated insulin receptor substrate further recruits and attaches with SH2 domain-containing molecule that in turn initiates two signaling pathways in the tissues. These can be characterized asPI3K (phosphatidylinositol-3-kinase)- Akt/PKB (protein kinase B) pathway that is termed as metabolic pathway while other is designated as MAPK- pathway (Ras-mitogen-activated protein kinase) and is referred as growth signaling pathway.

5.2. Phosphoinositide 3-kinase (PI3 kinase) and Phosphatidylinositol (3,4,5) Triphosphate (PIP3)

Activated IRS proteins are linked with downstream effector molecules for exhibition of metabolic actions of insulin through the PI3-kinase (PI3K) and Akt pathway.

5.2.1. Phosphoinositide 3-kinase (PI3 kinase)

PI3 Kinases belong to family of cytosolic molecular signal transducers (enzymes) that catalyze phosphorylation of hydroxyl group at 3rd position in the inositol ring of phosphatidylinositol [31]. The PI3 kinase is activated by tyrosine kinase receptors and G protein-coupled receptors [32]. There is conversion of phosphatidylinositol (4,5)-bisphosphate (PIP2) into phosphatidylinositol (3,4,5)-trisphosphate (PIP3) by activity of class I PI3 kinase [32]. The class I PI3 kinases are subdivided into IA and IB subsets based upon the similarity of amino acids sequence. These are heterodimeric compounds consisting of a catalytic subunit and a regulatory subunit. The class IA PI3 kinases are made up of p110 catalytic subunit and p85 regulatory subunit [33].

The regulatory subunit (p85) produces five variants namely p85α, p55α, p50α, p85β, and p55γ. The regulatory subunits namely p85α, p55α, and p50α are the splice variants of the gene Pik3r1, while the genes Pik3r2 and Pik3r3express regulatory subunits namely p85β, and p55γ, respectively. The p85α is the most abundant and highly expresse dregulatory subunit [33]. The catalytic subunit p110 also exhibit three variants namely p110α, p110β, and p110δ subunits. These variants are produced by genePik3ca, gene Pik3cb, and gene Pik3cd, respectively [32,33]. The catalytic subunits p110 α and p110 βare widely expressed in cells, however, p110δ is located on the surface of leukocytes only. In the class IB PI3 kinases, the catalytic p110γ subunit and regulatory p101 subunit are encoded by gene Pik3cg and gene Pik3r5, respectively [34]. The SH2 and SH3 domains are present in the p85 subunit. The SH2 domain exhibits selective affinity to bind with phosphorylated tyrosine amino acid residues in the sequence of Y-X-X-M [33,34]. When regulatory subunit attaches to catalytic subunit, there is enhanced stability of catalytic subunit leading to suppressed state of PI3 kinase, while attaching of regulatory subunit to a particular phosphorylated tyrosine motif present in the insulin receptor substrate could result in activation of PI3 kinase enzyme [35]. The insulin receptor substrate (IRS1/IRS2) recruit and activate PI3 kinase enzyme after attaching of two SH2 domains located in the regulatory subunits of PI3 kinase with phosphorylated IRS substraterelieves the catalytic subunit, that undergoes activation and catalyzes phosphorylation of posphatidylinositol 4,5-bisphosphate (PIP2) into phosphatidylinositol

(3,4,5)-triphosphate (PIP3) [36]. The latter serves as 2nd messenger and recruits Akt/protein kinase B to the cytosolic surface of plasma membrane of cell [37].

The Akt/protein kinase B is further phosphorylated and activated to affect the downstream molecules in insulin mediated metabolic signaling pathway [38]. Deletion of liver-specific p110α, and p110β subunits in mice models led to insulin resistance and glucose intolerance with hyperglycemia in mice models [39]. Furthermore, heterozygous deletion of regulatory subunits as p85α, and p85β or double deletion of p50α/p55α subunits resulted into enhanced tissues sensitivity to insulin [40]. Various pathways which are followed by reduced concentration of regulatory subunit p85 could be involved in the much better tissue sensitivity to insulin in insulin sensitive cells.

Study showed the involvement of p85α for activation of JNK pathway mediated by insulin or by tunicamycin (ER stress-mediating agent). Through a cdc42-MKK4 pathway mediated by insulin, the p85 regulatory subunit catalyzes the activation of JNK signaling pathway. Therefore, regulatory subunit p85could be essential for control of insulin sensitivity through activation of stress-activated protein kinases. The latter is linked with metabolic disorder, obesity and insulin resistance [41].

5.3. Activation of Downstream AGC Protein Kinase Family Members

The AGC protein kinase family belongs to subsets of Serine/Threonine protein kinases. These are named after the cAMP-dependent protein kinase (PKA), the cGMP-dependent protein kinase (PKG) and the protein kinase C (PKC) families of proteins [42]. The family members of AGC kinases have same structure and similar molecular pathways of activation that occur through phosphorylation of two ser/thr residues [43].

The PI3 kinase generated phosphatidylinositol (3,4,5)-trisphosphate (PIP3) is recruited to the plasma membrane and its effects on the downstream molecules are exercised by activation of subsets of family members of AGC protein kinases.

The 3-phosphoinositide-dependent protein kinase 1 (PDK-1) represents the kinase essential for phosphorylation and activation of the members of AGC kinases controlledby PI3 kinase [44]. The PH-domain is present in the PDK-1 that links with membrane-attached PIP3, activating PDK-1. Furthermore, the PDK-1 in turn phosphorylates ser/thr amino acid residues, namely Thr-308 for Akt/PKB [45].

The mammalian target of rapamycin complex 2 (mTORC2) catalyzes the phosphorylation at Ser-473 in Akt/PKB [46]. The isoforms of Akt/PKB possess a PH domain, thus interact with the PIP3 and are recruited to the plasma membrane in cells.The isoform Akt2 is abundantly expressed in insulin-sensitive tissues responsible for mediation of insulin actions in tissues. in a study with Akt2 deletion mice model, there occurs insulin resistance, glucose intolerance, hyperglycemia and features of type 2 diabetes mellitus [47].

The Akt/protein kinase B is essential enzyme controlling the metabolism of carbohydrates and lipids and is closely related with the ongoing research projects in the field of insulin resistance, diabetes and obesity. Multiple studies indicate the importance of Akt/PKB in maintaining healthy states of glucose metabolism and lipid metabolism.

Impaired Akt/PKB signaling pathways are coupled to the insulin resistance and metabolic syndrome and are the possible drug target in treatment of obesity and diabetes mellitus type 2 in patients.

Furthermore, after the DNA damage, the DNA-dependent protein kinase (DNA PK) is involved in activation of Akt/PKB and is essential in insulin mediated regulation of fatty acid synthase enzyme. Fatty acid synthase enzyme is closely related to lipogenesis and is activated transcriptionally in response to intake of food and insulin mediated signaling.

The DNA-dependent protein kinase exhibits role in the repair of DNA double-strand break through recognizing it initially followed by binding with the double strand break. The DNA-dependent protein kinase binds with regulatory (γ1) subunit of AMP-activated protein kinase enzyme. The AMPK serves as sensor to assess the energy status of tissues (fuel gauge) and is regulated by Ca^{2+}/calmodulin-dependent kinase kinase (CaMKK) and Liver Kinase B1 (LKB1).

Study point that loss of DNA-dependent protein kinase activity would lead to enhanced activity of AMP kinase in skeletal muscle. It has been studied that loss of DNA-dependent protein kinase activity offers protection against insulin resistance, diet mediated obesity, and mitochondrial dysfunction in cells [48]. Thus, inhibitors of DNA-dependent protein kinase can be valuable molecular targets of pharmacotherapy in type 2 diabetes mellitus.

5.4. Insulin receptor-mediated Signaling Downstream from AKT/PKB

The PDK-1 phosphorylates and activates Akt/PKB,that further activates the several downstream molecules. The activated Akt/PKB catalyzes phosphorylation of serine residue 939, serine residue 981, and threonine residue 1462 located in TSC2.[49]Furthermore, cytosolic anchoring protein 14-3-3are recruited to the TSC2, causing disruption of TSC1/TSC2 dimer. The events lead to loss of GAP activity of TSC2 and thus Rheb-GTP is sparred from hydrolytic action. Thus, mammalian target of rapamycin complex 1(mTORC1) is activated leading to anabolic effect on protein metabolism through insulin mediated signaling [50].

The mTORC1 complex further brings about phosphorylation and inactivation of 4E-binding protein 1, activation of ribosomal protein S6 kinases (S6K1 and S6K2) and Sterol regulatory element-binding transcription factor 1(SREBP1), and collectively regulates the transcription of genes involved in protein synthesis [51].

In the mTORC1 signalling negative feedback mechanism, the ribosomal protein S6 kinase beta-1 (S6K1) can phosphorylate the insulin receptor and reduce the insulin receptor's sensitivity, which results in insulin resistance. S6K1 is linked to eIF3 in the inactive state and undergoes dissociation after being phosphorylated by mTOR/Raptor. Its target is the S6 ribosomal protein, and phosphorylation of this protein causes ribosomes to synthesise proteins [52].

A study using a mouse strain inhibited for the S6K1 protein revealed that the development of fat tissues was postponed. The S6K1 protein may be the pharmaceutical target for the treatment of obesity, insulin resistance, and type 2 diabetes [53].

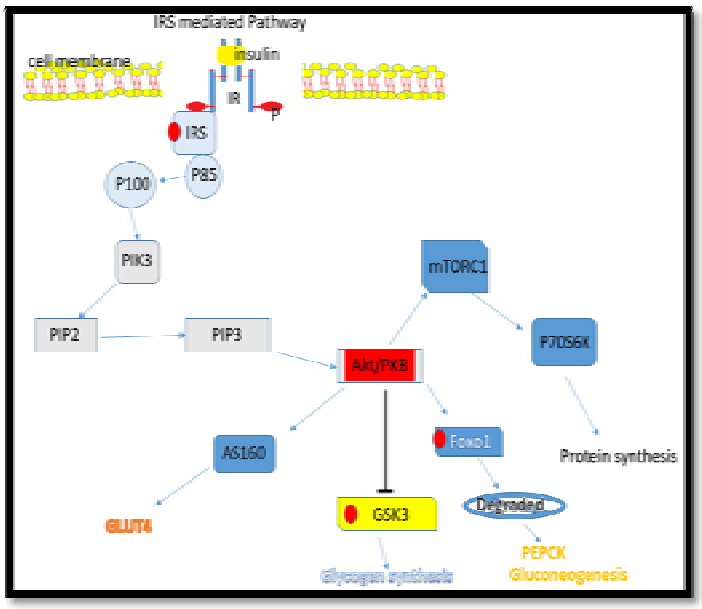

Fig.3: IRS mediated insulin signaling pathway.

The activated Akt/PKB phosphorylates Forkhead box O (Foxo) transcription factor that regulate the gluconeogenesis in liver and lipogenesis as in Figure 3. The phosphorylated Foxo is excluded from the nucleus and its transcriptional activity is inhibited [54]. Study involving transgenic mice models with knockout gene Akt1 and gene Akt2 demonstrated insulin resistance in liver and high gluconeogenesis in liver contributing to hyperglycemia and type 2 diabetes mellitus. After simultaneous ablation of Foxo1 in liver, the clinical manifestations were normalized.

Unexpectedly, mice were able to adjust to the fasting and fed states in the absence of Foxo 1 and Akt, and insulin's role was to regulate and inhibit hepatic gluconeogenesis in a typical pattern [55]. Therefore, hepatic Akt is crucial in controlling Foxo1 activity in liver cells. The body's hepatic gluconeogenesis is primarily mediated by insulin and glucose even when Foxo1 is not present [56].

5.5. Additional Effects of Insulin Receptor-mediated Signaling Pathway Downstream of AKT/PKB

The serine/threonine-specific protein kinase (AKT/PKB) enzyme that is associated with main roles as carbohydrate metabolism, cell proliferation, apoptosis, cell migration and transcription [57]. Murine double minute 2 (Mdm2) is an E3 ubiquitin ligase enzyme. It is phosphorylated by activated Akt/PKB enzyme leading to suppression of p53-induced apoptosis of cells contributing to tumorigenesis [58].

The enzyme Akt phosphorylates and inactivates Bad, Bax, and caspase-9, thus promoting cell survival [59]. The enzyme Akt brings about phosphorylation and activation of enzyme endothelial nitric oxide synthase (eNOS), that induces synthesis of nitric oxide

molecule which acts as inflammatory and vasodilator. It is implicated in the possible connection between insulin resistance, cardiovascular disease, and diabetes mellitus [60].

*Insulin hormone serves as mild tissue growth factor***.** Insulin upgrades growth of cells, cell division, migration, but it suppresses the apoptosis in cells. These actions of insulin are termed as mitogenic actions of insulin [61].

6. RAS-MAPK SIGNALING PATHWAY (MITOGENIC SIGNALING PATHWAY)

PI3 Kinase/Akt does not play a role in the Ras-MAPK signaling pathway. The IRS protein and the activated insulin receptor both have binding sites for Shc proteins and Growth factor receptor-bound protein 2 (Grb2), two adaptor molecules in the SH2 domain. Grb2's SH3 domain at its carboxy-terminal binds to GRB2-associated-binding protein 1 (Gab-1) and its SH3 domain at its amino-terminal binds to son-of-sevenless (SOS) protein's proline-rich domains [61].

A guanine nucleotide exchange factor (GEF) for the Ras protein is the SOS protein. It speeds up the transition from the GDP-bound (Ras-GDP) inactive form of Ras to the GTP-bound (Ras-GTP) active form of Ras [62].

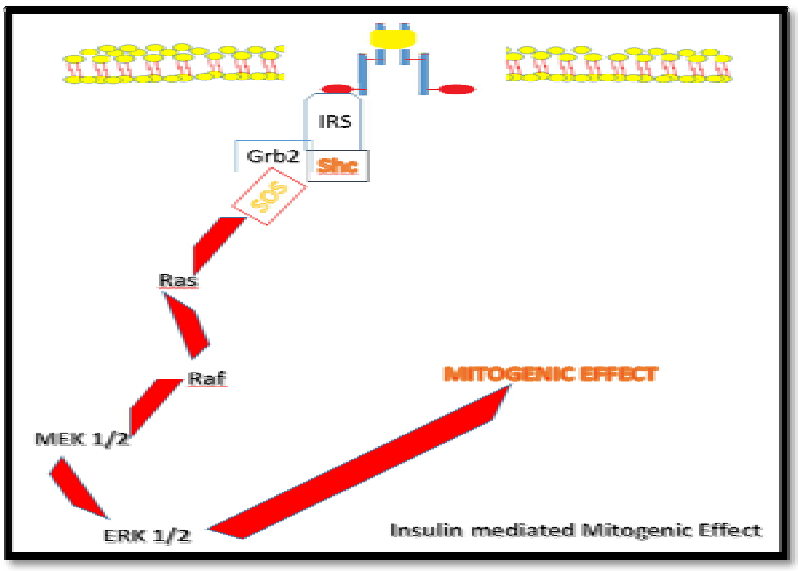

Fig. 4: Insulin mediated Mitogenic effect.

Activated Ras further activates downstream molecules namely Serine/Threonine kinase (Raf) that in turn activates MEK1 and MEK2 proteins which induces phosphorylation and activation of MAP kinases, ERK1 and ERK2 as in Figure 4. The activated ERK1/2 exerts its roles in cell proliferation, control of gene expression, reorganization of cytoskeleton via phosphorylation of their target molecules in the cytoplasm and nucleus [63].

7. NON-IRS-ACTIVATED SIGNAL TRANSDUCTION PATHWAYS

Insulin can also have metabolic and mitogenic effects on substrates other than the insulin receptor substrate. In the insulin signaling pathway, the insulin receptor phosphorylates the heterotrimeric G protein G alpha-q (Gq). GLUT4's insulin-stimulated glucose secretion and translocation are thought to be mediated by the protein Gq/11 [64].

Endogenous Gq action is necessary for insulin-mediated GLUT4 translocation, according to a study involving 3T3-L1 adipocytes. Through a signaling pathway that is dependent on PI3-kinase, the active Gq causes the uptake of 2-deoxy-D-glucose and the transfer of GLUT4 to another location. Phosphatidylinositol 3-kinase-independent phosphorylation of the proto-oncogene c-Cbl's tyrosine residues is necessary for glucose uptake [61,64].

The phosphorylation of Cbl's tyrosine residues by insulin led to its attachment to Cbl-associated protein (CAP) in muscle and adipose tissue in vivo, according to research on rat muscles and adipose tissues. In insulin-sensitive cells, the Cbl/CAP complex (c-cbl associated protein) can directly facilitate the uptake of glucose through the translocation of GLUT4 to the plasma membrane [63,64].

8. IMPAIRED INSULIN SIGNALING IN INSULIN RESISTANCE

The compromised biologic response of target tissues, particularly muscle, adipocytes, and liver tissues, to insulin hormone activation is known as insulin resistance. It slows down the metabolism of glucose, which leads to compensatory hyperinsulinemia and the development of type 2 diabetes. The pathology of insulin resistance is linked to a number of signaling pathways.

9. GENETICS IN IMPAIRED INSULIN SIGNALING

Insulin resistance can be caused by impaired insulin signaling caused by genetic mutations in several factors involved in insulin signaling.

9.1. Insulin Receptor Mutations

Insulin signaling is initiated by IRS protein tyrosine phosphorylation, which results in the activation of PI3 kinase, a downstream molecule. As a result, the metabolic effects of insulin are primarily dependent on the latter effector molecule [65]. The ubiquitin–proteasome-mediated signaling pathway accelerates insulin receptor decomposition in type 2 diabetes mellitus due to impaired serine residue phosphorylation of IRS proteins, which results in an improper or absent interaction between IRS proteins and insulin receptor. Insulin resistance and impaired insulin signaling are both brought about by these molecular events [65].

Mutations in genes that encode insulin receptor protein have been investigated in cases of severe insulin resistance. Leprechaunism, a rare disorder characterized by abnormal insulin resistance, necessitates a hundred times higher dose of insulin than typical diabetics [66].

Patients have missense or nonsense mutations in the insulin receptor's extracellular or tyrosine kinase domains, respectively. As a result of these mutations, the tyrosine kinase's activity is altered and the receptor's affinity for binding insulin is reduced. The majority of people with type 2 diabetes do not have mutations in their insulin receptor [67].

9.2. Insulin Receptor Substrate Mutations

In patients with type 2 diabetes mellitus, the Gly972Arg genetic polymorphism of insulin receptor substrate 1 has been linked to decreased PI3Kinase activity and decreased insulin-mediated signaling. The study [68] demonstrated that genetic polymorphisms in IRS-1 affect PI3 kinase activity. Insulin receptor substrates-1 is the primary substrate of the insulin receptor. The study utilized 32D(IR) cells (myeloid progenitor cell with overexpression of insulin receptor) that were transfected human variant of insulin receptor substrate 1 (IRS-1, Gly972Arg genetic polymorphism) in which amino acid residue, glycine at position 972 is replaced by amino acid residue, arginine.

According to the study, 32D(IR) cells exposed to insulin experienced a decrease in PI3 kinase activity of nearly 36% and a further 25% decrease in the regulatory subunit's binding affinity to insulin receptor substrate-1. The insulin receptor's tyrosine phosphorylation did not change as a result of these changes. Therefore, a Gly972Arg IRS1 genetic polymorphism may impair insulin-mediated signaling through impaired PI 3-kinase activity, resulting in patients developing insulin resistance and type 2 diabetes [68]. A study found a correlation between the presence of a Gly972Arg IRS1 genetic polymorphism and the development of insulin resistance and type 2 diabetes in the Mexican population [69].

The insulin receptor substrate 1 with single gene mutation (missense mutation, A T608R) led to decline in insulin mediated signaling and has been reported with clinical manifestation like insulin resistance in patients in type 2 diabetes, although it is rarely found in patients [70].

9.3. Phosphoinositide 3-Kinase Mutations

The Phosphatidylinositol 3-kinase has essential role in insulin mediated signaling in cells. Two genetic mutations have been reported in the regulatory p85 subunit of the enzyme namely Met-326Ile, and Asn-330Asp. Study reported that variant Met-326Ile of regulatory subunit, p85α can function normally in cytosolic signaling and differentiation of preadipocytes in humans but is associated with minor changes in expression of proteins molecules downstream of PI3 kinase that might influence the insulin mediated signaling in insulin sensitive cells [71].

9.4. Phosphatase and Tensin Homolog Mutations

Through its phosphatase activity on lipid and protein molecules, the phosphatase and tensin homolog (PTEN) protein plays a crucial role in tumor suppression. Through its phosphatase activity on the PIP3, the PTEN acts as a negative regulator of the insulin-mediated signaling pathway, resulting in the dephosphorylation of phosphatidylinositol

(3,4,5)-trisphosphate (PIP3) into phosphatidylinositol (4,5)-bisphosphate (PIP2) and the consequent suppression of PI3Kinase signaling [72].

In addition, loss-of-function mutations in the PTEN gene may enhance insulin sensitivity and insulin-mediated signaling in insulin-sensitive body tissues. As a result, insulin resistance and type 2 diabetes could be treated with drugs that target PTEN.

9.5. Mutations in 3-phosphoinositide-dependent Protein Kinase 1

Upstream effector molecules covered by protein kinases of the AGC family, which are activated in response to growth factors and insulin, are activated by the PDK1 (3-phosphoinositide-dependent protein kinase 1). Insulin's actions are triggered by the activation of protein kinase B (PKB)/Akt by PDK1. Phosphoinositides are bound by the pleckstrin homology (PH) domain of 3-phosphoinositide-dependent protein kinase 1.

Study involved generated knock-in mice that expresses mutant of PDK1 and the latter was unable to bind with phosphoinositides. This led to severe insulin resistance, and hyperinsulinemia in generated knock in mice due to failure of insulin mediated signaling via PDK1. Study reported reduction in action of PKB/AKT enzyme due to either failure or reduction in the PKB phosphorylation of threonine at position 308 in the molecule.

Overall, the events resulted into suppression of mTOR complex 1 and S6K1 mediated signaling in the cells [73]. Furthermore, feeding induced activation of p90 ribosomal S6 kinase or SGK1 behaved normally in response to mutation of PDK1 PH domain. Thus, binding between PDK1and phosphoinositide is essential element in phosphorylation of PKB in the insulin signaling to exhibit insulin mediated metabolic actions in cells [74].

9.6. Mutations in Protein Kinase B/AKT

Human type 2 diabetes mellitus has been linked for a long time to inherited flaws in the signaling pathways that follow the insulin receptor. We present the findings of a study that found a genetic mutation in the AKT2/PKBbeta gene, which was linked to patients' severe insulin resistance and type 2 diabetes mellitus and a pattern of autosomal dominant inheritance. In addition, co-expressed wild-type AKT activity was suppressed when the gene AKT2/PKBbeta was expressed in cultured cells, which resulted in impaired insulin-mediated signaling [75].

10. LIPOTOXICITY AND IMPAIRED INSULIN SIGNALING IN INSULIN RESISTANCE

Surplus accumulation of free fatty acids in plasma and tissues can contribute to pathogenesis of insulin resistance through multiple pathways. Rise in lipids concentration in non-adipose tissues and exaggerated hydrolysis of tissue due to over expression of tissue specific lipoprotein lipase, certainly are implicated in the pathology of insulin resistance [76]. Furthermore, rise in plasma free fatty acid content and rise in their transport in hepatocytes and cardiac muscle fibers could contribute to nonalcoholic fatty liver disease and lipotoxic cardiomyopathy [77].

Hepatocytes store excess free fatty acids and their metabolites, such as diacylglycerol (DAG) and long chain acyl-CoAs. Protein kinase C (PKC) isoforms 2 and 3 are activated by diacylglycerol [78]. According to a study conducted on transgenic rodents, activated PKC (serine/threonine kinase) can inhibit the pathogenesis of insulin resistance by reducing IRS-1 and IRS-2 tyrosine phosphorylation [79].

Thus, protein kinase C is implicated in the FFA and obesity induced suppression of insulin signaling in patients.

Lipotoxicity, obesity and oxidative stress could contribute to overexpression of c-Jun N-terminal kinase (JNK) and further phosphorylation of insulin receptor substrate-1 at serine307 residue leading to suppression of insulin mediated signaling and insulin resistance. Pharmacotherapeutic focused on the signaling pathway might be helpful in overcoming insulin resistance and type 2 diabetes mellitus [80].

As multiple mechanisms have been proposed to bring about insulin resistance, accumulated free fatty acids exhibit lipotoxicity and target the insulin receptor substrate (IRS). The use of linoleic acid—a free fatty acid—in obese mice decreased glucose uptake by 3T3-L1 adipocytes, according to the study. The decrease in glucose uptake was associated with a decrease in the amount of insulin receptor substrate 1, but the levels of IRS 2 and GLUT remained unchanged. Additionally, serine 307 phosphorylation of IRS 1 was mediated by c-JUN NH2-terminal kinase (JNK) and kappaB kinase (IKK) after IRS 1 content decreased [81].

11. OBESITY AND FREE FATTY ACIDS MEDIATED ACTIVATION OF JNK SIGNALING PATHWAYIN INSULIN RESISTANCE

Obesity and consumption of free fatty acids are linked to insulin resistance and the pathogenesis of Type 2 diabetes. The activation of c-Jun amino-terminal kinases (JNKs) in the pathology of insulin resistance has been characterized as the characteristic molecular pathway in cultured cells.

The mitogen-activated protein kinase (MAPK) belongs to the family of serine/threonine protein kinases. Tissues from mammals and humans frequently express these. The four categories of enzymes that make up the MAPK family are ERK5, JNK/stress-activated protein kinase (SAPK), extracellular signal-regulated kinase (ERK1/2), and p38. There are three JNK isoforms: JNKs 1, 2, and 3, all of which are expressed in cells. However, only pancreatic cells, the heart, brain, and testis contain the third isoform, JNK3 [82].

The MKK4 and MKK7 are located upstream of JNK protein and activate it. These MAP2 kinases (Both MKK4 and MKK7) can concomitantly catalyze phosphorylation of JNK at threonine-183 and tyrosine-185 positions. The JNK was designated as c-Jun N-terminal kinase due to its property of phosphorylation of c-Jun (nuclear transcription factor) [83]. After activation of JNK protein in signaling pathway, protein JNK is translocated from cytosol to nucleus where it mediates activation of the transcription factor, c-Jun through trans-phosphorylation of amino acid residues Ser63 and Ser73 located in the N-terminal domain of transcription factor, c-Jun [83].

The c-Jun transcription factor is activated, and it then binds to the binding site of the transcription factor activator protein-1 (AP-1) in the promoter region of the gene. This causes the expression of pro-inflammatory genes like IL-1, TNF, IL-8, and IL-6. Insulin resistance and the pathogenesis of type 2 diabetes mellitus may be caused by these pro-inflammatory molecules impairing insulin-mediated signaling in insulin-sensitive cells.

As a result, stress-induced activation of the JKN signaling pathway may be a drug target for the treatment of type 2 diabetes [84].

12. EFFECT OF JNK SIGNALING PATHWAY ON INSULIN SIGNALING PATHWAY IN INSULIN RESISTANCE

Akt/PKB, an insulin-mediated and insulin receptor-activated downstream molecule, is involved in insulin's metabolic actions by enhancing glycogen synthesis by influencing glycogen synthase kinase 3, promoting GLUT4 translocation, suppressing hepatic gluconeogenesis, and inhibiting free fatty acid oxidation in cells [85].

Insulin operates through downstream molecules as Akt/PKB catalyzes phosphorylation of PGC-1α (transcription factor); the cAMP level is reduced by insulin it suppresses protein kinase A enzyme essential for activation of hormone-sensitive lipase through PI3 Kinase and PKB dependent molecular pathways to suppress mobilization and hydrolysis of triglycerides in adipose tissues and thus inhibits lipolysis [86].

Several factors including free fatty acids activate JNK protein that further phosphorylate Ser/Thr residues in IRS 1 and IRS 2 and additionally catalyzes dephosphorylation of Tyr residues in insulin receptor substrate 1 that in turn materializes into inactivation of IRS 1 to activate downstream effector molecules mainly PI3Kinase enzyme [87].

Furthermore, the molecular event is associated with poor binding affinity of IRS 1 with upstream insulin receptor leading blockage of PI3 Kinase-AKT/PKB signaling pathway coupled with the insensitivity of target cells to insulin molecule.

Type 2 diabetes mellitus is characterized by hyperinsulinemia, hyperglycemia, and glucose intolerance, which are manifestations of this condition of insulin resistance [88]. It forces the pancreatic beta cells to produce a greater volume of insulin in order to regulate plasma glucose levels.

The study used a transgenic model of JNK1-deficient mice. Despite feeding mice a diet high in fat, the study found no insulin resistance in the liver. Adipose tissues and liver cells became more insulin-sensitive after the JNK1 gene was silenced.

It has been determined that silencing the JNK gene may result in an increase in tyrosine phosphorylation and a decrease in serine phosphorylation at the 307 position in IRS 1. Better insulin sensitivity was linked to these events [89]. *According to the study, silencing the JNK gene reduces the production of cytokines necessary for inflammation and suppresses the expression of genes that promote inflammation. Additionally, -tocotrienol may be able to reduce the transcriptional activity of factors like NF-B and AP-1, which are*

essential for the expression of pro-inflammatory genes, the manifestation of inflammation in adipose tissues, and the pathogenesis of insulin resistance in adipose tissues [90].

As a result, JNK activity can contribute to insulin resistance and mediate the activation of genes associated with inflammation. As a result, JNK is the drug target for insulin resistance and type 2 diabetes.

13. PPARγ SIGNALING PATHWAY AND JNK SIGNALING PATHWAY IN INSULIN RESISTANCE

The peroxisome proliferator-activated receptors (PPARs) represent transcription factors that are activated by ligands. The PPAR isoforms are PPARα, PPARβ/δ, PPARγ

Ligand induced activation of PPAR exists as heterodimer after binding with retinoic acid x receptor. This dimer translocate to nucleus where the complex binds with particular region of DNA to activate expression of specific genes. The PPARγ has been reported to enhance insulin sensitivity by activating metabolism of glucose and fatty acids in liver and skeletal muscle and improves the lipid diet mediated insulin resistance [91].

Free fatty acids, obesity and fat rich diet contribute to activity of TNF-α that further activate ERK and JNK mediated pathways. These pathways inhibit transcriptional activity of PPARγ through phosphorylation of Ser112 residue in PPARγ leading to suppression of transactivation of PPARγ [92].

Additionally, histone deacetylase 3 in the presence of TNF-α, attaches to heterodimer of PPARγ and retinoid X receptor leading to suppression of transcriptional activity of PPARγ [93].

Thus, PPARγ is an important factor in free fatty acids mediated inflammation, traverses with the JNK molecular signaling and controls expression of adiponectin, that could be indirectly implicated in the expression of anti-inflammatory genes helpful in alleviating insulin resistance.

14. NF-κB Signaling Pathway And JNK Signaling Pathway In Inflammation And Insulin Resistance

A member of the Rel family of proteins, nuclear factor kappa B (NF-B) is a transcription factor with a Rel domain (300 amino acid residues of sequence homology). In innate immunity, inflammation, and adaptive immunity, the NF-B is involved. In its inactive state, NF-B remains bound to proteins IB (the IKK complex includes subunits IKK and IKK), which prevent NF-B from transferring to the nucleus [94].

After exposure to multiple stimuli including free fatty acids, and fat accumulation, there is activation of IKK complex leading to phosphorylation of Ser32 and 36 amino acid residues in I*κ*B*α*.The phosphorylated I*κ*B*α* is disrupted exposing nuclear localization sequence in NF-*κ*B, and thus initiates the translocation of NF-*κ*B to nucleus where it activates transcription of specific genes for expression of inflammatory molecules namely IL-1*β*, TNF-*α*, and IL-6 [95].

Overexpression of IKK suppressed the expression of anti-inflammatory molecules like adiponectin and leptin in adipocytes, whereas deletion of IKK in a mouse model in adipocytes resulted in inhibition of free fatty acid mediated inflammation [96].

A novel drug target (NF-B pathway) for the treatment of type 2 diabetes mellitus and insulin resistance is provided by drug molecules that suppress NF-B activation and improve insulin sensitivity.

15. INFLAMMASOME SIGNALING PATHWAY IN INSULIN RESISTANCE

Several cytoplasmic protein complexes make up the inflammasomes, and they play a crucial role in mediating inflammation by triggering the release of IL-18 and IL-1. The inflammasomes may play a role in the spread of metabolic syndrome and insulin resistance [97].

NOD-like receptor proteins (NLRPs), apoptosis-associated speck-like protein (ASC), neutrophilic alkaline phosphatases (NALPs), and caspase-1 molecules make up the majority of the inflammasomes. Workers have conducted extensive research on the NLRP3's role in causing obesity-related inflammation, excess free fatty acids in tissues and plasma, and insulin resistance [98]. NLRP3, which serves as the link between fatty acid-induced inflammation in adipocytes, may be activated by the reactive oxygen species (ROS) produced by mitochondrial dysfunction [99].

The Toll-like receptors (TLR2) and (TLR4) that are found on adipocytes can be activated by saturated fatty acids in the diet, which in turn can activate the JNK, IRF3, and NF-B signaling pathways. Inflammation and insulin resistance in adipocytes are caused by transcriptional activation of pro-inflammatory genes in the nucleus [100].

16. IMPAIRED INSULIN SIGNALING IN ENDOTHELIAL CELLS

Insulin resistance and type 2 diabetes mellitus decrease skeletal muscle glucose uptake through insulin. Skeletal muscle has lower levels of IRS 2 expression and insulin-mediated phosphorylation of eNOS, both of which reduce insulin delivery and, as a result, muscle insulin-mediated glucose uptake. Additionally, insulin signaling in endothelial tissues is crucial for regulating skeletal muscle glucose uptake [101].

17. Impaired Insulin Signaling in Skeletal muscle Insulin Resistance

The primary risk factor in the aetiology of type 2 diabetes mellitus is skeletal muscle insulin resistance. The principal site of glucose absorption in response to insulin action following meal consumption occurs in the myofibers, which are the structural components of muscle. Ceramides, high free fatty acid levels, excess deposition of fat, and inflammatory cytokines may all play a role in the disruption of insulin signalling in muscles, resulting in decreased insulin sensitivity and insulin resistance.

Palmitate rich diet can impair functioning of ubiquitin-proteasomal system activity in cells. It leads to reduction in sequestration of unfolded proteins and their surplus accumulation

result into ER stress. Study was conducted in C2C12 myotubes (express contractile proteins and can contract). The ER stress contributed to increased levels of Thioredoxin-interacting protein and thus resulted into accumulation of ROS and release of inflammatory cytokines and characterizing inflammation in cells [102].

The STAT3 protein can be overexpressed as a result of the Thioredoxin-Interacting Protein, causing inflammation and insulin resistance in the muscle. In addition, ER stress in muscle and hepatocytes may be a factor in insulin resistance by activating c-Jun N-terminal kinases, suppressing PI3K activity, and impairing insulin-mediated signalling in muscle and the liver [103,104].

According to a study, eating a diet high in acute palmitate can cause L6myotubes to accumulate reactive oxygen species and misfolded proteins, whose diminished sequestration by UPS causes an unfolded protein response (ER stress). In L6 cells exposed to a diet high in palmitate, a study found a decrease in GLUT 4 expression [105,106].

The acute exposure to palmitate of L6 myotubes higher expression of IRE1a that can contribute to activation of nuclear factor kappa B. although basal activity of IKK is adequate to activate NFKB, but interaction in IRE1a, TRAF2 and IKK lead to activation of NFKB after 2 hours exposure of palmitate in L6 myotubes [107].

The activated NFKB now translocate to nucleus and binds with *Slc2a4* gene promoter in rat/mouse cells. The gene *Slc2a4* promoter sequence contains functional kB binding site and NFKB attaches with the site lading to down-regulation of the gene *Slc2a4* causing suppressed expression of GLUT 4 and thus diminishes the uptake of glucose leading to insulin resistance in l6 myotubes [108].

18. PROTEIN KINASE C MEDIATED IMPAIRED INSULIN SIGNALING

The protein kinase C is composed of several isoforms subdivided into three main groups namely classical PKC (cPKC), novel PKC (nPKC) and atypical PKC (aPKC) [109]. The cPKCs exhibits isoforms designated as α, βI βII, and γ which need essentially calcium ions, phospholipid, and DAG for activation, while nPKCs contains isoforms namely δ, ε, η, and θ and need only DAG for their activation. The aPKCs (along with protein kinase Mζ and ι/λ isoforms) need neither Calcium ions nor DAG for activation [110].

Study involving transgenic mice with intralipid/heparin infusion raised the serum free fatty acid content and elevated diacylglycerol content in muscle leading to activation of PKC-θand impaired insulin signaling and insulin resistance in muscle. The Mice without PKCθ showed normal insulin sensitivity in muscle [111].

In another study, after feeding PKC-θ knockout mice with high-fat diet for 14 weeks, it was reported that mice developed heaptic insulin resistance and obesity. So it was inferred that acute fat rich diet feeding could protect PKC-θ knockout mice from hepatic insulin resistance but chronic fat rich diet led to activation of other isoforms of nPKC and developed insulin resistance [112].

The PKCδ is additional isoform of PKC that is associated with hepatic insulin resistance. It was found in study in which transgenic mice after infusion of intralipid/heparin for 6hours, developed activation of PKCδ and manifestation of hepatic insulin resistance [113].

According to research conducted both in vivo and in vitro, the protein kinase C directly catalyzes the phosphorylation of the serine amino acid residues of IRS 1, as well as the decreased phosphorylation of the tyrosine residues in insulin receptor substrate 1. This results in the breakdown of IRS-1, as well as impaired insulin-mediated signaling and glucose intolerance. PKC isoforms, particularly PKCδ, have been shown to phosphorylate IRS-1's serine residue [114].

The activation of protein kinase C increases the potential of kinases namely inhibitor of κB kinase (IKK)-β, and Jun NH_2-terminal kinase (JNK) to induce serine residue-307 phosphorylation in IRS 1 which is the main controlling site located near to domain which binds with insulin receptor [115]. The PKC can also induce phosphorylation of serine residue-612 located upstream of kinases p42/44 MAPK and hence modulate activity of PI3K [116]. Latest studies reported that PKCθ has potential to induce phosphorylation of serine residue 1101 in IRS-1 that is linked with inhibiting insulin mediated tyrosine phosphorylation in IRS-1 [117,118,119].

19. CONCLUSION

The tyrosine kinase receptor is involved in insulin-mediated signaling, which initiates two signaling pathways: PI3K-Akt/PKB and the Grb2-SOS-Ras-MAPK pathways, which play a role in controlling cell proliferation, differentiation, and growth in living things like humans and regulate the metabolism of proteins, carbohydrates, and lipids. The insulin resistance, which is multifactorial in origin and is primarily involved in the pathogenesis of type 2 diabetes mellitus, impairs the signaling pathways. Additionally, the impaired signaling cascade's effector molecules may aid in the diagnosis of glucose intolerance.

New drug molecules that target impaired insulin signaling pathways may be able to repair an imminent defect, enhancing insulin sensitivity and reducing the symptoms of type 2 diabetes in patients.

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