Insulin and Insulin Resistance

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I dedicate this book to my wife

CONTENTS

PREFACE

Insulin is an endocrine peptide hormone composed of 51 amino acids. It is secreted mainly by pancreatic β-cells in the islets of Langerhans. The low concentration of this hormone is detected in certain neurons of the central nervous system. The synthesis and secretion of insulin are regulated by circulating glucose levels. It is involved in the regulation of glucose, lipid, and protein metabolism and its action is also associated with several other processes, including cell division, cell differentiation, cell growth, cytoskeletal reorganization, and more. Depending on the organ or tissue, it may play different roles. Insulin action is associated with two primary signaling pathways: the PI3K/AKT pathway and the MAPK signaling pathway.

Insulin resistance is associated with the impairment in insulin action of insulin-tergeting tissues. Insulin resistance causes compensatory insulin hypersecretion, resulting in hyperinsulinemia. Compensatory hyperinsulinemia may have adverse physiological outcomes in organs and tissues. Insulin resistance and related hyperinsulinemia may cause metabolic impairment and may be involved in several diseases, such as type 2 diabetes mellitus, cardiovascular disease, polycystic ovary syndrome, cancers etc.

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1

CHARACTERISTICS OF INSULIN

Insulin was isolated from a dog's pancreas in 1921 by Frederick Grant Banting and Charles Herbert Best, under the directorship of John James Rickard Macleod at the University of Toronto [Banting and Best, 2007; Lewis and Brubaker, 2021]. The purification of the discovered hormone was made possible by James Collip. For the discovery of insulin, the Nobel Prize in Physiology or Medicine was awarded jointly to F.G. Banting and J.J.R. Macleod in 1923 [Gerstein and Rutty, 2021]. In 1955, Frederick Sanger sequenced bovine insulin, identifying its composition of amino acids [Sanger and Thompson, 1953 a; Sanger and Thompson, 1953 b]; in 1958 he was awarded the Nobel Prize for Chemistry [Li et al., 2022]. In 1965 a crystalline bovine insulin that had full chemical property was synthesized [Kung et al., 1965]. As the next step, human insulin was produced, based on recombinant DNA methods and genetic modified bacteria [Goeddel et al., 1979]. All this work has played an important role in saving and improving the lives of many millions of patients worldwide [Gerstein and Rutty, 2021].

Insulin is an endocrine peptide hormone. It is composed of 51 amino acids. Insulin consists of two polypeptide chains, an A- and B-chain, linked together as a dimer by two disulfide bridges. In the A-chain, insulin contains a third intrachain disulfide bridge [Sanger, 1959]. The A-chain consists of 21 amino acids and the B-chain consists of 30 amino acids.

Biosynthesis and the Secretion of Insulin

Insulin is synthesized and secreted mainly by pancreatic β-cells in the islets of Langerhans. Previously, insulin was believed to be solely synthesized and secreted by pancreatic β-cells. Recent investigations revealed low concentrations of this hormone in certain neurons of the central nervous system [Csajbok and Tamas, 2016]. Both the synthesis and secretion of

insulin are regulated by circulating glucose levels. Based on the results obtained in animal studies, it was found that the initiation of these two processes depends on two different concentrations of glucose. The stimulation of its biosynthesis is due to fluctuations in circulating glucose levels between 2 mM and 4 mM. The initiation of insulin secretion is caused by glucose concentrations above 5 mM [Alarcon et al., 1993]. In the fasting state, the circulating insulin levels necessary to sustain life in a healthy adult typically range from approximately 25 pmol/L to 75 pmol/L [Kolb et al., 2020]. After meals, depending on carbohydrate content, insulin levels may rise to the range of approximately 300–800 pmol/l [Rijkelijkhizen et al., 2010].

The Human Insulin Gene

In humans, the insulin gene (*INS*) is located on chromosome 11p.15.5 [Støy et al., 2021]. The human insulin gene spans approximately 1.5 Kb. *INS* consists of three exons, separated by two introns. Exons two and three code protein [Steiner et al., 1985]. Exon two encodes the signal peptide, the B-chain, and the N-terminal part of the connecting peptide of preproinsulin (amino acids 1–62). Exon three encodes the remainder of the connecting peptide and the A-chain of preproinsulin (amino acids 63–110). Exon one plays a regulatory role in the transcription of the *INS* gene [Hay and Docherty, 2006]. Transcription of the *INS* gene is regulated by several transcription factors, such as PDX-1 (also known as insulin promoter factor-1), NEUROD1 (also known as Neuronal Differentiation 1), and MAFA in response to increased circulating glucose levels [Andrali et al., 2008].

In pancreatic β-cells, insulin is initially translated as preproinsulin (Figure 1.1). The next step in insulin synthesis takes place in the rough endoplasmic reticulum (RER). In the RER, the N-terminal signal peptide of insulin is cleaved by signal peptidase, resulting in the formation of proinsulin. Proinsulin contains C-peptide (also known as connecting peptide), a 31-amino-acid peptide that connects chain A and chain B in proinsulin molecule. In the RER, proinsulin is folded, and disulfide bonds are created between A and B chains. In the next step, in the Golgi complex proinsulin is sorted into immature secretory granules. In the granules, convertases (PCSK1 and PCSK2) cleave the C-peptide in prohormone that

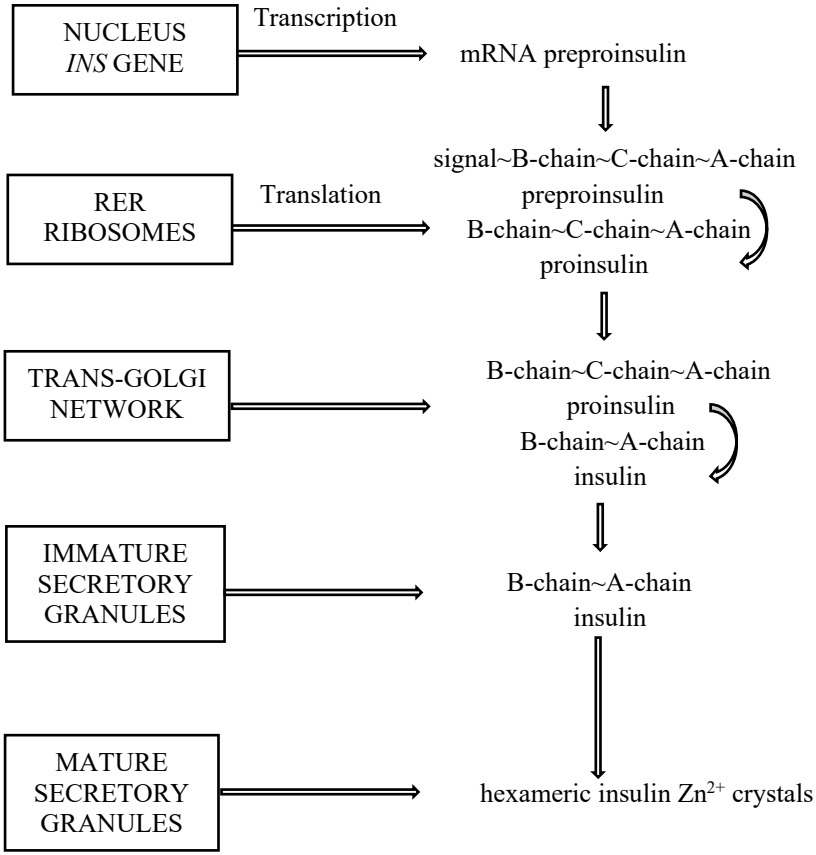

Figure 1.1. Insulin synthesis and maturation along the granule secretory pathway. mRNA preproinsulin is transcribed from insulin gene (*INS*) and translated to preproinsulin peptide. In rough endoplasmic reticulum (RER) and trans-Golgi network, the preproinsulin is processed to its mature form and then stored as hexameric insulin Zn^{2+} crystals within mature secretory granules [Goodge and Hutton, 2000; Infante, 2022; Tokarz et al., 2018].

is released into the bloodstream, as a by-product of proinsulin proteolysis. As mentioned above, mature insulin is composed of 51 amino acids, a result of carboxypeptidase E (CPE) activity, which removes C-terminal basic amino acids from the peptide chains. Both A and B chains are linked by two

disulfide bonds; however, within the A chain, another disulfide bond is formed [Goodge and Hutton, 2000; Infante, 2022; Tokarz et al., 2018]. In the mature secretory granules, the zinc cation (Zn^{2+}) co-crystalizes with insulin, forming a hexamer crystal. The transport of the insulin hexamer into secretory granules is associated with zinc transporter 8 (ZnT8). It has been suggested that other zinc transporters may also be involved in this process [Taniguchi et al., 2006].

Secretion of Insulin

The secretion of insulin induced by glucose is biphasic. The first phase of insulin secretion, characterized by a rapid, early peak, is rapidly triggered by elevated cytosolic Ca^{2+} levels and is mainly driven by the exocytosis of primed (readily releasable) insulin granules. The second phase of insulin secretion, slower, with a gradually rising peak, is stimulated by cytosolic Ca^{2+}, ATP cytosolic adenosine monophosphate (cAMP) production. This phase is due to the subsequent supply of new granules of insulin for release. Induction of insulin secretion by glucose may be modulated by amino acids, free fatty acids, as well as incretin hormones, growth factors and neurotransmitters [Park et al.; 2021].

As mentioned above, glucose-stimulated insulin secretion (GSIS) is a biphasic process. All the steps of the process of insulin secretion are regulated by signals initiated by glucose. Glucose is the primary regulator of pancreatic β-cell function. However, the role of GLUT proteins in human pancreatic β-cells remains controversial. In human β-cells GLUT2 is present at background levels: therefore, it is suggested that β-cells uptake of glucose through GLUT2 [Sun et al., 2023; Wonderline et al., 2024]. On the other hand, increased evidence suggests that GLUT1 and GLUT3 are predominant in human β-cells [Berger and Zdzieblo, 2020]. Glucose enters the β-cells via glucose transporter 1, which is expressed on the cell membrane [McCulloch et al., 2011]. In β-cells glucose is rapidly phosphorylated by glucokinase to glucose-6-phosphate (G6P). In mitochondria, G6P undergoes oxidation in the processes of glycolysis, generation of pyruvate, mitochondrial tricarboxylic acid cycle (TCA), also known as the Krebs cycle, leading to the generation of adenosine triphosphate (ATP) from adenosine diphosphate (ADP) by ATP synthase,

causing an increased cytosolic ATP/ADP ratio. The increased ratio ATP/ADP subsequently leads to the closure of ATP-sensitive K^+ (K_{ATP}) channels, causing cell membrane depolarization. Depolarisation of the cell membrane results from the conversion of chemical signals into electrical signals. Exceeding threshold potential (-55 mV to -50 mV) activates Na^+ and Ca^{2+} channels and allows the entry of Ca^{2+} via the opening L-type voltage-dependent Ca^{2+}, resulting in the elevation of intracellular levels of Ca^{2+}. Elevated levels of calcium stimulate the fusion of the insulin granule bilayer with the plasma membrane, inducing exocytosis of secretory granules which contain insulin/proinsulin from pancreatic β-cells [Infante, 2022; Park et al., 2021].

Clearance of Insulin

The pharmacological half-life of insulin is estimated to be between five and eight minutes. The activity of insulinase cleaves it mainly within several organs and tissues, such as the liver, kidney, and some other tissues [Park et al., 2021]. Two physiological processes mainly control the levels of plasma insulin: insulin secretion and insulin clearance. An imbalance between these processes may cause hyperinsulinemia. Insulin secretion has been extensively studied, whereas insulin clearance is less investigated [Kurauti et al., 2016]. The main organ responsible for insulin clearance is the liver; however, other tissues also play a minor role in the process of insulin clearance. Organs and tissues involved in insulin clearance include the kidneys, adipose tissue, fibroblasts, monocytes, lymphocytes, and a few other tissues [Duckworth et al., 1998].

There are three steps in insulin clearance: binding of insulin to its receptor, internalization of the insulin–insulin receptor complex, and degradation of the insulin by insulin-degrading enzyme (IDE) [Duckworth et al., 1998; Leroux et al., 2021]. An ethnic difference was found in insulin clearance [Bergman et al., 2022]. Insulin clearance may also be altered in obesity, non-alcoholic fatty liver disease, and type 2 diabetes mellitus. This process also depends on age [Ghadich et al., 2023; Najjar et al., 2023]. There are several questions and hypotheses which need further investigation. For example, several mechanisms are proposed which explain the difference in insulin clearance depending on age. There is also a suggested role of lower

insulin degradation in pathogenesis of type 2 diabetes mellitus and dysregulated metabolism, an association between decreased insulin clearance and the risk of development diabetes mellitus, a link between insulin resistance (IR) and insulin degradation, and many other factors [Bergman et al., 2022; Ghadieh et al., 2023; Najjar et al., 2023]. Animal studies revealed that acute exercise may restore insulin degradation in obese mice, induced by diet [Kurauti et al., 2016].

Insulin Degrading Enzyme

The role of IDE in insulin degradation was described more than seventy years ago [Mirsky and Broh-Khan, 1949; Mirsky et al., 1950]. But for many years, the precise role of IDE in the degradation of insulin remained controversial, as was the location of this process [Authier et al., 1996; Hersh, 2006]. There have been several recent studies on IDE [Hulse et al., 2009]. IDE (EC 3.4.24.56), also known as insulysin or insulinase [Amata et al.; 2009; Hulse et al., 2009] is a neutral 110 kDa Zn^{2+}-metallo-endopeptidase, ubiquitously expressed in insulin-responsive and insulin-non-responsive cells [González-Casimito et al., 2021]. This enzyme is ancient in evolutionary terms. Its homologs are detected in phylogenetically diverse organisms, such as bacteria, fungi, plants, and animals [Farris et al., 2005]. Its primarily localization was suggested to be cytosol, but its presence was described in peroxisomes, mitochondria, and endosomes [González-Casimito et al., 2021]. It was also detected on the surface of cerebrovascular endothelial cells [Bulloj, et al., 2008; Hulse et al., 2009; Li et al., 2006]; however, the nature of the attachment membranes remains unclear.

The IDE gene is located on human chromosome 10q23-q25 [Leissring et al., 2021]. The open frame of human IDE RNA and rodent *Ide* RNA contains two possible translational initiation sites: the longer (Met^{1}-IDE) and shorter form (Met^{42}-IDE). The shorter isoform is predominantly expressed in tissues and cells, whereas the longer isoform is detected in mitochondrion [Leissring et al., 2004]. IDE hydrolyzes several small (<12 000 MW) peptides [Cordes et al., 2011], such as amylin, glucagon, and somatostatin. IDE also has greatest affinity for insulin, but it also degrades amyloid β (Aβ), a peptide associated with the pathogenesis of Alzheimer's

disease (AD), and chemokine ligand [Amata et al., 2009; Hulse et al., 2009; Leissring et al., 2021]. Several studies have revealed an association between IDE, degradation of Aβ and Alzheimer's disease [Amata et al., 2009]; therefore, IDE was suggested as a therapeutic target for Alzheimer's disease [Pivovarova et al., 2016]. This suggestion is based on the role of IDE in the central nervous system (CNS). Animal studies revealed that this enzyme is involved in brain functionality [Kuo et al., 1993; Sánchez-Cruz et al., 2022]. During brain aging, the expression of IDE is reduced, probably associated with dysregulation of insulin metabolism and accumulation of some amyloidogenic proteins. These amyloidogenic proteins are found in healthy-aged individuals [Kochkina et al., 2015; Press et al., 2019; Sousa et al., 2021]. It was found that IDE plays a role in Aβ clearance [Qiu and Folstein, 2006; Pivovarova et al., 2016]. One of the multiple mechanisms of pathogenesis of AD is associated with the accumulation of Aβ and hyperphosphorylation tau protein in amyloid plaques in selected areas of the brain [Crous-Bou et al., 2017; Selkoe and Hardy, 2016; Wei et al., 2021]. As mentioned above, IDE is the primary enzyme among the proteases involved in Aβ clearance in the cytosol of human brain lysates and in cerebrospinal fluid [Kurochkin et al., 2018]. It regulates Aβ levels, and factors that influence the expression and activity of IDE can increase its concentration [Farris et al., 2003; Miners et al., 2008]. IDE expression on the developmental phase of AD was also found [Tundo et al., 2023]. Under physiological conditions, the expression of IDE is controlled and regulated by the insulin signalling pathway. In patients with T2DM, the level of IDE is reduced. Decreased levels of IDE cause IR because of slower insulin turnover, leading to hyperinsulinemia. Desensitization of the insulin receptor (INSR) in peripheral tissues is an effect of these disturbances, and is causing progression of T2DM [Rezende et al., 2014; Zhao et al., 2004]. In humans, the significant reduction of IDE levels in diabetic patients is observed in the muscles and liver, whereas its level is increased in the serum [Fernández-Gamba et al., 2009; Kullenberg et al., 2022; Pivovarova et al., 2016]. Based on the role in the insulin catabolism, it is suggested that its inhibitors may be used as a new antidiabetic therapy [Broh-Kahn et al., 1950]. There are several controversies. For example, the inhibition of IDE may induce chronic hyperinsulinemia, causing IR over time. The inhibition of IDE may not be beneficial because IDE plays different biological roles

in organism. Therefore, further studies are needed to investigate the association between IDE inhibition and glucose metabolism [Tundo et al., 2023].

Hepatic Clearance of Insulin

The liver is the primary site of insulin clearance. Approximately 50% of insulin is degraded in the first-pass hepatic clearance, and in the second-pass hepatic clearance25% of insulin is degraded [Infante, 2022]. However, according to other authors, the liver degrades about 60–70% of insulin [Najjar et al., 2023]. From the portal circulation, insulin diffuses into the space between the sinusoids and the hepatocytes (the perisinusoidal space). Then insulin interacts with the surface of hepatocytes. The main mechanism of hepatic insulin clearance is receptor-mediated insulin uptake [Najjar and Perdomo, 2019]. Insulin receptors are located on the microvilli of the hepatocyte membrane. Insulin binds the INSR. The insulin–insulin receptor complex is then internalized through clathrin-mediated endocytosis [Hall et al., 2020]. A high level of the enzyme carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1) is expressed in hepatocytes. It is a transmembrane glycoprotein that is phosphorylated by INSR. This enzyme is associated with the formation of an insulin–insulin receptor-CEACAM1 complex [Horst et al., 2018]. This process causes upregulation processes of endocytosis and degradation of insulin. When insulin binds to the hepatocyte's membrane, extracellular IDE partly degrades insulin [Yokono et al., 1982]. Then, insulin is internalized into hepatocytes and degraded by IDE in endosomes, as well as through proteolysis in lysosomes [Tokarz et al., 2018]. For more details, see Infante [2022].

Kidney Clearance of Insulin

The liver degrades approximately 60–70% of insulin. Approximately 25% of undegraded insulin is degraded in the kidney. From the systemic circulation, the kidney clears insulin via two main mechanisms: glomerular filtration and proximal tubular reabsorption and degradation [Duckworth et al., 1998; Hysing et al., 1989; Pina et al., 2020; Rabkin et al., 1984]. Cleared insulin analogs are also cleared by the kidney [Kruse et al., 1997], circulating proinsulin and C-peptide [Rabkin et al., 1984]. The primary site

of renal insulin clearance is glomerular filtration, followed by proximal tubular reabsorption and intracellular degradation of insulin [Rabkin et al., 1984]. Glomerular clearance of insulin is due to nonspecific diffusion and specific receptor-mediated transport. Insulin is filtered freely across the capillaries of the glomerulus, entering the luminal space, reaching the proximal tubule. After passage through the glomerular filtration barrier, more than 99% of the filtered hormone is reabsorbed by proximal tubular epithelial cells. This step is mainly caused by endocytosis and is mediated by scavenger receptors. Less than 1% of filtered insulin appears in the urine [Rubenstein et al., 1976; Rabkin et al., 1970; Tokarz et al., 2018]. Insulin receptors expressed on the membrane of the epithelial cells bind insulin and transport insulin intracellularly for degradation [Nielsen et al., 1987; Rabkin et al., 1984]. The process of endocytosis causes insulin to be taken into proximal tubular cells within endocytotic vesicles. The internalization of insulin into endosomes causes the start of insulin degradation [Surmacz et al., 1988]. Then vesicles with insulin fuse with lysosomes [Bordeau et al., 1973]. Renal lysosomes play a greater role in insulin degradation in comparison with the liver. Most of the endosomal insulin and partially degraded fragments of insulin that are delivered to lysosomes are degraded completely [Duckworth et al., 1998].

Other Tissues Associated with Insulin Removal

The liver and kidneys are not the only organs involved in insulin degradation; it is suggested that all insulin-sensitive cells may also take up and break down insulin to some extent [Duckworth et al., 1998]. Insulin that was not removed by the liver and kidneys may be removed by other tissues and cells, such as adipocytes [Jochen et al., 1989], fibroblasts [Stentz et al., 1985], monocytes [Powers et al., 1980], lymphocytes [Buffington et al., 1986], gastrointestinal cells [Bai et al., 1995], and other tissues [Duckworth et al., 1988] However, these tissues play a minor role in this process [Duckworth et al., 1998]. For example, liver degrades ~60–70% of insulin, kidney ~25%, adipose tissue ~7–14%, and muscle degrade ~6% of insulin [Najjar et al., 2023]. Obtained values (mL/5 min/g tissue) of insulin clearance in animal studies were in: kidney 4.3, liver 0.64, brain (cortex) 0.07, skeletal muscle 0.07 (Meijer and Barrett, 2021]. There have been

studies of the role of muscle in insulin removal [Duckworth et al., 1979]. In muscles, as with the liver and kidneys, insulin receptors play a key role as major mediators in initiating insulin clearance from the plasma and its uptake by muscle [Meijer and Barrett, 2021]. An acute exercise also reestablishes insulin clearance. It has been proposed that the reduction of hyperinsulinemia due to exercise is caused primarily by decreased insulin secretion [Aarnio et al., 2001]. More recent animal studies have revealed that an increase in insulin degradation as an effect of acute exercise is also involved in the reestablishment of plasma insulin levels in hyperinsulinemic, diet-induced obese mice. Authors suggest that increased insulin clearance is probably caused by the increased activity of IDE in skeletal muscle [Kurauti et al., 2016]. Therefore, it is suggested that the skeletal muscle plays a role in insulin degradation during exercise. However, insulin clearance occurs primarily in the liver [Kurauti et al., 2016].

As mentioned earlier, adipocytes also degrade insulin. It was found that insulin clearance depends on the localization of adipose tissue. Adipocytes from the visceral site degrade more insulin compared with adipocytes localized subcutaneously [Fawcett et al., 2010]. It was also found that insulin degradation may be carried out by mononuclear cells from peripheral blood. Authors have observed that these cells degrade insulin in a time- and temperature-dependent fashion. Obtained results have also revealed that homogenized monocytes degrade approximately fourfold more insulin in comparison with lymphocytes. Based on the obtained results, researchers also suggest that the main site of insulin clearance in mononuclear peripheral blood cells is cytosol [Powers et al., 1980].

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2

INTRACELLULAR INSULIN SIGNALING PATHWAY

Insulin is an anabolic hormone and is involved in the regulation of glucose, lipid, and protein metabolism. Its action is also associated with several other processes, such as cell division, cell differentiation, cell growth, cytoskeletal reorganization and so on. In the fasted state, glucose secreted by the liver into blood maintains euglycemia and provides fuel for tissues, which use it as a source of energy. This process, called hepatic glucose production (HGP), is associated with the breakdown of hepatic glycogen (glycogenolysis) and *de novo* synthesis of glucose (gluconeogenesis), using non-carbohydrate substrates, such as fatty acid and glycerol. These substrates are derived from adipose tissue [Rizza, 2010]. After food intake, increased blood glucose levels stimulate pancreatic β-cells for secretion of insulin, promoting anabolic and suppressing catabolic processes. Secreted insulin stimulates glucose-consuming tissues, such as skeletal muscle and adipose tissue, for uptake of glucose. The hormone also stimulates the synthesis of glucagon and lipid in the liver, skeletal muscle, and adipose tissue [Moore et al., 1991]. Insulin also inhibits the expression of gluconeogenic genes, resulting in the suppression of HGP. It also suppresses the process of lipolysis in adipose tissue [Petersen and Shulman, 2018] (Figure 2.1). Further, it suppresses the secretion of glucagon from pancreatic α-cells [Asplin et al., 1981] and decreases appetite, involving the central nervous system. The action of insulin begins when the hormone binds to the extracellular α-subunit of the insulin receptor (INSR), resulting in conformational changes.

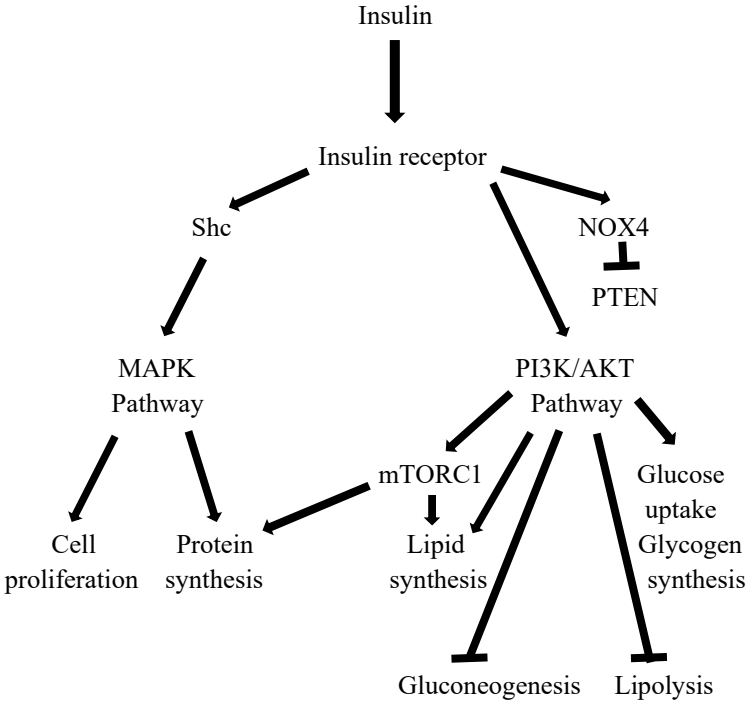

Figure 2.1. Insulin signaling pathways. The MAPK insulin signaling pathway is involved in cell proliferation and synthesis of proteins. The PI3K/AKT pathway is associated with synthesis of proteins, lipid and glycogen and uptake of glucose. This pathway also inhibits processes of gluconeogenesis and lipolysis. Another insulin signaling pathway involves NOX4, inhibits phosphatase and tensin homolog (PTEN), an inhibitor of the PI3K/AKT pathway [Kolb et al., 2020].

Conformational changes induce the autophosphorylation of several tyrosine residues present in the β-subunit of INSR. Autophosphorylated β-subunits form the binding sites for insulin receptor substrate (IRS) proteins, which contain phosphotyrosine binding (PTB) domains or for Shc adapter proteins, containing Src-homology (SH2) domains. The human insulin receptor gene (*INSR*) is located on chromosome 19. There are two INSR isoforms: INSR-A and INSR-B. These isoforms differ in the length, depending on the inclusion (INSR-B) or exclusion (INSR-A) of exon 11 that contains the 36 base pairs [De Meyts and Whittaker, 2002]. The INSR-

A isoform is highly expressed in fetal tissues. It enhances the effect of insulin-like growth factor-2 (IGF2) during embryogenesis and fetal development [Louvi et al.; 1997]. In adults, its expression is detected in the brain [Pomytkin et al., 2018], spleen [Mosthaf et al., 1990], ovary [Phy et al., 2004], and testis [Neuvians et al., 2005]. The upregulation of INSR-A is detected in several cancers, caused by its mitogenic effects [Vella et al., 2018]. INSR-B is the predominant isoform in the the liver, adipose tissue, and kidney [Seino and Bell, 1989; Sesti et al., 1994]. The INSR can form two types of receptors: homodimers, which consist of two identical α-β subunit pairs (either INSR-A/INSR-A or INSR-B/INSR-B), and heterodimers, which contain one INSR-A and one INSR-B α-β subunit pair (INSR-A/INSR-B). There are also hybrid receptors which contain subunits of INSR and subunits of insulin-like growth factor-1 (IGF1R). In this case, they may be INSR-A/IGF1R and INSR-B/IGF1R. Hybrid receptors are expressed in all tissues and cells which contain INSR and IGF1R [Bailyes et al., 1997; Boucher et al., 2014]. Insulin can bind to homo-, hetero-, and hybrid-receptors. The best described substrates of insulin receptors are members of insulin receptor substrate (IRS). IRS family proteins are referred to as IRS1 through IRS6 [Boucher et el., 2014].

There are two primary insulin signaling pathways: the PI3K/AKT pathway and the MAPK signaling pathway (Figure 2.1).

The PI3K/AKT Signaling Pathway

The important pathway associated with cellular function and energy metabolism is the PI3K/AKT signaling pathway [Haeusler et al., 2018]. The activation of PI3K depends on the binding of the two SH2 domains p85 and p55 in the regulatory subunits to tyrosine-phosphorylated IRS1 and IRS2 [Shaw, 2011], resulting in the activation of the PI3K catalytic subunit, p110. The activation of the catalytic subunit rapidly catalyzes phosphorylation phosphatidylinositol 4,5-biphosphate (PIP_2) to generate phosphatidylinositol 3,4,5-triphosphate (PIP_3) [Cantley, 2002; Shepherd, et al., 1998). PIP_3 is the lipid, an important second messenger. It is involved in the recruitment of 3-phosphoinositide dependent protein kinase-1 (PDK-1) and AKT to the plasma membrane. Phosphorylation of PDK-1 activates the serine/threonine residues of AKT [Taniguchi et al., 2006]. AKT, activated by

phosphorylation induces downstream signaling, in which AKT plays a role in four, critical downstream processes [Boucher et al., 2014; Rahman et al., 2021]. AKT plays a role in the regulation of protein synthesis. This action is due to the mammalian target of rapamycin complex 1 (mTORC1), a serine/threonine kinase. The mTORC1 phosphorylates and inhibits eukaryotic translation initiation factor 4E-binding protein 1, also known as 4E-BP1, and activates p70S6K1 and p70S6K2 (p70 ribosomal protein S6 kinase 1 and S6 kinase 2). The mTORC1 also activates sterol regulatory binding proteins (SREBPs) which are translocated to the nucleus, influencing regulation of genes associated with synthesis of fatty acid and cholesterol [Boucher et al., 2014; Harris and Lawrence, 2003; Rahman et al., 2021]. Transcription factors, such as forkhead box protein O1 (FOXO1), regulate the expression of genes involved in gluconeogenesis and lipogenesis. In the absence of insulin, FOXO1 translocates to the nucleus. In the nucleus it activates the expression of genes associated with gluconeogenesis, such as phosphoenolopyruvate carbokinase (PEPCK) [Taniguchi et al., 2006]. Phosphorylation of FOXOs by AKT causes their exclusion from the nucleus, which inhibits their transcriptional activity [Tzivion et al., 2011]. AKT is also involved in the regulation of glycogen synthesis. This is due to phosphorylation and inactivation glycogen synthase kinase 3 (GSK3), causing activation glycogen synthase, resulting in synthesis and accumulation of glycogen in the liver [Cohen and Frame, 2001; Kim, et al., 2004]. AKT is also associated with the phosphorylation of peroxisome proliferator-activated receptor-gamma coactivator 1α (PGC-1α), leading to disturbances in PGC-1α's ability to stimulate gluconeogenesis and fatty acid oxidation [Li et al., 2007]. Phosphorylation of phosphodiesterase 3B (PDE3B) due to AKT causes its activation, resulting in decreased levels of cyclic AMP (cAMP). It is involved, together with insulin, in the inhibition of lipolysis in adipocytes and the secretion of insulin by β-cells [Degerman et al., 2011]. AKT also regulates the expression and activity of several proteins, such as enzymes, transcription factors, cell cycle regulating proteins, and other factors. Studies in animal models have shown that Akt phosphorylates murine double minute 2 (Mdm2), which inhibits p53-mediated apoptosis and promotes tumorigenesis [Cheng et al., 2010]. Phosphorylating cell cycle inhibitors, such as p21Cip1/WAF1 and p27Kip1, Akt contribute to cell growth and

inhibit apoptosis [Zhou et al., 2001]. The inhibition of Bax, Bad, and caspase-9, due to their phosphorylation by Akt, promotes cell survival [Boucher et al., 2014; Gardai et al., 2004]. The activation of endothelial nitric oxide synthase (eNOS) by AKT catalyzes the production of the vasodilator and anti-inflammatory nitric oxide (NO) [Yu et al., 2011]. The PI3K/AKT signaling pathway is also associated with the translocation of the insulin-sensitive glucose transporter4 from intracellular vesicles of muscle cells and adipocytes to the plasma membrane, stimulating glucose uptake by these cells. This action of AKT is associated with the phosphorylation of AS160 (160-kDa AKT substrate). It is a GTPase-activating protein that activates RAB, a member of the small G protein family involved in membrane trafficking, by blocking the exchange of GDP for GTP [Sano et al., 2003].

The MAPK Signaling Pathway

The MAPK pathway is a second key pathway essential to insulin signaling. It is activated independently of PI3K/AKT signaling pathway. The activated insulin receptors and IRS proteins contain docking sites for adaptor molecules which contain SH2 domains, such as growth factor receptor-binding protein 2 (Grb2) and Shc. The activation of the MAPK signaling pathway begins when Grb2 binds to tyrosine-phosphorylated Shc, or when SH2 binds to the insulin receptor. The carboxy-terminal SH3 domain of Grb2 binds to proline-rich regions of proteins, such as son-of-sevenless (SOS) that is a guanine nucleotide exchange factor (GEF) for Ras. SOS catalyses the activation of Ras, a membrane-bound, low-molecular-weight GTPase belonging to the small GTPase family, by facilitating the exchange of GDP for GTP, converting Ras from its inactive GDP-bound form (Ras-GDP) to its active GTP-bound form (Ras-GTP) [Boucher et al., 2014; Rahman et al., 2021; Skolnik et al., 1993]. The activated Ras-GTP interacts with and can stimulate downstream effectors, such as serine/threonine kinase Raf that phosphorylates and it stimulates its downstream targets mitogen-activated protein kinase kinase 1 and 2 (MEK1 and MEK2), which stimulate and activate the mitogen-activated protein kinases (MAPKs): extracellular signal-regulated kinase 1 and 2 (ERK1 and ERK2). The activated ERK1 and ERK2 are translocated to the nucleus, where their