Charles University
Faculty of Science

Study Programme: Molecular Biology and Biochemistry of Organisms

Pavlína Tesařová

Insulin Signaling Pathways: From Peripheral Tissues to Emerging Functions in the Neurons
Signální dráhy inzulínu: Od periferních tkání k novým funkcím v nervových buňkách

Bachelor's thesis

Supervisor:
Mgr. Blanka Holendová, Ph.D.

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Declaration

I declare that I wrote this thesis independently and that I have appropriately cited all literature and other sources of information used. This thesis, or any significant portion of it, has not been submitted to obtain any other academic degree. In this work, I have used artificial intelligence tools for linguistic and stylistic editing of the text. These tools helped me improve clarity, grammatical accuracy, and the overall quality of the text. All ideas and content remain my own.

In Prague, 24. 4. 2025 Signature

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Abstract

Insulin and insulin-like growth factors (IGFs) signaling play crucial roles in regulating glucose homeostasis and energy metabolism in peripheral tissues. According to the latest findings, this signaling is also important in neurons in the brain. The insulin and IGFs signaling cascades are initiated when these molecules bind to specific receptors on the surface of target cells, triggering a series of phosphorylation events that activate various proteins and transcription factors. In the liver, insulin and IGF-1 suppress hepatic glucose production by inhibiting glycogenolysis and gluconeogenesis while promoting glycogenesis. Additionally, in skeletal muscle and adipose tissue, they facilitate glucose uptake. These mechanisms are essential for maintaining normoglycemia and modulating lipid and protein metabolism. In the brain, insulin and IGFs signaling significantly influence neuronal health, development, and function. Dysregulation of these signaling pathways in both peripheral tissues and the brain has been associated with metabolic disorders and neurodegenerative diseases.

This thesis summarizes the current understanding of insulin and IGFs signaling in peripheral tissues and neurons, highlighting its impact on cellular processes and their role in the development of metabolic and neurodegenerative disorders.

Key words: insulin, IGFs, insulin signaling, muscle, adipose tissue, liver, neurons

Abstrakt

Signální dráhy inzulínu a inzulínu podobným růstovým faktorům (IGFs) hrají klíčovou roli v regulaci glukózové homeostázy a energetického metabolismu v periferních tkáních. Podle nejnovějších poznatků je tato signalizace neméně důležitá v nervových buňkách v mozku. Inzulínové a IGFs signální kaskády jsou iniciovány navázáním těchto molekul na specifické receptory na povrchu cílových buněk, což spouští řadu fosforylačních kroků vedoucích k aktivaci dalších proteinů a transkripčních faktorů. V játrech inzulín a IGF-1 potlačují jaterní produkci glukózy prostřednictvím inhibice glykogenolýzy a glukoneogeneze a zároveň stimulují glykogenezi. V kosterním svalstvu a tukové tkáni podporují příjem glukózy. Tyto mechanismy jsou nezbytné pro udržení normální hladiny glukózy v krvi a regulaci metabolismu lipidů a proteinů. V centrálních nervovém systému tyto signální dráhy zásadně ovlivňují zdraví, vývoj a funkci neuronů. Poruchy v regulaci inzulínové a IGFs signalizace v periferních tkáních a v mozku jsou spojovány s rozvojem metabolických poruch a neurodegenerativních onemocnění.

Tato práce shrnuje současné poznatky o signalizaci inzulínu a IGFs v periferních tkáních a neuronech, s důrazem na jejich vliv na buněčné procesy a jejich úlohu ve vzniku metabolických a neurodegenerativních poruch.

Klíčová slova: inzulín, IGFs, inzulínová signalizace, sval, tuková tkáň, játra, nervové buňky

List of abbreviations

Aβ	amyloid-β
AD	Alzheimer's disease
AgRP	agouti-related peptide
AMPK	adenosine 5'-monophosphate-activated protein kinase
APP	amyloid precursor protein
ATF6	activating transcription factor 6
ATP	adenosine triphosphate
BBB	blood-brain barrier
ChREBP	carbohydrate response element binding protein
CNS	central nervous system
CREB	cyclic AMP response element binding protein
CSF	cerebrospinal fluid
DAGs	diacylglycerols
DNL	*de novo* lipogenesis
ERK	extracellular signal-regulated kinase
FAHFAs	fatty acid hydroxy fatty acids
FAs	fatty acids
FOXO (1)	forkhead box O (1)
G6P	glucose 6-phosphate
GAB	Grb2-associated binder
GABA	γ-aminobutyric acid
GAP	GTPase-activating protein

GCK	glucokinase
GDP	guanosine diphosphate
GLP-1R	glucagon-like peptide-1 receptor
GLUT (1-4)	glucose transporter (1-4)
Grb2	growth factor receptor-bound protein 2
GSK-3 (β)	glycogen synthase kinase 3 (β)
GSVs	GLUT4 storage vesicles
GTP	guanosine triphosphate
HGP	hepatic glucose production
IDE	insulin degrading enzyme
IGFs	insulin-like growth factors
IGF-1R	insulin-like growth factor 1 receptor
IGF-2R	insulin-like growth factor 2 receptor
IL-6	interleukin 6
IR	insulin receptor
IRE-1	inositol-requiring enzyme-1
IRKO	insulin receptor knockout
IRS (1-6)	insulin receptor substrate (1-6)
JNK	c-Jun N-terminal kinase
MAPK	mitogen-activated protein kinase
MEK	MAPK/ERK kinase
mTOR	mechanistic target of rapamycin
mTORC1	mechanistic target of rapamycin complex 1

NFTs	neurofibrillary tangles
NGFC	neurogliaform cells
NIRKO	neuronal insulin receptor knockout
NPY	neuropeptide Y
O-GlcNAcylation	O-linked N-acetylglucosamine modification
PAHSAs	palmitic acid esters of hydroxy stearic acids
PCK1	phosphoenolpyruvate carboxykinase 1
PD	Parkinson's disease
PDK1	3-phosphoinositide-dependent kinase-1
PERK	PKR-like ER kinase
PI3K	phosphoinositide 3-kinase
PI3K-C2γ	class II PI3K isoform γ
PIP2	phosphatidylinositol 4,5-bisphosphate
PIP3	phosphatidylinositol 3,4,5-trisphosphate
PKC	protein kinase C
PM	plasma membrane
POMC	proopiomelanocortin
PP2A	protein phosphatase 2A
PTEN	phosphate and tensin homologue
SH2	Src homology 2
Shc	Src homology 2 domain-containing
SOS	Son of Sevenless
STAT3	signal transducer and activator of transcription 3

T2D	type 2 diabetes
TCPTP	T-cell tyrosine phosphatase
TGs	triacylglycerols
TNF- α	tumor necrosis factor-α
UPR	unfolded protein response
VAMP-2	vesicle-associated membrane protein 2

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1 Introduction

Insulin is a peptide hormone produced by the pancreatic β-cells in the islets of Langerhans that profoundly contributes to maintaining glucose homeostasis. In response to increased glucose levels, insulin is secreted into the bloodstream and transmitted to the peripheral tissues, such as the liver, skeletal muscle, and adipose tissue. There it binds to its receptor, initiating a cascade of phosphorylation events that lead to the activation of downstream signaling pathways essential for glucose metabolism. The two primary cascades involved are the PI3K/Akt and the MAPK pathways, which control various cellular functions, including glucose uptake, energy storage, and cell growth and proliferation.

In addition to insulin, insulin-like growth factors (IGFs), produced primarily in liver but also in other extrahepatic tissues, contribute to the insulin signaling pathways as well, assisting in glucose metabolism and promoting cell growth and proliferation. In peripheral tissues, insulin and IGFs facilitate glucose uptake through specialized glucose transporters, especially in the muscle and adipose tissue, and reduce hepatic glucose production (HGP) in the liver to enhance glycogenesis. Disruptions in these pathways can negatively affect insulin action, leading to insulin resistance and contributing to metabolic disorders such as type 2 diabetes (T2D).

Apart from classical actions of insulin and IGFs in peripheral tissues, the brain has also been recognized as a target of insulin and IGFs due to the expression of insulin and IGF receptors. Activation of these receptors in the brain triggers signaling cascades similar to those in peripheral tissues, influencing glucose uptake by neurons and other neuronal functions. Furthermore, it has been demonstrated that neurons can synthesize their own insulin. The function of insulin and IGFs in the brain is critical, and any alterations in their signaling pathways can lead to the progression of neurodegenerative diseases.

This thesis aims to provide an understanding of how insulin and IGFs signaling impacts glucose metabolism across different tissues and influences neuronal health in the brain.

2 Insulin and Insulin-like Growth Factors as Signaling Molecules

Insulin and IGFs are essential signaling molecules that regulate glucose metabolism, cell growth, and proliferation. Insulin primarily regulates glucose homeostasis, while IGFs, especially IGF-1, influence growth and development (Morrione *et al.*, 1997; Huat *et al.*, 2014). Furthermore, IGF-2 has a more important role in the brain compared to other tissues, particularly in memory formation (Chen *et al.*, 2011), and neuroprotection (Martín-Montañez *et al.*, 2017).

Insulin is a peptide hormone that binds to the insulin receptor (IR). The IR is a glycoprotein characterized by its tetrameric structure and composed of four subunits: two α subunits and two β subunits (Massague *et al.*, 1980; Kasuga *et al.*, 1982). Both subunits are generated from a single large precursor through the process of proteolytic cleavage and are connected by a disulfide bond (Hedo *et al.*, 1983). The α subunits are positioned extracellularly and contain the insulin binding site. This structure allows the receptor to recognize and bind insulin (Massague *et al.*, 1980; Kasuga *et al.*, 1982). The β subunits extend across the cell membrane, each containing a tyrosine kinase domain (Saltiel, 2021)*. IR has two isoforms: IR-A, which lacks exon 11 near the C-terminus of the α subunit, and IR-B, which includes exon 11. These isoforms are the result of an alternative splicing of the *INSR* gene (Mosthaf *et al.*, 1990).

IGF-1 is a hormone composed of a single-chain polypeptide that is structurally similar to insulin (Rinderknecht & Humbel, 1978b). IGF-1 binds to insulin-like growth factor-1 receptor (IGF-1R), which shares structural homology with the IR. IGF-1R contains two α-binding subunits and two β subunits with tyrosine kinase activity (Massague & Czech, 1982).

In the brain and peripheral tissues, IR and IGF-1R can form five distinct dimer combinations through ligand-induced interactions, including both homodimers and heterodimers. The heterodimers, known as hybrid receptors, are able to bind both insulin and IGF-1, with higher affinity for IGF-1. The concentration of these receptors in tissues influences whether they form homodimers or heterodimers (Pandini *et al.*, 2002).

IGF-2, like IGF-1, is a single-chain polypeptide hormone structurally similar to insulin (Rinderknecht & Humbel, 1978a), that can bind to insulin-like growth factor-2 receptor (IGF-2R) with high affinity, but also to IGF-1R and IR with lower affinities. Unlike IGF-1R and IR, IGF-2R is distinct from these two receptors as it lacks the tyrosine kinase activity (Massague & Czech, 1982).

2.1 Insulin/IGF-1 Receptor Activation

Insulin binds to the α subunits of the IR (Boucher *et al.*, 2014)* at sites 1 and 2 (Uchikawa *et al.*, 2019)*. This interaction triggers conformational changes within the β subunits (Boucher *et al.*, 2014)*, transforming them from an inverted V structure into a T shape (Uchikawa *et al.*, 2019)*, which leads to the autophosphorylation of tyrosine residues on the β subunits. This activation of the receptor's tyrosine kinase activity creates docking sites for downstream signaling molecules such as insulin receptor substrate (IRS) and Src homology 2 domain-containing (Shc) proteins. These adaptor proteins are recruited through their Src homology 2 (SH2) domains, activating the downstream signaling cascades. Similarly, IGF-1 binds to the α subunits of its receptor, triggering autophosphorylation of tyrosine residues on the β subunits and recruiting adaptor proteins like IRS and Shc, thus initiating downstream signaling cascades. Other direct substrates recruited by phosphorylated IR/IGF-1R include Grb2-associated binder (GAB) proteins, Cbl and APS (SH2B2) (Boucher *et al.*, 2014)*.

The IRS family includes six proteins, from IRS-1 to IRS-6, with IRS-1 and IRS-2 being the most important in insulin signaling pathways in humans (X. J. Sun *et al.*, 1991, 1995; Lavan, Fantin, *et al.*, 1997; Lavan, Lane, *et al.*, 1997; D. Cai *et al.*, 2003). While IRS-1 primarily plays a crucial role in glucose metabolism, it also influences lipid metabolism to some extent. On the contrary, IRS-2 has a more significant role in lipid metabolism but also contributes to glucose metabolism (Bouzakri *et al.*, 2006). IRS-4 is also expressed in humans, but at lower levels compared to IRS-1 and IRS-2, primarily found in the brain, skeletal muscle, heart, and liver (Lavan, Fantin, *et al.*, 1997).

Additionally, IGF-2 is able to bind to IR and IGF-1R, activating similar pathways as insulin and IGF-1. However, its binding abilities to these receptors are much lower compared to insulin and IGF-1 (Massague & Czech, 1982), which makes it less effective in classical insulin signaling. Therefore, the discussion of signaling pathways in peripheral tissues will focus primarily on insulin and IGF-1. In contrast, IGF-2 plays a more significant role in the brain, which will be discussed in chapter 7.

2.2 Insulin/IGF-1 Signaling Pathways

Upon insulin/IGF-1 binding to their receptors on the target cell membranes, the receptor substrates are phosphorylated, activating two main signaling pathways: the phosphoinositide 3-kinase (PI3K)/Akt pathway and the mitogen-activated protein kinase (MAPK) pathway, promoted by the recruitment of adaptor proteins that interact with the receptors through their SH2 domains (Saltiel, 2021)* (Fig. 1).

2.2.1 The PI3K/Akt Signaling Pathway

Phosphorylated IRS recruits PI3K to the membrane. Once bound, PI3K is activated and catalyzes the conversion of phosphatidylinositol 4,5-bisphosphate (PIP2) in the plasma membrane (PM) to phosphatidylinositol 3,4,5-trisphosphate (PIP3). PIP3 acts as a second messenger that recruits 3-phosphoinositide-dependent kinase-1 (PDK1) to the membrane, where PDK1 phosphorylates and activates Akt, also known as protein kinase B (Saltiel, 2021)*. In turn, Akt phosphorylates TBC1D4 (also known as AS160), a Rab GTPase-activating protein (GAP). The TBC1D4 phosphorylation inactivates its GAP domain, which acts as a negative regulator of Rab GTPases, leading to the activation of specific Rab GTPases associated with glucose transporter type 4 (GLUT4) vesicles. Under basal conditions, most of GLUT4 is stored intracellularly, mainly in two types of organelles: recycling endosomes and GLUT4 storage vesicles (GSVs), with GSVs being the main reservoir for GLUT4 (Brumfield *et al.*, 2021). The activation of the Rab proteins is essential for the mobilization of GLUT4 vesicles to the PM (Sano *et al.*, 2003). As a result, GLUT4 is recruited to the membrane, facilitating glucose uptake, especially in muscle and adipose tissue (Saltiel, 2021)* and promoting glycogen synthesis by inactivating glycogen synthase kinase 3 (GSK-3) in the liver (Cross *et al.*, 1995). Moreover, Akt is involved in protein synthesis by activating the mechanistic target of rapamycin (mTOR) complex (Dan *et al.*, 2014). It also regulates gene expression by inhibiting the forkhead box O (FOXO) transcription factor. By inhibiting FOXO, Akt promotes cell survival and proliferation by suppressing the apoptosis induced by FOXO activity (Bloedjes *et al.*, 2020) (Fig. 1).

2.2.2 The MAPK Signaling Pathway

The MAPK pathway (Fig. 1) is a signaling cascade involved in cell growth, proliferation, and differentiation (Yunn *et al.*, 2023)*. It is initiated by interaction between IRS and growth factor receptor-bound protein 2 (Grb2). This interaction initiates a cascade of events that leads to the activation of Ras. Son of Sevenless (SOS) protein is pivotal in this process. It binds to Grb2 and facilitates GDP-GTP exchange on Ras, effectively activating it (Rahman *et al.*, 2021)*. Farnesylation is a post-translational modification where a farnesyl group is added to a protein by farnesyltransferase. This modification is critical for translocation of Ras to the PM, ensuring that Ras can engage in downstream signaling (Kato *et al.*, 1992). Once activated, Ras recruits the c-Raf, which phosphorylates MAPK/ERK kinase (MEK). MEK, in turn, phosphorylates extracellular signal-regulated kinase (ERK), which moves into the nucleus. Within the nucleus, Erk can phosphorylate and activate other transcription factors, for instance, ELK1, which promotes cell proliferation (Rahman *et al.*, 2021)*.

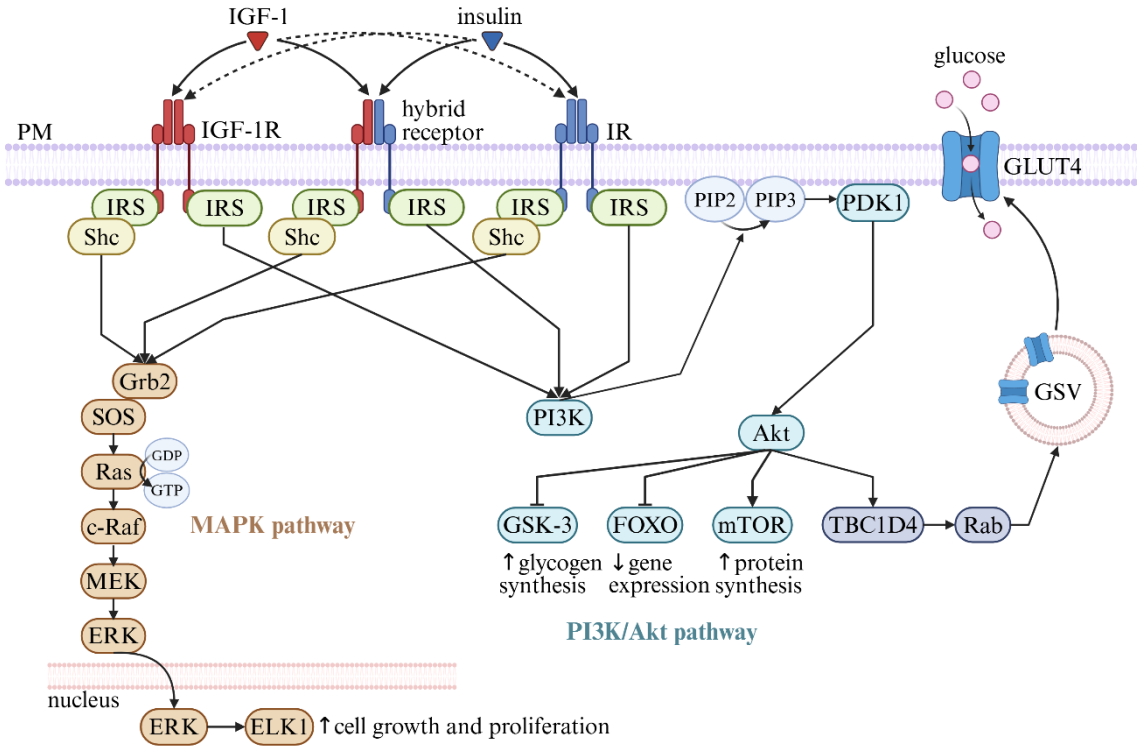

Figure 1. Insulin/IGF-1 signaling pathways. Insulin and insulin-like growth factor 1 (IGF-1) bind to their receptors on the plasma membrane (PM), including insulin receptor (IR), IGF-1 receptor (IGF-1R), and hybrid receptors. This interaction triggers autophosphorylation of the receptors, leading to the recruitment of adaptor proteins such as insulin receptor substrates (IRS) and Src homology 2 domain-containing (Shc) proteins. These adaptors initiate the PI3K/Akt and MAPK pathways. In the PI3K/Akt pathway, IRS recruits phosphoinositide 3-phosphate (PI3K) to the PM, where it converts phosphatidylinositol 4,5-bisphosphate (PIP2) to phosphatidylinositol 3,4,5-trisphosphate (PIP3). PIP3 then recruits 3-phosphoinositide-dependent kinase-1 (PDK1), which phosphorylates and activates Akt. Akt subsequently inhibits glycogen synthase kinase 3 (GSK-3), which enhances glycogen synthesis in the liver. Additionally, Akt suppresses forkhead box O (FOXO) transcription factor, thereby inhibiting the gene expression mediated by FOXO. Akt also activates the mechanistic target of rapamycin (mTOR) complex, which promotes protein synthesis. Furthermore, Akt phosphorylates TBC1D4, activating Rab GTPases associated with glucose transporter 4 (GLUT4). This activation facilitates the translocation of GLUT4 from intracellular GLUT4 storage vesicles (GSVs), where the majority of GLUT4 is stored, to the PM. Consequently, IRS and Shc interact with growth factor receptor-bound protein 2 (Grb2), activating the MAPK pathway. Grb2 binds to Son of Sevenless (SOS), which promotes Ras activation by exchanging GDP for GTP. Activated Ras recruits c-Raf, which phosphorylates MAPK/ERK kinase (MEK). MEK then phosphorylates extracellular signal-regulated kinase (ERK), leading to its translocation into the nucleus, where it activates transcription factor ELK1, which promotes cell growth and proliferation. Created in https://BioRender.com.

3 Insulin Metabolic Functions in the Liver

The liver plays a key role in insulin signaling pathways as it regulates plasma glucose levels and also maintains glucose homeostasis in both fasting and postprandial states through glycogen storage and HGP. These regulations are mediated by insulin, so the stable blood glucose levels can be sustained (Guerra & Gastaldelli, 2020)*. Additionally, the liver is in charge of clearing insulin from the body. Approximately 60% of the insulin released by the pancreas is broken down by the liver during the initial passage. By preserving appropriate levels of insulin in the blood, this clearance mechanism contributes significantly to overall metabolic balance (Eaton *et al.*, 1983). Furthermore,

the liver produces hepatokines and lipids that can act as modulators of insulin sensitivity through their autocrine and paracrine functions (Guerra & Gastaldelli, 2020)*.

Insulin is an important hormone in the liver with its vital role in promoting glucose uptake and storage (Sharabi *et al.*, 2015)*. When nutrients are abundant, pancreatic β-cells secrete insulin, which is carried to the liver through the portal vein, where it stimulates hepatic glucose uptake (Matveyenko *et al.*, 2012). This process causes absorption of glucose but also promotes the conversion of absorbed glucose into glycogen and excess glucose into fatty acids (FAs) (Sharabi *et al.*, 2015)*.

Glucose transport into hepatocytes is primarily facilitated by GLUT2 (Fukumoto *et al.*, 1988), a transporter that works independently of insulin and exhibits low affinity for glucose (Ciaraldi *et al.*, 1986). GLUT2 not only facilitates the glucose uptake but also controls the export of glucose from the liver. Aside from glucose, this transporter enables the transfer of other saccharides, such as fructose, mannose, and galactose. Once glucose enters the liver through GLUT2, it is phosphorylated by glucokinase (GCK), a hexokinase found in the liver, forming glucose 6-phosphate (G6P), which cannot be further exported. G6P can then either undergo glycolysis or be stored as glycogen (B. Sun *et al.*, 2023)*. However, genetic mutations affecting GLUT2 and GCK can disrupt this balance and lead to T2D (Mueckler *et al.*, 1994).

Insulin also regulates glucose production by the liver, alongside glucagon, which acts as a counter-regulator. During fasting, when glucose levels in the blood are low, the liver contributes to the release of glucose to maintain normoglycemia. This is achieved through two main processes, glycogenolysis and gluconeogenesis (Pan *et al.*, 2024) (Fig. 2).

In the postprandial state, when blood glucose levels rise after a meal, insulin secretion increases, suppressing HGP while promoting glucose uptake from the bloodstream and its storage as glycogen. HGP suppression is primarily mediated through the PI3K/Akt signaling cascade, which inhibits glycogen phosphorylase, an enzyme necessary for glycogenolysis. Gluconeogenesis is inhibited as well by insulin, allowing the control of the release of glucose from the liver into the bloodstream (Sharabi *et al.*, 2015)* (Fig. 2).

Depending on glucose levels in the bloodstream, the activation of the Akt pathway can also promote glycogenesis by activating glycogen synthase while inhibiting GSK-3 (Cross *et al.*, 1995) and FOXO1, leading to decreased HGP (Langlet *et al.*, 2017) and enhanced glycogen storage (Braccini *et al.*, 2015). Additionally, insulin signaling facilitates protein and lipid synthesis through the mechanistic target of rapamycin complex 1 (mTORC1) (Titchenell *et al.*, 2016).

Insulin not only regulates glucose metabolism but also modulates the activity of key enzymes involved in gluconeogenesis and glycolysis in the liver. Among these enzymes are GCK

(Nozaki *et al.*, 2020) and phosphoenolpyruvate carboxykinase 1 (PCK1) (Pan *et al.*, 2024). Certain PI3K subclasses contribute individually to these metabolic pathways. Class II PI3K isoform γ (PI3K-C2γ) can associate with Rab5-GTP, a small GTPase that plays a role in the formation and maturation of early endosomes. This causes PI3K-C2γ to be directed to the early endosomes, enhancing the accumulation of PIP2. This lipid is an important component in prolonging the activation of Akt2, a signaling molecule involved in insulin's metabolic actions, ultimately leading to glycogen synthesis (Braccini *et al.*, 2015). Unlike class II PI3K, class I PI3K catalyzes the conversion of PIP2 to PIP3 (Vanhaesebroeck *et al.*, 2010)*, which initiates downstream signaling cascades in the liver by recruiting Akt to the PM. This pathway regulates cell growth through the activation of mTOR and FOXO (Vanhaesebroeck *et al.*, 2012)*.

In summary, the liver's metabolic functions are regulated largely by insulin and are essential for maintaining glucose homeostasis by managing the balance between glucose production through glycogenolysis and gluconeogenesis and promoting glucose storage in the form of glycogen (Fig. 2).

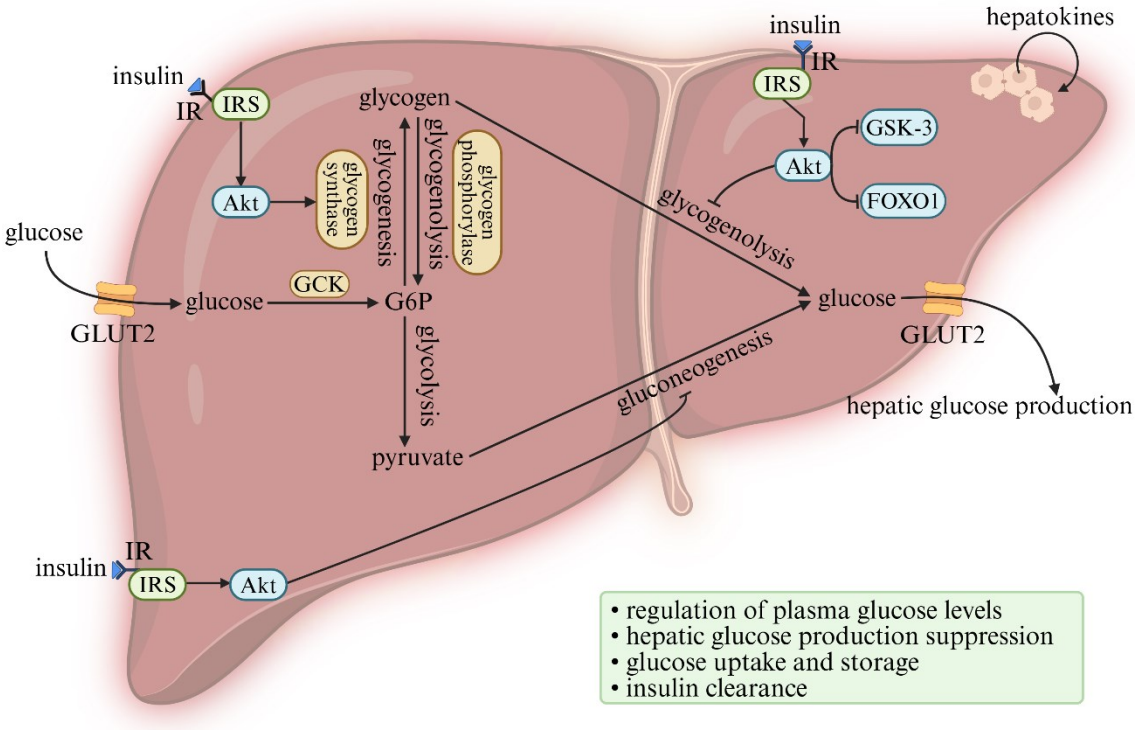

Figure 2. Insulin signaling in the liver. Glucose transport into the liver is facilitated by the bidirectional, insulin-independent glucose transporter 2 (GLUT2). Once inside hepatocytes, glucose is phosphorylated by glucokinase (GCK) to form glucose 6-phosphate (G6P). G6P can then undergo glycolysis to produce pyruvate or be converted to glycogen through glycogenesis, catalyzed by glycogen synthase. Glycogen can later be broken down into G6P through glycogenolysis, catalyzed by glycogen phosphorylase. When blood glucose levels are low, the liver produces glucose through hepatic glucose production, which involves both glycogenolysis and gluconeogenesis. Insulin mediates several functions in the liver by binding to the insulin receptor (IR) on hepatocytes, activating the PI3K/Akt pathway. Akt plays a vital role in enhancing glycogen synthase activity, thereby promoting glucose storage as glycogen. Additionally, Akt inhibits glycogenolysis and gluconeogenesis by suppressing glycogen synthase kinase 3 (GSK-3) and forkhead box O1 (FOXO1), both of which are involved in liver metabolism. Furthermore, hepatocytes release hepatokines that influence liver metabolism through autocrine signaling. Created in https://BioRender.com.

4 Insulin Metabolic Functions in Skeletal Muscle

Skeletal muscle contributes significantly to glucose homeostasis, serving as a major site for glucose disposal and being responsible for approximately 80% of glucose uptake in the presence of insulin (Ferrannini *et al.*, 1988). This is important, particularly during exercise, when the muscles rely on energy from glucose and glycogen. The process of glucose uptake in muscles begins when insulin binds to its receptor, activating IRS-1 (Saltiel, 2021)*. This in turn leads to PI3K activation, which triggers two main signaling cascades: one mediated by serine-threonine kinase Akt (Wang *et al.*, 1999), especially Akt2 (Bouzakri *et al.*, 2006), and the other by Rac1, a Rho family GTPase (Ueda *et al.*, 2008) (Fig. 3).

Akt phosphorylates TBC1D4, a protein that regulates Rab GTPases. In its unphosphorylated state, TBC1D4 inhibits specific Rab GTPases (Thong *et al.*, 2007). However, when TBC1D4 is phosphorylated by Akt, it facilitates the activation of these Rab GTPases, which promotes vesicle mobilization (Sano *et al.*, 2003). Simultaneously, Rac1 facilitates cortical actin remodeling, which is vital for GLUT4 vesicle insertion to the PM (Tong *et al.*, 2001). Studies have shown that inhibition or loss of Rac1 significantly impairs insulin-stimulated glucose uptake (Chiu *et al.*, 2010).

These signaling events result in GLUT4 translocation from intracellular compartments to the cell surface, leading to increased glucose entry into the cell (Bouzakri *et al.*, 2006). Once glucose enters the cell, it is phosphorylated to G6P by hexokinase II, thereby maintaining a concentration gradient for prolonged uptake (Osawa *et al.*, 1995). This process ensures sustained glucose transport into muscle cells. This is an important step for storing glucose as glycogen, which serves as a quick energy source, especially during exercise (Nedachi & Kanzaki, 2006).

Exercise promotes a rapid increase in glucose uptake through both insulin-dependent and independent pathways (Lund *et al.*, 1995). During physical activity, skeletal muscle contraction triggers immediate glucose uptake and induces insulin sensitivity after exercise through insulin-dependent pathway, leading to more efficient glucose uptake and storage (Richter *et al.*, 1982). Exercise activates adenosine 5'-monophosphate-activated protein kinase (AMPK) (Kjøbsted *et al.*, 2017), which subsequently phosphorylates TBC1D4 as a response to insulin stimulation (Treebak *et al.*, 2014). TBC1D4 then facilitates the translocation of GLUT4 (Mîinea *et al.*, 2005) (Fig. 3).

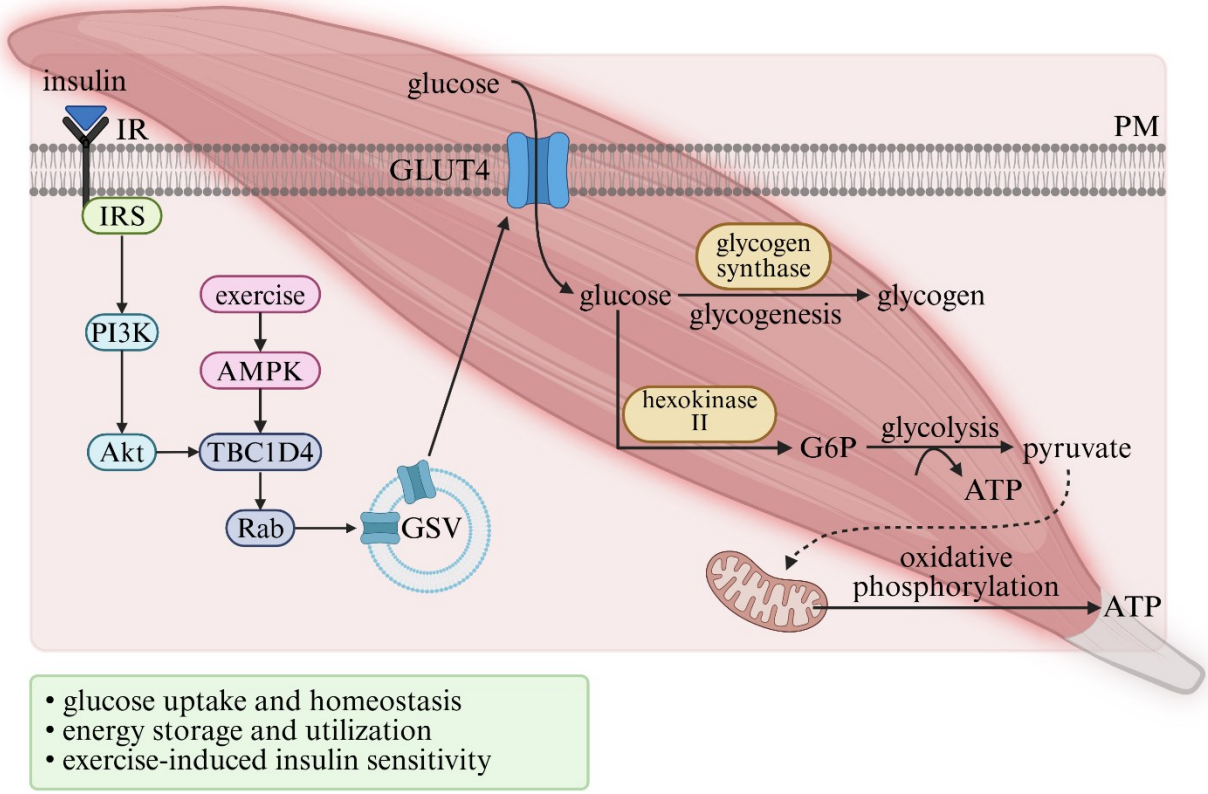

Figure 3. Insulin signaling in skeletal muscle. Glucose uptake in the skeletal muscle is mediated primarily by the insulin-dependent glucose transporter 4 (GLUT4). After insulin binds to the insulin receptor (IR) on the plasma membrane (PM), insulin receptor substrates (IRS) are recruited and phosphorylated by the activated IR. Phosphorylated IRS activates phosphoinositol-3-phosphate (PI3K), which in turn phosphorylates and activates Akt. Akt phosphorylates TBC1D4, a protein that regulates Rab GTPases. Activated Rab GTPases facilitate the translocation of GLUT4 from intracellular GLUT4 storage vesicles (GSVs) to the PM, enabling glucose uptake into muscle cells. Inside the cell, glucose is converted to glucose 6-phosphate (G6P) by hexokinase II. G6P enters glycolysis, generating pyruvate, which is used in oxidative phosphorylation in mitochondria to produce ATP. Additionally, glucose can be stored as glycogen through glycogenesis catalyzed by glycogen synthase. During exercise, glucose uptake occurs primarily through the insulin-independent pathway by activating adenosine 5'-monophosphate-activated protein kinase (AMPK), which phosphorylates TBC1D4, promoting GLUT4 translocation to the PM. Created in https://BioRender.com.

5 Insulin Metabolic Functions in Adipose Tissue

Insulin plays a vital role in adipose tissue as it enhances glucose uptake and consequently converts the excess glucose into FAs through a process called lipogenesis. Additionally, insulin inhibits lipolysis, which is essential for preventing excessive fat loss during postprandial states (Krycer *et al.*, 2020). This inhibition is important for HGP, as glycerol and FAs produced from lipolysis serve as substrates for glucose production in the liver, particularly in fasting states (Santoro *et al.*, 2021)*. Together, these processes help manage energy storage in the body (Fig. 4).

Glucose uptake in adipocytes is facilitated by translocation of GLUT4 to the PM in response to increased insulin levels (Suzuki & Kono, 1980). The mechanism is similar to its action in muscles. However, a considerable portion of glucose is redirected to lipid metabolism instead of being stored

as glycogen. FAs are stored as triacylglycerols (TGs) in adipose tissue, although less than 5% of glucose is converted to glycogen, and up to 50% can be metabolized into TGs via *de novo* lipogenesis (DNL) (Krycer *et al.*, 2020). The synthesis of TGs in adipocytes relies on insulin, as glucose uptake and its catabolism provide glycerol-3-phosphate for fatty acid esterification (Goldberg *et al.*, 2009) (Fig. 4).

Lipids derived from glucose by DNL act as signaling molecules that modulate metabolic and inflammatory processes in other tissues (Santoro *et al.*, 2021)*. Carbohydrate response element binding protein (ChREBP) is a transcription factor that plays a crucial role in regulating the DNL process in response to glucose (Iizuka *et al.*, 2004). Insulin does not directly activate ChREBP, but it facilitates its regulation by promoting glucose uptake (Ishii *et al.*, 2004). ChREBP is also involved in glycolysis (Iizuka *et al.*, 2004) and the pentose phosphate pathway, providing substrates for DNL (Kabashima *et al.*, 2003).

Additionally, there is a strong link between GLUT4 expression and ChREBP levels in white adipose tissue (Herman *et al.*, 2012). Increasing GLUT4 levels in adipocytes increase the expression of ChREBP and DNL, whereas removing ChREBP inhibits the insulin-stimulated movement of GLUT4 from GSVs to the PM. This is significant because GLUT4 is crucial for insulin-stimulated glucose uptake (Vijayakumar *et al.*, 2017). ChREBP activation also enhances the production of helpful signaling lipids, such as fatty acid hydroxy fatty acids (FAHFAs). Palmitic acid esters of hydroxy stearic acids (PAHSAs), a subfamily of FAHFAs, can be found mostly in white adipose tissue and brown adipose tissue (Yore *et al.*, 2014). These lipids enhance insulin action, improve glucose homeostasis, and reduce HGP by suppressing lipolysis in adipocytes (P. Zhou *et al.*, 2019). Besides, adipocytes synthesize other signaling lipids, like diacylglycerols (DAGs) and ceramides, which can modulate insulin action and influence cellular metabolism (Santoro *et al.*, 2021)*.

Moreover, adipocytes contribute to overall insulin sensitivity and inflammation by releasing hormones and cytokines (Santoro *et al.*, 2021)*, including adiponectin, an anti-inflammatory adipokine (Yokota *et al.*, 2000) that promotes insulin sensitivity and is typically lower in obese individuals (Hotta *et al.*, 2001).

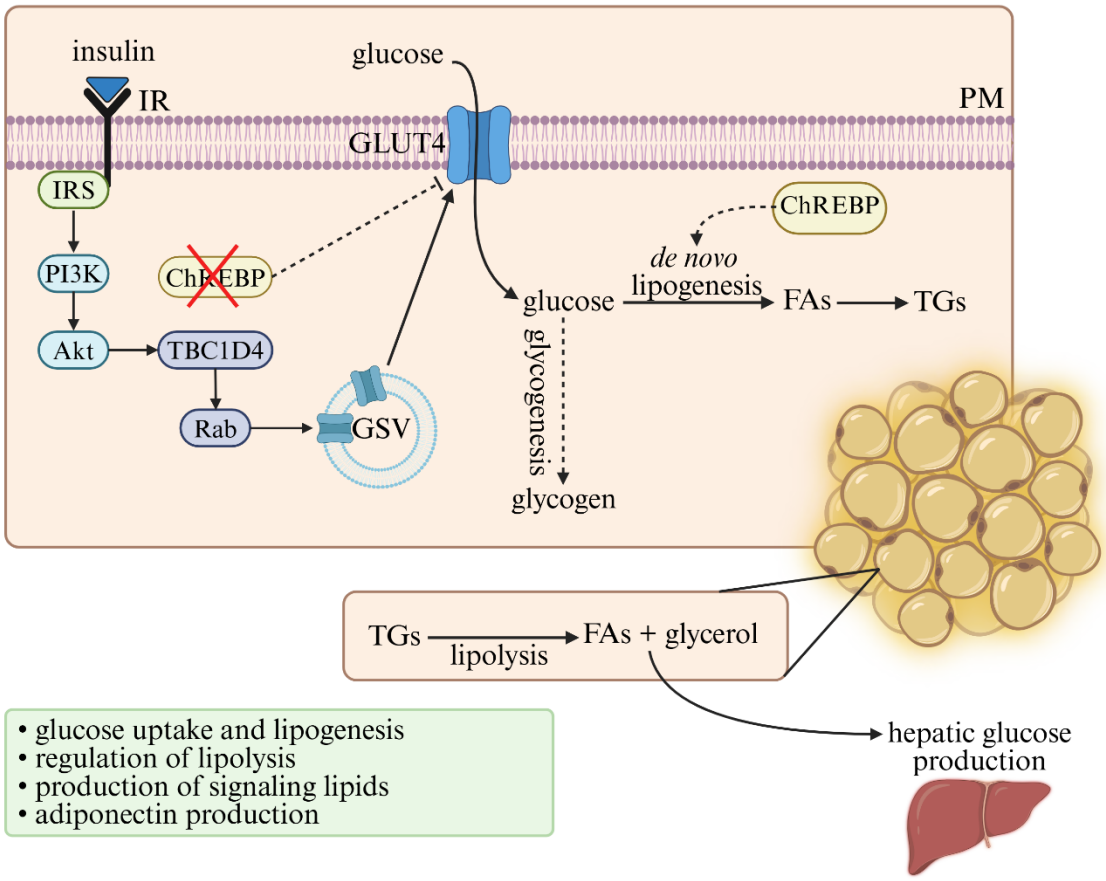

Figure 4. Insulin signaling in adipose tissue. Glucose uptake in the adipose tissue is mediated by the insulin-dependent glucose transporter 4 (GLUT4). After insulin binds to the insulin receptor (IR) on the plasma membrane (PM), insulin receptor substrates (IRS) are recruited and phosphorylated by the activated IR. Phosphorylated IRS activates phosphoinositol-3-phosphate (PI3K), which in turn phosphorylates and activates Akt. Akt phosphorylates TBC1D4, a protein that regulates Rab GTPases. Activated Rab GTPases facilitate the translocation of GLUT4 from intracellular GLUT4 storage vesicles (GSVs) to the PM, enabling glucose uptake into adipocytes. Inside the cell, excess glucose is primarily directed toward *de novo* lipogenesis, where it is converted into fatty acids (FAs) and incorporated into triacylglycerols (TGs) for storage. A small portion of glucose can be stored as glycogen through glycogenesis, however, most glucose is redirected to lipid metabolism. The transcription factor carbohydrate response element binding protein (ChREBP) is essential in regulating *de novo* lipogenesis. Deletion of ChREBP disrupts GLUT4 translocation to the PM, highlighting its importance in insulin-stimulated glucose transport and lipid metabolism into adipocytes. Created in https://BioRender.com.

6 The Role of Insulin Resistance and Its Contribution to Metabolic Disorders

Insulin resistance is a metabolic condition in which cells become less responsive to the normal insulin levels, which further leads to elevated blood glucose levels. In a healthy individual, insulin, produced by the pancreas, facilitates glucose uptake into cells for energy production. In insulin resistance, insulin-sensitive tissues like skeletal muscle, adipose tissue, and liver fail to respond effectively to insulin. As a result, the pancreas produces more insulin to compensate for this state, resulting in hyperinsulinemia. In the final stage, the β-cells get exhausted, dedifferentiate, and fail

to produce insulin, resulting in high blood glucose levels (hyperglycemia) and, eventually, T2D (S. H. Lee *et al.*, 2022)*.

Insulin resistance is one of the major factors contributing to the development of T2D, along with obesity and other risk factors (B. C. Martin *et al.*, 1992). Chronic inflammation caused by excess fat accumulation in obese individuals (Hotamisligil *et al.*, 1993), ectopic lipid accumulation (Turinsky *et al.*, 1990), and ER stress (Brown *et al.*, 2020) are among the main mechanisms causing insulin resistance in tissues like skeletal muscle, adipose tissue, and liver.

Chronic inflammation plays a significant role in obesity-related insulin resistance, characterized by changes in cytokine levels and the activation of inflammatory signaling pathways (Hotamisligil *et al.*, 1993). Two cell types are important in inducing proinflammatory response, namely adipocytes and macrophages. Both can secrete proinflammatory cytokines (Kamei *et al.*, 2006), such as tumor necrosis factor-α (TNF-α), which is capable of inducing serine phosphorylation of IRS-1, which interferes with the tyrosine phosphorylation induced by insulin, leading to impaired insulin signaling (Kanety *et al.*, 1995). Adipose tissue also contributes to insulin resistance as it secretes adipokines such as leptin (Finucane *et al.*, 2009), resistin, and adiponectin (Lu *et al.*, 2006). Leptin influences feeding behavior, with higher levels found in obese individuals (Heini *et al.*, 1998). Resistin has also been associated with insulin resistance because it is secreted by macrophages and other immune cells (Patel *et al.*, 2003).

Furthermore, insulin resistance caused by ectopic lipid accumulation occurs when excess lipids, especially FAs, are deposited in tissues other than adipose, such as liver and skeletal muscle. Some lipid metabolites are involved in inducing insulin resistance, in particular, DAGs and ceramides (Turinsky *et al.*, 1990). Increased levels of DAGs in insulin-sensitive tissues activate protein kinase C (PKC). Specific isoforms, such as PKCε of the liver (Petersen *et al.*, 2016) or PKCθ of the muscle tissues (Schmitz-Peiffer *et al.*, 1997), disrupt the insulin signaling pathways, causing impaired insulin receptor signaling and glucose uptake (Schmitz-Peiffer *et al.*, 1997; Petersen *et al.*, 2016). Ceramides inhibit the Akt activity, which is essential for glucose uptake, thus contributing to insulin resistance. They activate protein phosphatase 2A (PP2A), which dephosphorylates Akt (Teruel *et al.*, 2001), and promote Akt phosphorylation at Thr-34 by PKCζ, leading to decreased binding of PIP3 to Akt (Powell *et al.*, 2003). This inhibition impairs the translocation of GLUT 4 to the PM, leading to decreased glucose uptake (Teruel *et al.*, 2001).

Moreover, the ER stress in the insulin-responsive tissues also contributes to the development of insulin resistance. The ER is responsible for protein synthesis and their proper folding and maturation. However, ER stress disrupts this process, leading to inadequate maturation of insulin receptors and interfering with its translocation to the PM, resulting in depletion of the IR

on the surface (Brown *et al.*, 2020). Moreover, ER stress activates stress kinase c-Jun N-terminal kinase (JNK), which phosphorylates serine residues of IRS-1, leading to altered insulin signaling (Özcan *et al.*, 2004). In a state of overnutrition, like in obesity, the accumulation of unfolded proteins in the ER leads to activation of the unfolded protein response (UPR). In response to an excess of unfolded proteins in the ER, inositol-requiring enzyme-1 (IRE-1), PKR-like ER kinase (PERK), and activating transcription factor 6 (ATF6) are activated, initiating an adaptive response leading to increased production of ER chaperones and inhibition of protein translation, resulting in a reduction of unfolded proteins (Okada *et al.*, 2002).

7 Introduction to Insulin Receptor in the Brain: Neuronal Insulin/IGFs Signaling Mechanisms

The brain is recognized as an insulin-sensitive organ due to the expression of IR across various brain regions. These receptors are selectively distributed throughout the central nervous system (CNS) but are expressed more in certain areas such as the olfactory bulb, cerebellum, cortex, and hypothalamus (Hopkins & Williams, 1997). Neurons predominantly express IR-A isoform (Spencer *et al.*, 2018) in contrast to glial cells, such as astrocytes, primarily expressing the IR-B isoform (Garwood *et al.*, 2015).

The majority of insulin in the brain comes from the pancreas. In order to reach its receptors in the brain, the transport of insulin between blood and the brain is mediated through the blood-brain barrier (BBB) and cerebrospinal fluid (CSF) barrier (Milstein & Ferris, 2021)*. Insulin primarily enters the brain through an insulin receptor-mediated transport mechanism (Saura *et al.*, 1993). However, emerging evidence suggests that insulin can cross the BBB through mechanisms independent of IR (Rhea *et al.*, 2018), though more research is needed to clarify this alternative mechanism.

Glucose uptake in the brain primarily occurs through the insulin-independent transporters GLUT1 and GLUT3. GLUT1 is abundantly expressed by astrocytes and endothelial cells of the BBB, while GLUT3 is predominantly expressed by neurons (Milstein & Ferris, 2021)*. Additionally, brain glucose uptake is facilitated by insulin-sensitive GLUT4, but the expression of this transporter is restricted to specific areas like the hippocampus and hypothalamus, with a similar mechanism to that in peripheral tissues (Leloup *et al.*, 1996).

Once in the brain, insulin/IGF-1 binds to its receptors (IR and IGF-1R), which leads to a cascade of phosphorylation events (White *et al.*, 1988). The process begins with the autophosphorylation of the IR and IGF-1R in neurons and glial cells, which leads to IRS-1 and IRS-2 recruitment (Milstein & Ferris, 2021)*, with IRS-2 being more prominently expressed, especially in the hypothalamus,

where its role is essential for insulin signaling (Torsoni *et al.*, 2003). These substrates then undergo tyrosine phosphorylation, thus inducing the activation of the PI3K/Akt/mTOR signaling pathway, which partakes in synaptic plasticity, neuronal survival, or neurotransmitter trafficking (Milstein & Ferris, 2021)*. Additionally, insulin can influence cell growth and proliferation through the Shc/Ras/ERK/MAPK pathway, which is primarily activated by IGF-1R signaling and, to a lesser extent, by IR signaling. This pathway is less actively involved in neurons (W. Cai *et al.*, 2017). The PI3K/Akt/mTOR pathway is primarily activated by the IR-B isoform, while the IR-A isoform preferentially activates the Shc/Ras/ERK/MAPK pathway (Milstein & Ferris, 2021)*.

Overall, insulin signaling in neurons focuses on regulating neurotransmission and energy homeostasis, which are important for neuroprotection and cognitive functions.

7.1 *De Novo* Insulin Synthesis in the Brain – Historical Perspective

Insulin is not only produced in the pancreas but is also expressed in the CNS, specifically in neurons. The discovery started in the early 1980s with the detection of insulin immunoreactivity in cultured neuronal cells from rat (Weyhenmeyer & Fellows, 1983) and mouse fetal brains (Birch *et al.*, 1984). The presence of insulin was confirmed by the following studies in cultured neurons (Devaskar *et al.*, 1994), but it was not detected in glial cells (Schechter *et al.*, 1988). Insulin secretion from neuronal cultures was also observed when membrane depolarization was induced by release of K^+ and Ca^{2+} (Clarke *et al.*, 1986).

These findings were supported by more recent studies conducted *in vitro*. For instance, research by Molnár *et al.* (Molnár *et al.*, 2014) demonstrated that expression of *Ins2* mRNA occurs in the cytoplasm of GABAergic interneurons in the rat cerebral cortex. Their study found that reducing glucose levels to those typical of brain extracellular fluid during hypoglycemia resulted in a decrease in *Ins2* mRNA (Molnár *et al.*, 2014). In rodents, the ancestral *Ins2* gene, which is homologous to the human *Ins* gene, is expressed in the brain throughout life, but the duplicated *Ins1* gene is expressed differently (Shiao *et al.*, 2008).

Another study demonstrated the role of glucagon-like peptide-1 receptor (GLP-1R) in insulin expression, where the activation of GLP-1R mediates the glucose-induced insulin expression in neurogliaform cells (NGFC). The study showed the co-expression of *Ins2* mRNA and *Glp1r* mRNA in inhibitory interneurons and identified high extracellular glucose concentrations as the regulatory mechanism that upregulates *Glp1r* expression (Csajbók *et al.*, 2019).

Additionally, like in neocortical neurons, insulin expression in the hypothalamic neuronal cell lines was shown to be stimulated by high glucose concentrations (Madadi *et al.*, 2008). A study focusing on the N39 hypothalamic cells revealed more regulatory molecules involved in insulin

expression. Treatment with Wnt3a, a secreted glycoprotein (J. Lee *et al.*, 2016) and a crucial component of the Wnt signaling pathway involved in the development of the pancreas (Papadopoulou & Edlund, 2005) and the brain (C. J. Zhou *et al.*, 2004), led to increased *Ins2* gene expression and insulin secretion. Not only the Wnt3a promoted insulin production but also induced the transcription factor NeuroD1 (J. Lee *et al.*, 2016), which participates in insulin gene transcription in the pancreas (Naya *et al.*, 1995) and also has an ability to promote the differentiation of glial cells into neurons (Wei *et al.*, 2023). Furthermore, inhibiting GSK-3 resulted in increased insulin and NeuroD1 expression (J. Lee *et al.*, 2016).

Havrankova *et al.* were the first to report the presence of insulin in the rat brain in 1978 (Havrankova *et al.*, 1978). These findings led to later confirmations of insulin immunoreactivity in various brain regions in rodents, particularly rats, or humans (Devaskar *et al.*, 1994).

A study on neonatal rabbits showed a positive correlation between brain and CSF insulin concentrations, suggesting that the brain independently contributes to CSF insulin levels (Schechter *et al.*, 1992). In addition, the insulin mRNA presence demonstrated that insulin is synthesized in the brain during all developmental stages (Devaskar *et al.*, 1993; Schechter *et al.*, 1996; Mehran *et al.*, 2012).

Other studies on mice revealed insulin expression occurring throughout various phases of brain development and its presence, reported at the levels of *Ins2* mRNA, mature insulin, and C-peptide, in regions such as the cerebellum, cerebral cortex, olfactory bulb, and especially hippocampus. Notably, although hippocampal insulin concentrations were lower compared to those in the pancreas, the close contacts between neurites (axons and dendrites) and neurons suggest an autocrine or paracrine function of the insulin derived from the brain as a signaling molecule (Mehran *et al.*, 2012).

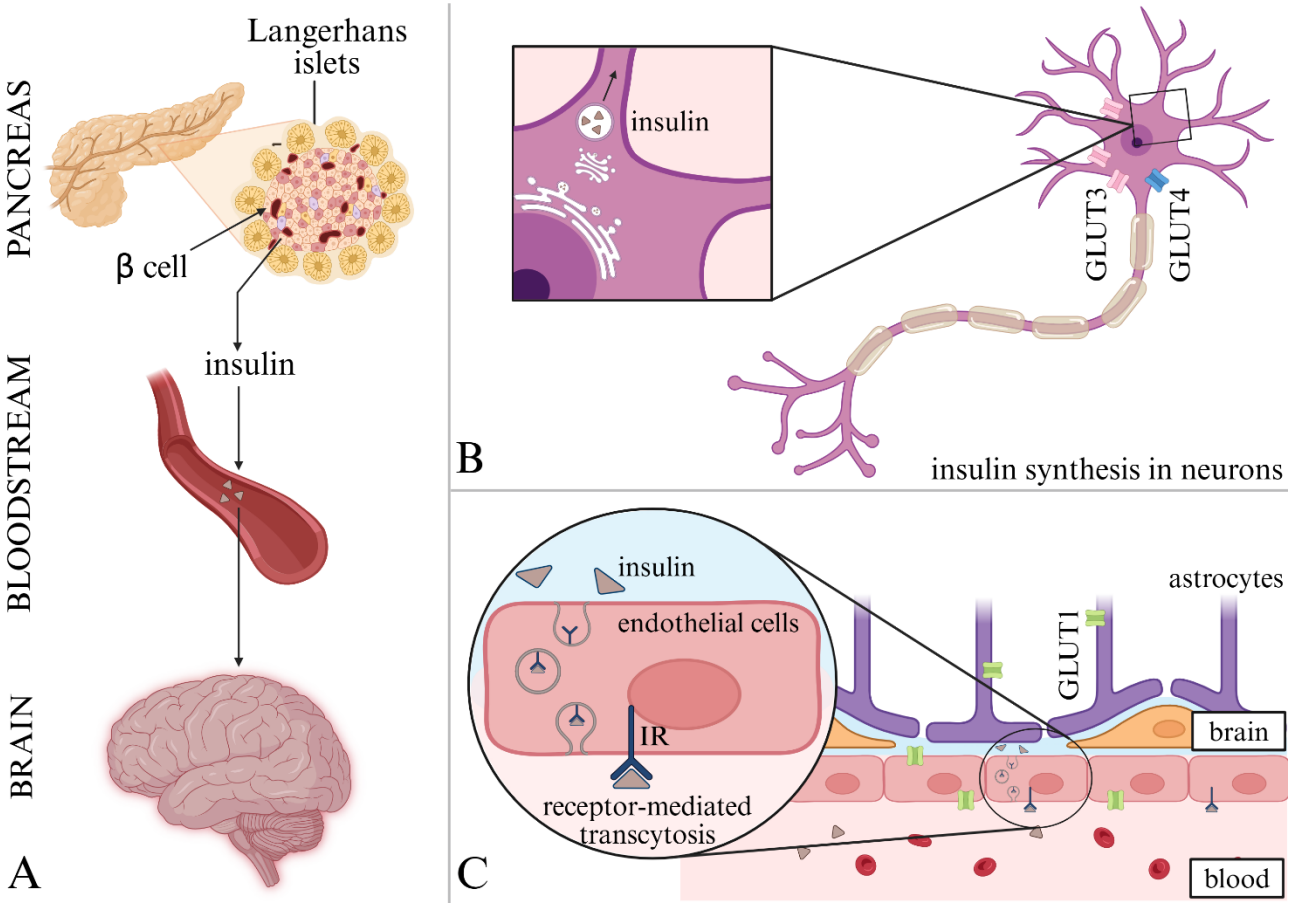

Figure 5. Insulin transport into the brain and *de novo* insulin synthesis. A. Insulin, produced by pancreatic β-cells within the Langerhans islets, is transported to the brain through bloodstream. It crosses the blood-brain barrier (BBB) through a saturable transport system, which involves receptor-mediated transcytosis, where insulin binds to insulin receptors on brain endothelial cells and is transported into the brain, where it is released. **B.** While most brain insulin originates from the pancreas, neurons also synthesize insulin. **C.** Glucose uptake in the brain occurs predominantly through insulin-independent glucose transporters (GLUT1 and GLUT3). Created in https://BioRender.com.

7.2 The Role of Insulin/IGFs in the Brain

After the discovery of the IR presence in the CNS and transport of insulin across the BBB, researchers have been investigating insulin's effects and role in the brain. Consequently, many insulin actions in the CNS have been identified. Insulin plays an important role as a regulator of food intake, cognitive processes, neuroprotection etc.

7.2.1 Neuroprotective Effects

Brain-derived insulin has a role in promoting distribution of neurofilaments, as well as axon development through MAPK phosphorylation, as demonstrated on cultured rat fetal neurons (Schechter *et al.*, 1999). When these neurons were cultured in an environment without insulin, their somata, axons, and dendrites displayed neurofilament immunoreactivity, using neurofilament specific antibodies targeting neurofilament proteins in these structures. This suggests that insulin

originating in the brain is sufficient to support neurofilament expression and distribution (Schechter *et al.*, 1998). When treated with insulin antibodies for insulin detection and its neutralization, the neurons showed hypertrophy and vacuolation, followed by shortening of their neurites. Additionally, inhibiting MAPK led to neurite retraction and neuron rounding (Schechter *et al.*, 1999).

Moreover, both insulin and IGF-1 exhibit neuroprotective effects (Fig. 6), as they can reduce neuronal apoptosis caused by oxidative stress. This neuroprotection likely results from the restoration of IR and IGF-1R signaling (Duarte *et al.*, 2008). One mechanism involves IGF-1 activation of the IGF-1/PI3K/Akt pathway, which leads to the phosphorylation of survival factors like cyclic adenosine monophosphate response element binding protein (CREB) and inactivation of FOXO1 and a pro-apoptotic effector GSK-3β (Leinninger *et al.*, 2004; Chin *et al.*, 2005).

Additionally, protection against apoptosis is provided through the PI3K pathway (Ryu *et al.*, 1999) (Fig. 6). Oxidative stress leads to oxidation of lipids and proteins, such as GLUT3, which can disrupt their function. This leads to impaired glucose uptake or further causes issues like lactate build-up, leading to acidosis and mitochondrial dysfunction (Blázquez *et al.*, 2014)*. Insulin can reduce oxidative stress through enhanced glucose uptake, promotion of pyruvate production, or restoration of ATP levels (Duarte *et al.*, 2006). When oxidative stress occurs, it also affects the uptake of neurotransmitters like γ-aminobutyric acid (GABA) and glutamate, which can later accumulate outside synapses. This can be prevented by the administration of insulin (Duarte *et al.*, 2003).

Furthermore, IGF-2 is also involved in mediating neuroprotection through its interaction with IGF-2R, which was demonstrated in the study, where oxidative damage was induced by corticosterone. The interaction of IGF-2 and IGF-2R led to reduced oxidative stress, improved mitochondrial function, and resulted in restoration of redox balance in the neurons (Martín-Montañez *et al.*, 2017) (Fig. 6).

7.2.2 Feeding Behavior

The effects pf insulin on body weight and glucose homeostasis (Fig. 6) are mediated through its interaction with the IR on specialized neuronal populations of neurons in the arcuate nucleus of the hypothalamus that express proopiomelanocortin (POMC), agouti-related peptide (AgRP) (Benoit *et al.*, 2002), GABA, and neuropeptide Y (NPY) (Krashes *et al.*, 2013).

Food intake is regulated by AgRP neurons, whose activity is inhibited by the interaction between insulin and IR (Könner *et al.*, 2007). This fact is further supported by an experiment

demonstrating that the elimination of AgRP neurons in adult mice leads to reduced food consumption (Gropp *et al.*, 2005).

Additionally, AgRP neurons are involved in regulating HGP. In mice lacking IR in AgRP neurons, the suppression of HGP by insulin was disrupted. These mice also exhibited reduced expression of interleukin 6 (IL-6) (Könner *et al.*, 2007), which is essential for activating the transcription factor signal transducer and activator of transcription 3 (STAT3). Activation of STAT3 reduces HGP by suppressing the expression of gluconeogenic enzymes, such as glucose 6-phosphatase (Inoue *et al.*, 2006), the enzyme necessary for conversion of G6P to glucose in the liver.

The IR signaling in AgRP neurons may be inhibited by the T-cell tyrosine phosphatase (TCPTP) (Dodd *et al.*, 2018). This enzyme can, among other functions, dephosphorylate IRS and affect glucose metabolism and insulin signaling (Galic *et al.*, 2003). It diminishes the ability of insulin to inhibit AgRP neurons, leading to weight gain. Conversely, the deletion of TCPTP in AgRP neurons enhances insulin sensitivity and reduces HGP (Dodd *et al.*, 2018).

Moreover, high glucose levels reduce AgRP expression and stimulate POMC expression. Glucose administration further increases POMC expression and decreases AgRP and NPY expression, resulting in decreased food intake (Zhang *et al.*, 2015). IR signaling in POMC neurons is also essential for regulating lipolysis in adipose tissue, as demonstrated in mice lacking IR in POMC neurons. These mice exhibited impaired suppression of lipolysis by insulin, leading to elevated TG levels in the liver (Shin *et al.*, 2017).

7.2.3 Learning and Memory

It has been proposed that insulin plays a role in learning and memory (Fig. 6). Studies have shown that systemic insulin and IGF-1 administration can improve cognitive functions, such as memory and learning, in rats (Lupien *et al.*, 2003) and intranasal insulin administration may improve cognitive functions in healthy individuals under controlled conditions when blood glucose levels are stabilized (Kern *et al.*, 2001). Moreover, intranasal insulin administration has been found to increase insulin levels in CSF (Born *et al.*, 2002), suggesting a direct influence of insulin on brain function.

Some studies suggested the importance of IR in learning and memory, in which rats trained on learning tasks had an increased IR mRNA expression in some areas of the hippocampus, accompanied by the rise in IR protein levels (W. Zhao *et al.*, 1999).

Administration of streptozotocin, a drug that is, among other things, used in experiments to destroy pancreatic β-cells, caused significant impairments in learning and memory in rats (Biessels *et al.*, 1998). In another study, the administration of streptozotocin caused interference

in insulin binding to its receptor, leading to altered insulin action and impairments in memory (Lannert & Hoyer, 1998).

To investigate the effects of chronic glucocorticoid exposure on neurological function, rats were treated with corticosterone over an extended period. This treatment aimed to evaluate behavioral changes and alterations in gene expression related to the insulin signaling pathway and Tau protein regulation in the cerebral cortex. This treatment resulted in reduced insulin and IR levels in cortical tissue. Simultaneously, it increased Tau mRNA expression in the same regions, which correlated with the observed memory deficits. These findings suggest that chronic corticosterone exposure disrupts insulin-related neuroprotective mechanisms while promoting Tau-associated pathology, potentially contributing to cognitive decline (Osmanovic *et al.*, 2010). These results align with the association of Alzheimer's disease (AD) where insulin deficiency and insulin resistance occur in the brain and show the importance of brain-derived insulin in maintaining cognitive function, which can be altered by impaired transport across BBB (Leclerc *et al.*, 2023) or impaired function of IR (Steen *et al.*, 2005).

Both peripheral and central insulin administration have positive effects on memory and learning (Park *et al.*, 2000), through the increase in IR expression and its signal transduction pathways in the hippocampus (W. Zhao *et al.*, 1999).

Compared to these findings, neuronal insulin receptor knockout (NIRKO) mice do not show any impairments in spatial learning (Schubert *et al.*, 2004). According to that, the role of IR signaling might not be as important in memory formation, and other mechanisms might compensate for its absence.

The Shc-mediated signaling pathway is believed to play an important role in learning and memory formation, as rats that underwent water maze training exhibited higher Shc tyrosine phosphorylation (W. Zhao *et al.*, 1999). The Shc-mediated signaling pathway involves the Shc protein, which comprises a phosphotyrosine-binding domain and SH2 domain, and connects the IR to the Grb2/SOS complex, which acts as an adaptor protein. After binding to the IR, Shc undergoes phosphorylation by the receptor's tyrosine kinase activity, allowing it to associate with the Grb2/SOS complex. This interaction between phosphorylated Shc and the Grb2/SOS complex activates Ras, further leading to the activation of MAPK cascade (Dikic *et al.*, 1995). More subsequent studies further support the involvement of the MAPK cascade in learning and memory (Selcher *et al.*, 1999) and in synaptic plasticity (English & Sweatt, 1997).

Furthermore, IGF-2R is widely expressed in neurons, especially in the pericytes, cells that help maintain the BBB and regulate blood flow in the hippocampus (Pandey *et al.*, 2023). In hippocampal neurons, this receptor plays a crucial role in memory formation, including memory retention,

preventing forgetting in between the learning and recall phase (Chen *et al.*, 2011). IGF-2 signaling drives the formation of long-term memory through the cooperation between neurons and pericytes. Neuronal activity increases during learning and enhances IGF-2 production in pericytes, contributing to the formation of long-term memory. This is supported by evidence that pericyte-specific *Igf2* knockout has disrupted the formation of long-term memory (Pandey *et al.*, 2023).

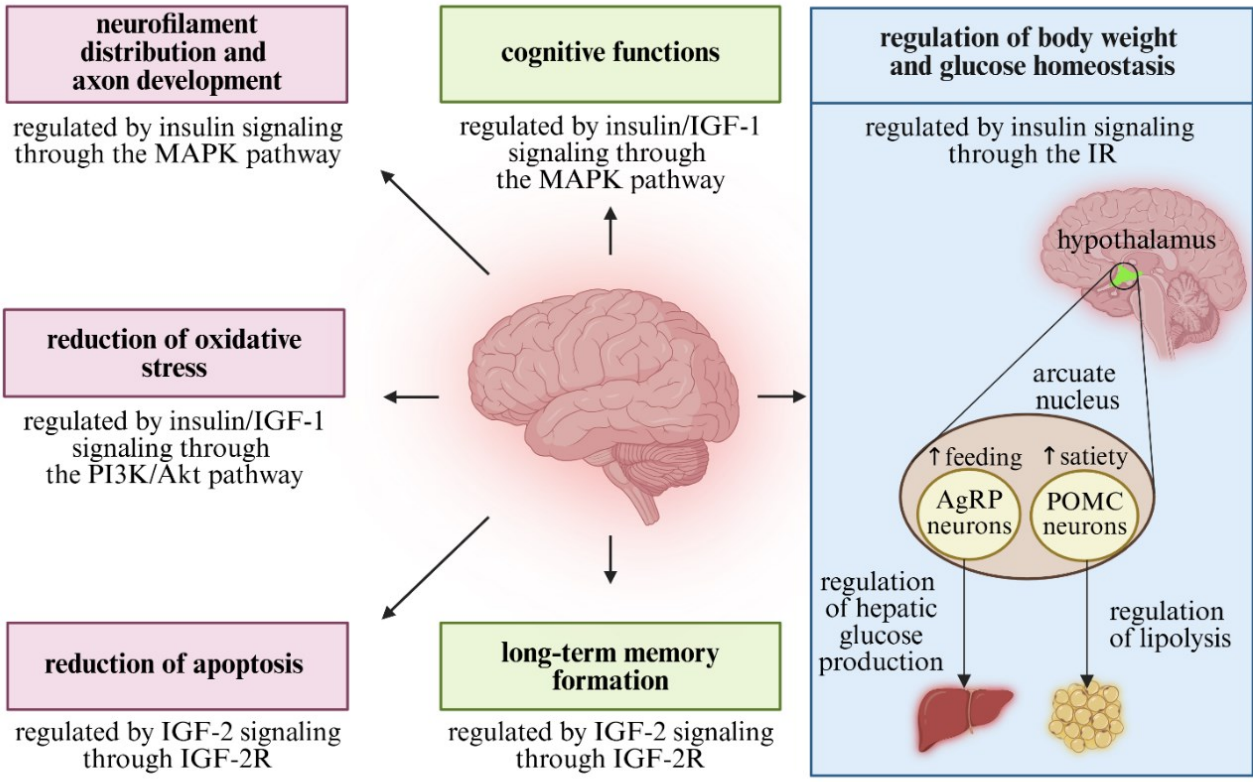

Figure 6. The role of insulin/IGFs in the brain. Insulin and IGFs regulate many functions in the brain, including neuroprotection, cognitive functions, and feeding behavior. Insulin promotes neurofilament distribution and axon development through the MAPK cascade. Both insulin and IGF-1 reduce oxidative stress through the PI3K/Akt pathway, and IGF-2 signaling further contributes to neuroprotection by reducing apoptosis through interacting with its receptor (IGF-2R). Beyond neuroprotection, insulin and IGFs modulate cognitive functions and long-term memory formation. Cognitive functions are regulated by insulin and IGF-1 signaling through MAPK activation, whereas long-term memory formation involves IGF-2 interaction with IGF-2R. Insulin also regulates body weight and glucose homeostasis through specialized neurons in the arcuate nucleus of the hypothalamus, namely AgRP and POMC neurons. Insulin inhibits AgRP neurons, which are involved in stimulating food intake. Additionally, insulin signaling in AgRP neurons suppresses hepatic glucose production. POMC neurons, which promote satiety, are activated by insulin, and insulin signaling in these neurons enhances regulation of lipolysis in adipose tissue. Created in https://BioRender.com.

7.3 Insulin in Neurodegenerative Diseases

Insulin impacts neuronal health and function in neurodegenerative diseases like AD and Parkinson's disease (PD), where alterations in insulin signaling are associated with cognitive decline and neuronal degeneration (Blázquez *et al.*, 2014)*.

7.3.1 Alzheimer's Disease

AD is a progressive neurodegenerative disorder that leads to significant cognitive decline and memory loss, primarily affecting older adults (Blázquez *et al.*, 2014)*. AD has been increasingly linked to insulin resistance and impaired insulin signaling in the brain. This connection is clear through several factors. AD patients show decreased binding of insulin to its receptor, leading to altered insulin signaling (Steen *et al.*, 2005). Insulin sensitivity is also reduced, especially at the IRS-1 levels, along with disrupted IRS-1/PI3K signaling (Talbot *et al.*, 2012). Interestingly, both insulin levels and IR densities in some cortical regions of the brain decrease with age. In AD patients, lower insulin levels are observed, however, these individuals often exhibit an upregulation of IR densities in specific areas, such as the occipital cortex, compared to age-matched controls. Nonetheless, these IR densities are still lower compared to those found in middle-aged controls. This suggests a compensatory upregulation of IR in the brains of AD patients due to impaired insulin signaling. In contrast, IGF-1R densities remain stable (Frölich *et al.*, 1998). Additionally, plasma insulin levels are higher and CSF insulin levels are lower in individuals with AD (Craft *et al.*, 1998). However, insulin administration was shown to improve memory in AD patients, which seems to work independently of glucose metabolism (Craft *et al.*, 1999). Furthermore, insulin influences amyloid-β (Aβ) plaques and Tau neurofibrillary tangles (NFTs), a characteristic feature of AD (Blázquez *et al.*, 2014)*.

Insulin regulates the amyloid precursor protein (APP), which further affects the Aβ metabolism (Rad *et al.*, 2018)* (Fig. 7). APP can be processed through the non-amyloidogenic pathway, which is promoted by insulin affecting the phosphorylation of the APP and is predominant, and through the amyloidogenic pathway, by which only a small part is processed. Impaired insulin signaling can thus lead to increased Aβ levels (Pandini *et al.*, 2013).

In the non-amyloidogenic pathway, APP is cleaved by α-secretase, which prevents the formation of Aβ, and the products generated do not contribute to plaque formation. On the other hand, the amyloidogenic pathway generates Aβ fragments by cleavage of APP by β- and γ-secretases. These Aβ fragments are then released into the extracellular environment, where they aggregate into oligomers and eventually form Aβ plaques, which are associated with AD (Rad *et al.*, 2018)*.

Low insulin levels can subsequently lead to increased Aβ levels that contribute to Aβ plaques in the brain (Rad *et al.*, 2018)*. Insulin degrading enzyme (IDE) plays a crucial role in Aβ degradation as it targets both insulin and Aβ levels (Farris *et al.*, 2003) and an alteration in the insulin signaling cascades decreases the degradation of amyloid (W. Q. Zhao *et al.*, 2009). Insulin affects Aβ metabolism and increases extracellular Aβ levels by modulating secretion and degradation by IDE (Pérez *et al.*, 2000) (Fig. 7). Conversely, the actions of insulin can be disrupted by Aβ, which competes with insulin for the binding to the IR or reduces the affinity of insulin for the receptor (Xie *et al.*, 2002). There is a correlation between reduced IDE expression and increased plaque formation in the hippocampus of individuals with AD (Bernstein *et al.*, 1999). In addition, IDE abnormal expression leads to elevated insulin levels and elevated Aβ levels in the brain (Leal *et al.*, 2006).

Similar to its regulation of Aβ metabolism, insulin also modulates Tau phosphorylation (Hong & Lee, 1997). Tau protein has been discovered to be part of NFTs found in the brain in the AD patients (Wischik *et al.*, 1988) (Fig. 7). It is a microtubule-associated protein (Wischik *et al.*, 1988) important for the formation and stability of microtubules (Kadavath *et al.*, 2015). Its activity is regulated by phosphorylation at serine, threonine, and tyrosine sites, which together comprise 85 phosphorylable residues (Hanger *et al.*, 2007).

In AD patients, Tau protein is hyperphosphorylated (Köpke *et al.*, 1993), because of an imbalance between kinases that contribute to the phosphorylation (Sędzikowska & Szablewski, 2021)*. One of the main contributors to the development of AD and the formation of NFTs is the kinase GSK-3β, which phosphorylates Tau at several sites (Chakraborty *et al.*, 2023) and is regulated by Akt, which phosphorylates GSK-3β on serine 9, leading to inhibition of its activity (Summers *et al.*, 1999). Inhibition of the PI3K/Akt pathway, caused by altered insulin signaling, can lead to enhanced Tau phosphorylation due to increased GSK-3β activation (Baki *et al.*, 2004) (Fig. 7). Another factor contributing to Tau hyperphosphorylation is reduced Tau O-GlcNAcylation that results from insulin resistance and decreased glucose metabolism (F. Liu *et al.*, 2009).

Once Tau protein is hyperphosphorylated, it undergoes conformational changes and can no longer effectively bind to microtubules that mediate the transport of misfolded Tau monomers. This results in their accumulation, oligomerization, and aggregation in neuronal cell bodies. As a result, the Tau protein adopts a beta-sheet conformation and forms filaments, which can be stored in NFTs (Alonso *et al.*, 1994).

In the brain, insulin signaling is influenced by Tau protein, as demonstrated in studies conducted on animals. Deletion of Tau leads to reduced response to insulin in the hippocampus, which is further worsened by alterations in the actions of IRS-1 and the negative regulator of the PI3K/Akt pathway,

phosphate and tensin homologue (PTEN). Impairments in energy metabolism within the hypothalamus can be observed in Tau knockout mice, suggesting that insulin resistance in the brain may be caused by the loss of Tau function (Marciniak *et al.*, 2017). Additionally, research indicates that in AD, insulin tends to accumulate in neurons with hyperphosphorylated Tau. This insulin buildup is further linked to insulin resistance as well as decreased IR levels (Rodriguez-Rodriguez *et al.*, 2017).

7.3.2 Parkinson's Disease

PD ranks as the second most prevalent neurodegenerative disorder, impacting individuals over the age of 60 worldwide. The likelihood of developing PD rises with age. The primary feature is the degeneration of dopaminergic neurons in the nigrostriatal system. This leads to typical motor symptoms such as stiffness (Athauda & Foltynie, 2016)*.

Recent studies indicate that disturbance in glucose and energy metabolism may occur early in the development of PD (Dunn *et al.*, 2014), similar to those seen in T2D, suggesting that these two disorders might have common underlying causes (Athauda & Foltynie, 2016)*. Increasing evidence suggests that a form of insulin resistance is present in the brains of PD patients, further indicating that impaired insulin signaling might play a role in the progression of PD (D'Amelio *et al.*, 2009).

In addition to motor symptoms, PD is also associated with cognitive impairments and dementia, which are significant aspects of PD (Rosenthal *et al.*, 2010). Like individuals with AD, half of the PD patients show the presence of Aβ plaques and NFTs (Compta *et al.*, 2011), with both conditions being influenced by insulin's action.

Some studies indicated a correlation between PD and T2D, with the risk of developing PD increasing with the severity of T2D (Han *et al.*, 2023). However, fewer than half of T2D patients show an increased risk of developing PD (Q. Xu *et al.*, 2011).

The most significant risk factor for PD is age. As people age, the sensitivity of peripheral IR tends to decline, just like the levels of IR mRNA in the brain (Athauda & Foltynie, 2016)*. However, PD patients exhibit a more pronounced decline in insulin signaling (Morris *et al.*, 2014). Inhibition of insulin signaling occurs when the phosphorylation of IRS at serine residues increases, which can be observed in brain parts such as the substantia nigra (Moroo *et al.*, 1994). The phosphorylation of IRS-1 on serine residues disrupts insulin signaling, as it prevents the activation of downstream signaling pathways by blocking the insulin and IGF-1 from binding to the IR (Moloney *et al.*, 2010). It has been shown that levels of IRS-1 phosphorylation on serine were increased in patients with PD (Gao *et al.*, 2015) (Fig. 7).

The substantial evidence suggests the significance of Akt in PD, as its impaired signaling may play a role in PD pathogenesis (Timmons *et al.*, 2009). Reduced Akt phosphorylation and decreased Akt levels were observed in individuals with PD after *postmortem* analysis (Timmons *et al.*, 2009). Blocking Akt signaling can result in the death of dopaminergic cells (Y. Xu *et al.*, 2014), and improper regulation of Akt signaling may influence the α-synuclein expression in PD (Kim *et al.*, 2011) (Fig. 7).

α-synuclein is a small protein found predominantly in the brain (Jakes *et al.*, 1994), particularly at presynaptic sites (Iwai *et al.*, 1995), where it participates in synaptic transmission through its interaction with synaptic vesicles (Kahle *et al.*, 2000) and synaptic proteins, such as vesicle-associated membrane protein 2 (VAMP-2) (J. Sun *et al.*, 2019). α-synuclein can be degraded by IDE, which is influenced by insulin signaling. IR activation stimulates PI3K activation, allowing IDE to prevent the formation of α-synuclein fibrils by blocking the conversion of α-synuclein oligomers into fibers (Sharma *et al.*, 2015). α-synuclein aggregation can be prevented by activation of insulin signaling through IGF-1 (Kao, 2009). Similarly, IGF-2 treatment has been shown to have a neuroprotective effect in PD patients, where it reduces α-synuclein aggregation, which is associated with toxicity in PD models. The neuroprotective mechanism involves the interaction of IGF-2 with IGF-2R, leading to α-synuclein secretion. Additionally, this treatment can prevent dopaminergic neuronal loss (Arcos *et al.*, 2023).

Akt activation leads to inactivation of GSK-3β through its phosphorylation on serine (Moore *et al.*, 2013). GSK-3β is important as it promotes expression of α-synuclein (Credle *et al.*, 2015), inflammation (M. Martin *et al.*, 2005) or mitochondrial dysfunction (S. A. Martin *et al.*, 2018). Individuals with PD exhibiting α-synuclein aggregation show elevated GSK-3β expression (Wills *et al.*, 2010) (Fig. 7).

Autophagy malfunction is also involved in PD. Autophagy is regulated by the mTOR kinase, an important component of the insulin IR/PI3K/Akt signaling pathway, which is activated by Akt. mTOR prevents excessive autophagy, allowing cells to grow. However, PD patients exhibit irregularities in the regulation of this pathway, which leads to altered autophagy (Heras-Sandoval *et al.*, 2014)* (Fig. 7). mTORC1, a component of mTOR, can be inhibited by rapamycin. This compound can also reduce accumulation of α-synuclein in PD models and Aβ accumulation and Tau misfolding in AD models (Caccamo *et al.*, 2010).

α-Synuclein enhances IRS phosphorylation on serine, thereby inhibiting insulin signaling (Fig. 7). Although the mechanism is not fully understood, it is proposed that α-synuclein causes prolonged activation of mTORC1, which intensifies the negative feedback loop on insulin signaling, leading to increased IRS-1 degradation (Gao *et al.*, 2015). Additionally, α-synuclein

influences microglia by directly activating them (Béraud *et al.*, 2013), resulting in the release of pro-inflammatory cytokines as observed in PD patients (Reale *et al.*, 2009) and has been associated with decreased motor functions and cognitive ability (Williams-Gray *et al.*, 2016).

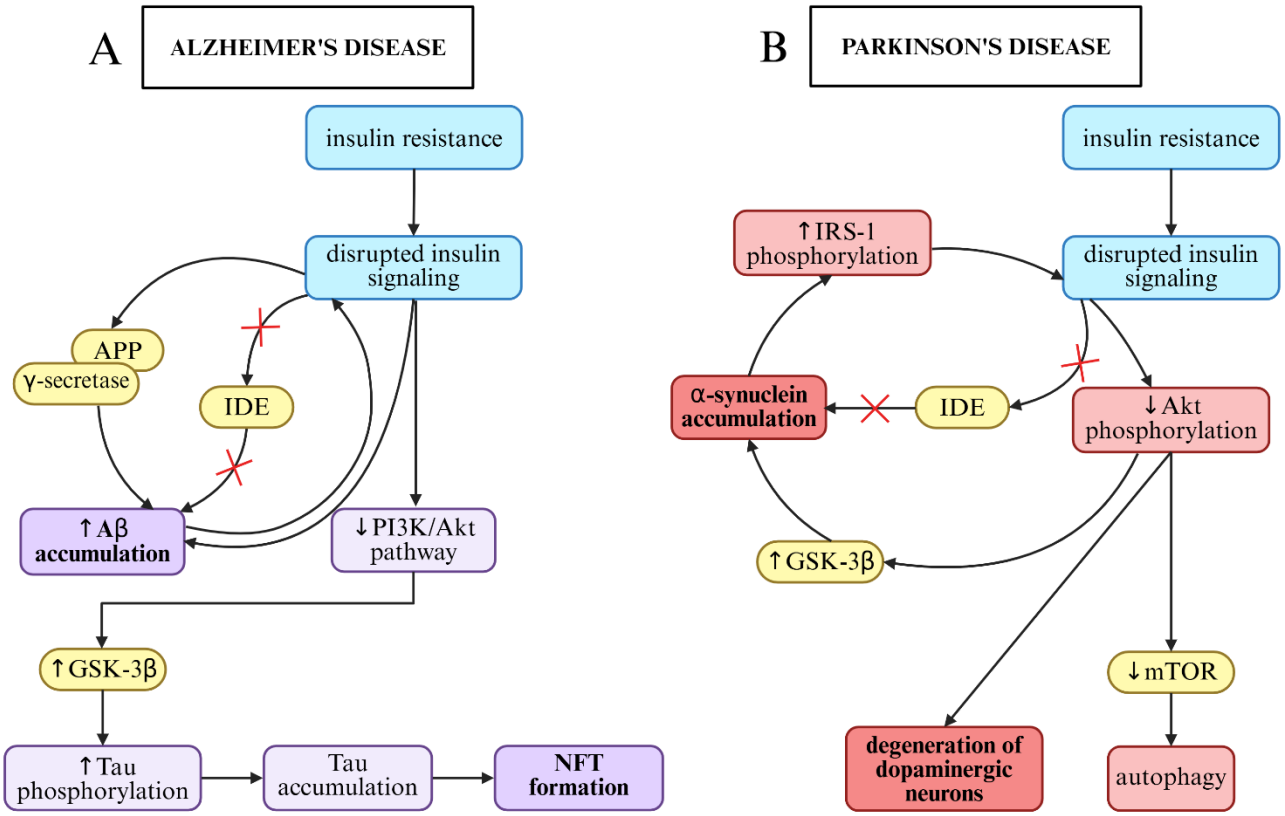

Figure 7. The role of insulin in neurodegenerative disorders. A. In Alzheimer's disease (AD), insulin resistance contributes to disrupted insulin signaling, leading to increased accumulation of amyloid-β (Aβ). Insulin plays a role in regulating amyloid precursor protein (APP) processing, and impaired insulin signaling may contribute to the amyloidogenic pathway involving γ-secretase, further increasing the Aβ production. Under normal conditions, insulin degrading enzyme (IDE) helps clear Aβ by degrading it, however, altered insulin signaling suppresses IDE function, thus worsening Aβ aggregation. Additionally, Aβ accumulation can impair insulin signaling, creating a feedback loop that worsens the condition. Simultaneously, altered insulin signaling disrupts the PI3K/Akt pathway, reducing Akt activation, which normally inhibits glycogen synthase kinase-3β (GSK-3β). Increased GSK-3β activity promotes Tau hyperphosphorylation and neurofibrillary tangle (NFT) formation. **B.** In Parkinson's disease (PD), insulin resistance similarly disrupts insulin signaling, leading to reduced Akt phosphorylation and increased GSK-3β activity. This promotes α-synuclein aggregation, significantly contributing to serine phosphorylation of insulin receptor substrate 1 (IRS-1), impairing its function and disrupting insulin signaling. Suppressed IDE function due to insulin resistance also hinders α-synuclein clearance. Furthermore, reduced Akt activity inhibits the mechanistic target of rapamycin (mTOR), impairing autophagy. Decreased Akt phosphorylation also advances the degeneration of dopaminergic neurons. Created in https://BioRender.com.

8 Conclusion

Insulin signaling pathways are essential for mediating glucose utilization in peripheral tissues and neurons. Under physiological conditions, glucose is either used as an immediate energy source or stored as glycogen in skeletal muscles. In hepatocytes, insulin stimulates glycogenesis while inhibiting HGP. In adipocytes, insulin promotes the conversion of glucose into FAs, which are then stored as TGs, enhancing fat storage and inhibiting lipolysis. These processes are crucial for maintaining glucose homeostasis, and disruptions in insulin signaling significantly contribute to metabolic disorders such as insulin resistance, which can further lead to T2D.

Moreover, insulin and IGFs signaling in the brain deserve greater attention, as they not only mediate neuronal processes but also influence peripheral tissues, where they control several metabolic functions. Neurons rely on both blood-derived insulin and *de novo* insulin production to maintain neuronal health and function.

The roles of insulin and IGFs are clinically significant in AD pathology, where impaired insulin signaling promotes Aβ accumulation and Tau hyperphosphorylation. Similarly, PD models exhibit insulin resistance, which worsens dopaminergic neuron loss and α-synuclein aggregation.

Given the emerging evidence of the role of insulin signaling in both metabolic and neurological health, targeting these pathways presents promising potential for therapeutic strategies. Furthermore, insulin and IGFs administrations may offer protection against metabolic and neurological disorders.

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