REVIEW
Biomedical Engineering Communications 2025;4(2):10. https://doi.org/10.53388/BMEC2025010

Overview of insulin: more than a hormone for controlling glucose level

Izmailova Regina[1, 2], Fang Tong[1, 2*]

[1]Wound Prevention and Treatment Institute, Shanghai University of Medicine & Health Sciences, Shanghai 201318, China. [2]Department of Physiology, School of Fundamental Medicine, Shanghai University of Medicine & Health Sciences, Shanghai 201318, China.

[*]**Correspondence to:** Fang Tong, Department of Physiology, School of Fundamental Medicine, Shanghai University of Medicine & Health Sciences, No. 279, Zhouzhu Highway, Shanghai 201318, China. E-mail: fytongfang@gmail.com.

Author contributions

Regina I: Conceptualization, Writing – original draft preparation, and Visualization. Tong F: Funding acquisition, Conceptualization, Writing – review and editing, and Supervision. All authors have reviewed the manuscript.

Competing interests

The authors declare no conflicts of interest.

Acknowledgments

This project was supported by grants from National Natural Science Foundation of China (Grant No. 82301577).

Peer review information

Biomedical Engineering Communications thanks Yun Xiao, Ning Sun and other anonymous reviewers for their contribution to the peer review of this paper.

Abbreviations

IRS, insulin receptor substrate; SH3, SRC Homology 3; Grb2, growth factor receptor-bound protein 2; Sos, son of Sevenless; PI3K, phosphatidylinositol-3 kinase; PIP3, phosphatidylinositol triphosphate; AKT, activates serine/threonine kinase; EC, endothelial cells; VSMC, vascular smooth muscle cells; eNOS, endothelial nitric oxide synthase.

Citation

Regina I, Tong F. Overview of insulin: more than a hormone for controlling glucose level. *Biomed Eng Commun*. 2025;4(2):10. doi: 10.53388/BMEC2025010.

Executive editor: Jian Jia.

Received: 14 November 2024; **Revised:** 8 January 2025; **Accepted:** 21 January 2025; **Available online:** 24 January 2025.

© 2025 By Author(s). Published by TMR Publishing Group Limited. This is an open access article under the CC-BY license. (https://creativecommons.org/licenses/by/4.0/)

Abstract

Insulin is a peptide hormone secreted by pancreatic β-cells, which plays a key role in regulating glucose metabolism and is the only hormone in the body capable of lowering blood glucose level. The development of insulin preparations has undergone nearly 100 years of history, from early animal insulin extraction to modern synthetic insulin and insulin analogs, which have greatly advanced the treatment of diabetes. The insulin receptor has a wide distribution in the body, and its activation leads to intracellular signaling mainly through two pathways, PI3K/Akt and Ras/MAPK. Clinically, insulin is crucial in the treatment and management of diabetes and its complications, especially in the cases where oral medications fail to control blood glucose. The role of insulin is not limited to the regulation of blood glucose but has a wide range of functions throughout the body, such as regulation of mitochondrial function and metabolism, the promotion of protein synthesis, adipogenesis, and cellular proliferation. However, insulin overdose may lead to severe hypoglycemia, which, if left untreated, poses the risk of irreversible neurological damage or even fatality. In this paper, we review the history of the development of insulin preparations, the molecular structure of insulin, the biological processes initiated by insulin and insulin deficiency/resistance. The overview of side effects from insulin is also included in this review. We assume that future research could focus on refining insulin analogs for greater therapeutic precision, minimizing side effects, and extending benefits beyond glycemic control. Exploring insulin's additional effects may unlock potential applications in treating multiple diseases.

Keywords: insulin; insulin preparation; insulin receptor; biological process of insulin; functions of insulin; insulin deficiency and resistance; side effects of insulin

The well-known functions and applications of insulin

The structure of endogenous insulin

Insulin is a peptide hormone secreted by pancreatic β-cells, which plays an important role in regulating glucose metabolism and is the only glucose-lowering hormone in the body, with a relative molecular mass of 5,807.69. Insulin is produced from the insulin source (proinsulin) by the preprotein convertases 1 and 2 (Proprotenin convertas, pc1, pc2), which cut down the intermediate part, C peptide, of the insulin source within the rough endoplasmic reticulum. The carboxy-terminal part, A-chain, and amino-terminal part, B-chain, of the insulin source are bound together by three disulfide bonds to form insulin.

Synthesis and degradation of insulin

The synthesized mature insulin is stored in secretory vesicles in pancreatic β-cells as a hexamer coordinated with two zinc ions [1]. In response to external stimuli, insulin is released from the secretory vesicles into the bloodstream, where it binds specifically to the insulin receptor (IR) as a monomer and exerts its physiological effects.

Insulin is degraded after its action mainly by insulin degrading enzyme (IDE), whose active site contains zinc ions, which are essential for the catalytic reaction of the enzyme and initiates the degradation process by binding to specific sites of the insulin molecule [2]. It recognizes and binds to a variety of substrates, including insulin and even β-Amyloid (Aβ) [3]. Nevertheless, the mechanism of IDE is still unclear, and it has been hypothesized that the first step of the reaction is a nucleophilic attack on the carbon substrate by the zinc-bound hydroxide root to form the intermediate INT1, in which the bound zinc hydroxide is completely transferred to the carbonyl carbon of the substrate due to the breakage of the Zn^{2+}-OH bond. In TS2, the Glu111 residue rotates to a suitable position and forms two hydrogen bonds with the amide nitrogen and the -OH group attached to the carbon atom of the substrate, thus acting as a hydrogen donor and acceptor at the same time. The formation of the second priming bond facilitates the re-establishment of the Zn^{2+}-OH bond that had been previously broken in the INT1 layer. The nucleophilic addition and protonation of amide nitrogen of the peptide is a very rapid process. Due to the proton transfer of the Glu111 to amide nitrogen of the substrate in TS3, N-C bond of the peptide is broken, and insulin is finally degraded to prodrug (ProD) [4].

Promotion of the conversion of glucose to glycogen

The main mechanism of insulin glucose lowering is to promote glucose phosphorylation and conversion to hepatic glycogen and myoglycogen [5]. At the beginning of the reaction, glucose needs to consume 1 ATP for phosphorylation, generating glucose-6-phosphate (G-6-P), and then catalyzed by phosphoglucose mutase, converted to glucose-1-phosphate (G-1-P), and then through UDP-glucose pyrophosphorylase (UDP-G). Finally, UDP-G is catalyzed by glycogen synthase to polymerize to form glycogen, and -OH- on carbon 1 and H^+ on carbon 4 of glucose are dehydrated and condensed to form a 1,4-glycosidic bond. -glycosidic bond [6]. In turn, glycogen-branching enzyme (GBI) catalyzes the formation of the 1,6-glycosidic bond. In this reaction, glycogenin acts as both primer and priming enzyme [6].

Promotion of the glucose utilization

Glycolysis, the cellular breakdown of glucose into pyruvate and the production of ATP, is a ten-step process in which insulin plays a catalytic role in several key steps [7, 8]. First, insulin activates hexokinase, which phosphorylates glucose to glucose-6-phosphate, initiating glycolysis9. Next, insulin activates phosphofructokinase-1 by increasing the production of fructose-2,6-bisphosphate, accelerating the conversion of fructose-6-phosphate to fructose-1,6-bisphosphate, the rate-limiting step of glycolysis [9]. Finally, insulin up-regulates the activity of pyruvate kinase, which promotes the conversion of phosphoenolpyruvate to pyruvate and the generation of ATP [9]. Through these mechanisms, insulin significantly improves the efficiency of sugar metabolism and promotes cellular energy production.

Clinical applications of insulin

Insulin is the only glucose-lowering hormone in the human body and is widely used in clinical practice. It is mainly used for the treatment of diabetes mellitus, a chronic disease characterized by impaired blood glucose homeostasis, which is becoming increasingly prevalent worldwide. For patients with type 1 diabetes, insulin is the only necessary therapeutic agent, when the pancreas is unable to produce enough insulin [10]. In patients with type 2 diabetes, insulin can be used as an important tool for glycemic management when oral hypoglycemic agents are not effective in controlling blood glucose [11]. In addition, insulin is effective in the management of acute hyperglycemic complications such as diabetic ketoacidosis [12]. Insulin also plays a key role in the treatment of gestational diabetes and other insulin-resistant diseases [13]. Insulins are classified as rapid, intermediate and long-acting insulins based on their rate of action and duration to meet the individualized management of different patients and therapeutic needs.

Development and evolution of insulin preparations

Today, insulin and diabetes are inextricably linked. Diabetes was documented by ancient Greek physicians as early as the first century A.D., while the discovery of insulin was not made until a thousand years later in 1869 by Paul Langelhans, and the link between insulin and diabetes was not discovered until 20 years later in 1889 by Oskar Minkowski and Josef von Merrill, until 1901, when Eugene Opie first proposed that diabetes was due to partial or total pancreatic islet damage caused by damage to the pancreas [14]. This section will introduce the history of insulin discovery and development in detail (Figure 1).

The first generation of insulin: animal-refined insulin

In 1921 Frederick Banting and John Macleod collaborated to extract insulin from the pancreas of dogs for the first time, and successfully applied to clinical treatment, the two also won the Nobel Prize in Physiology and Medicine in 1923 for the discovery of insulin. However, the amino acid sequences and structures of insulin molecules from different races of mammals differ slightly, with porcine insulin being the closest to that of humans, which led to the earliest application of porcine insulin in the treatment of diabetes [15]. However, the sequence of porcine insulin differs from that of human insulin by one to four amino acids, so it is susceptible to immune reactions, leading to atrophy or hyperplasia of subcutaneous fat at the injection site, and is prone to insulin resistance, and in severe cases, even anaphylaxis. In the following decades, the development of molecular biology brings people more understandings on the structure and function of insulin, and in 1955 Frederick Sanger elucidated the amino acid sequence of the insulin molecule for the first time, for which he was awarded the Nobel Prize in Chemistry in 1958. However, insulin preparations at that time still suffered from low purity, unstable yield along with strong side effects, and needed further improvement.

The second generation of insulin: synthetic insulin

In 1965, under the leadership of Academician Wang Yingjian, Chinese scientists Zou Chenglu, Du Yucang, Wang You, Xing Qiyi, Niu Jingyi, Gong Yuting and others were the first to successfully synthesize insulin. Highly pure synthetic human insulin was produced through E. coli bacteria, and its structure is the same as that of insulin secreted by the human body. In 1969, Dr. Dorothy Hodgkin successfully analyzed the crystal structure of insulin for the first time by X-ray crystallography, and in 1971 and 1972, a number of Chinese scientists jointly analyzed the crystal structure of insulin with a resolution of 2.5 Å and 1.8 Å in Beijing, the latter of which was called by Dr. Hodgkin

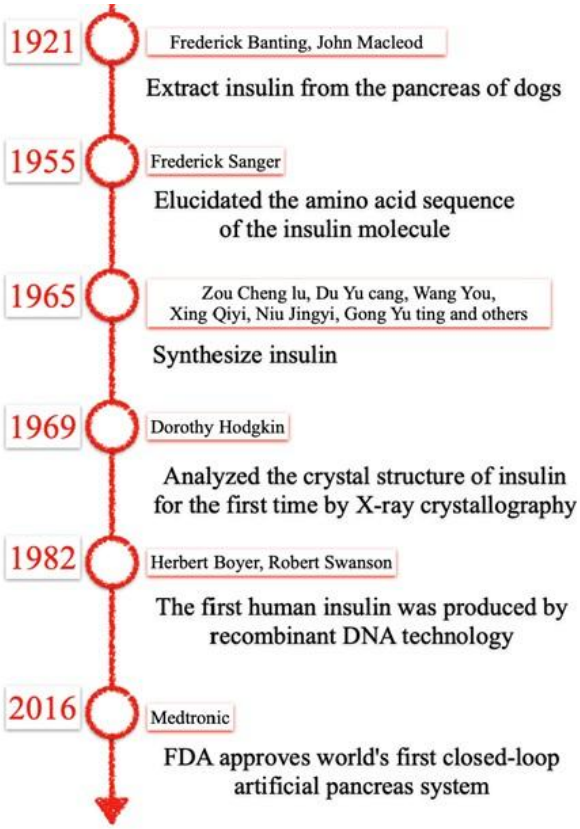


Figure 1 Milestones in the history of insulin discovery and development. (This timeline highlights key achievements in the study of insulin from its discovery to the analysis of its molecular structure. In 1921, Frederick Banting and John Macleod extracted insulin from the pancreas of dogs, marking a pivotal moment in diabetes treatment. In 1955, Frederick Sanger elucidated the amino acid sequence of the insulin molecule, advancing understanding of its structure. In 1965, Chinese scientists, including Zou Chenglu and Du Yucang, successfully synthesized insulin. In 1969, Dorothy Hodgkin analyzed the crystal structure of insulin using X-ray crystallography, providing detailed insights into its molecular configuration. In 1982, the first human insulin was produced by Herbert Boyer and Robert Swanson through recombinant DNA technology. Furthermore, nowadays, the development of insulin analogues, and the development of insulin pumps and automated insulin injection systems.)

to be the most accurate structure of insulin at that time.

Third generation insulin: insulin analogs

In the in-depth study of the structure and composition of human insulin, it is found that the peptide chain of insulin analogs can be modified by using genetic engineering technology, such as changing the amino acid combinations of certain parts of the insulin peptide chain, changing the isoelectric point, increasing the strength of hexamers, replacing zinc ions by cobalt ions, and adding fatty acid chains in the molecule to strengthen the binding with albumin, so that insulin analogs can be more suitable for the physiological needs of the human body.

In 1989, the Declaration of St. Vincent was issued in Italy, which signaled a new stage in the ball's emphasis on diabetes prevention and treatment, and the overall improvement of diabetes management and prevention through the setting of clear goals, the promotion of international cooperation, the enhancement of the quality of life of patients and the facilitation of the efforts of governments in the formulation of policies and the investment of resources [16].

Insulin delivery methods

A closed-loop system is defined as a system that is capable of autonomous regulation, typically comprising sensors, controllers, and actuators. It is distinguished by its capacity to adjust the output of the system by monitoring feedback in real time, thereby achieving automation and precise control [17]. In the context of diabetes management, a closed-loop system reduces manual intervention by monitoring blood glucose levels in real time in conjunction with an insulin pump that automatically delivers insulin [17].

A wearable insulin pump is a device that assists diabetic patients in accurately administering insulin. It possesses two primary characteristics: firstly, it delivers insulin continuously, emulating the natural secretion pattern of the human pancreas and ensuring consistent infusion around the clock by determining the basal rate and meal-time dose [18]; secondly, it is lightweight and portable, with a small size and light weight, facilitating daily use and significantly enhancing the quality of life for patients [18].

The integration of modern wearable insulin pumps with continuous glucose monitoring (CGM) devices, along with advanced control algorithms that utilize artificial intelligence or mathematical modeling to collect blood glucose data, calculate insulin infusion requirements, and automatically adjust the pump's output, has led to the development of an artificial pancreas system [19]. This system represents a significant advancement in diabetes management, as it enables precise regulation of insulin dosing, thereby preventing hypoglycemia caused by excess insulin and maintaining blood glucose levels within a more stable range. Additionally, it reduces the need for manual adjustments to insulin dosage, enhancing convenience and safety [20].

The biological process initiated by insulin

Characteristics of insulin receptors

When insulin activates IRs, it initiates multiple signaling pathways that lead to the production of various key proteins [21]: (1) structural proteins, such as actin and microfilament proteins, which are crucial for maintaining cellular shape and providing mechanical support; (2) enzymes, including glycogen synthase and fatty acid synthase, which play vital roles in energy metabolism and biosynthesis; (3) hormone receptors, like the insulin receptor itself and others, essential for

cellular signaling and regulatory mechanisms within energy metabolism and cellular communication.

Through these mechanisms, insulin not only maintains the normal functions of cells and the structural stability of tissues, but also supports the cell growth, tissue repair and overall metabolic homeostasis. IRs are widely distributed in the peripheral system, including the liver, skeletal muscle, adipose tissue, heart, kidney, and reproductive system, with the main functions of maintaining energy and regulating blood glucose24. In the central nervous system (CNS), IRs are widely distributed in the hypothalamus, hippocampus, cortex and midbrain [22, 23].

Receptor tyrosine kinase signaling of the insulin receptor

Binding of insulin to the IR activates the receptor tyrosine kinase (RTK) signaling pathway. The structure and activation mechanism of the IR is illustrated in Figure 2. First, the activated receptor phosphorylates its own tyrosine, and then one of the phosphorylated tyrosines is recruited through the phosphotyrosine-binding domains (PTB) of the insulin receptor substrate (IRS). An IRS acts as a docking protein, and then the Pleckstrin homology domain (PHD) of the IRS also binds to specific phosphatidylinositols on the inner surface of the plasma membrane. The activated receptor phosphorylates tyrosine fragments of IRS, and one of the phosphorylated tyrosines binds to the SRC Homology 3 domain (SH3) of the adaptor protein growth factor receptor-bound protein 2 (Grb2) [24]. Grb2 uses one of its two SH3 domains to bind to the proline-rich region of a protein called son of Sevenless (Sos), which activates the Ras protein by acting as a downstream signaling guanine nucleotide exchange factor (GEF). Sos also binds to phospholipids in the plasma membrane through its PH domain. grb2 utilizes another SH3 domain to bind to proline-rich sequences in scaffold proteins. The scaffold protein binds to several other signaling proteins, and other phosphotyrosines on the IRS recruit other signaling proteins with SH3 domains [25].

Insulin receptor substrates

IRS can be divided into two subtypes: IRS1 and IRS2, which differ in their distribution and biological functions. IRS1 is highly expressed mainly in muscle and adipose tissues and is involved in insulin-mediated glucose uptake and metabolism [26]. Regulation of insulin sensitivity in peripheral tissues, such as muscle and adipose, is particularly important for glucose uptake and metabolism. Deficiency of IRS1 leads to severe peripheral insulin resistance [27]. It is more directly regulated by insulin signaling and its expression and function are susceptible to nutritional status and insulin levels. IRS2 is highly expressed in the liver, pancreatic β-cells, and CNS, and is primarily involved in the regulation of gluconeogenesis and insulin secretion in the liver [28]. IRS2 regulates the production and release of glucose in the liver, insulin secretion in the pancreatic β-cells, and is involved in brain regulation of energy homeostasis. IRS2 is regulated not only by insulin signaling, but also by other hormones (e.g., growth hormone and glucocorticoids) and nutritional status [29]. Deficiency of IRS2 leads to insufficient insulin secretion and hepatic insulin resistance [30].

Major molecular pathways related to insulin

PI3K/Akt pathway. The PI3K (phosphatidylinositol-3 kinase)/Akt pathway is involved in fundamental cellular processes, including protein synthesis, proliferation and survival [31]. Insulin first binds to the IR on the cell surface, leading to autophosphorylation and activation of the IR. Activated IR recruits and phosphorylates IRS proteins, which in turn recruit and activate PI3K. PI3K catalyzes the generation of cell membrane phospholipids to produce phosphatidylinositol triphosphate (PIP3), and the generation of cell membrane phospholipids to produce PIP3. PIP3 further activates serine/threonine kinase (AKT), which is the core molecule of this signaling pathway and regulates multiple downstream effectors upon activation. The specific process is shown in Figure 3.

Ras/MAPK pathway. The Ras/MAPK pathway is a key signaling pathway that controls cell proliferation, differentiation and death [32]. Insulin binds to cell surface IR and activates Ras, which in turn activates a MAPK module. This three-part module starts with a MAPKKK called Raf, which then activates MAPKK (Mek), which in turn activates MAPK (Erk). Erk in turn phosphorylates a variety of downstream proteins, including other protein kinases and transcriptional regulators in the nucleus. The resulting changes in protein activity and gene expression lead to complex changes in cellular functions. The specific process is shown in Figure 4. It has been posited that insulin may also regulate mitochondrial function and metabolism through activation of MAPK, which plays a role in mitochondrial biosynthesis, oxidative phosphorylation, oxidative stress, and apoptosis, and has synergistic effects on cancer progression and energy homeostasis [33].

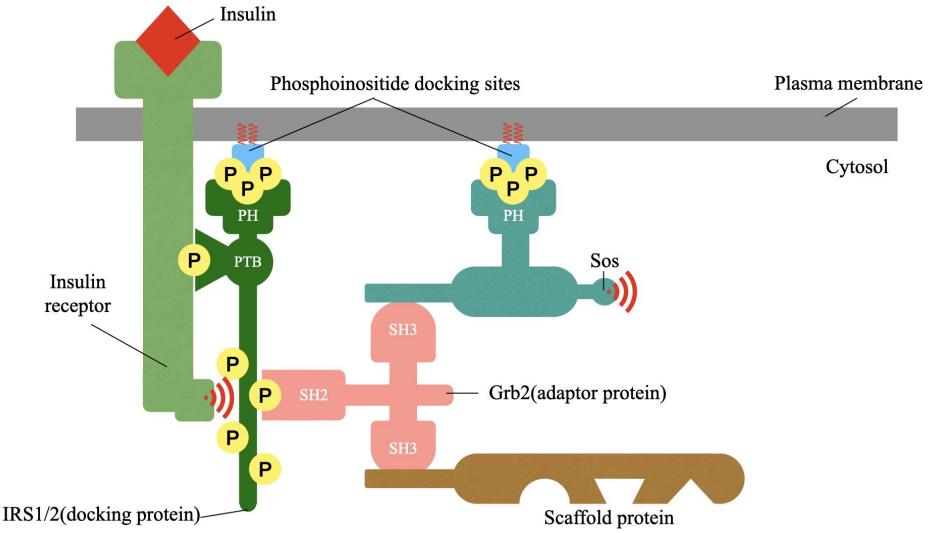


Figure 2 Mechanism of signal transduction through an activated receptor and adaptor proteins. (This diagram illustrates the signal transduction pathway initiated by insulin binding to IR on the cell membrane. The receptor's activation leads to the phosphorylation of docking sites within the cytosolic domain, attracting various adaptor and scaffold proteins. The IRS1/2 docking protein binds via PTB and PH domains to these phosphoinositide docking sites. Grb2, an adaptor protein with SH2 and SH3 domains, links the receptor to Sos, which acts as a guanine nucleotide exchange factor, facilitating downstream signaling. This complex assembly, supported by scaffold proteins, ensures specificity and efficiency in cellular response to external signals.)

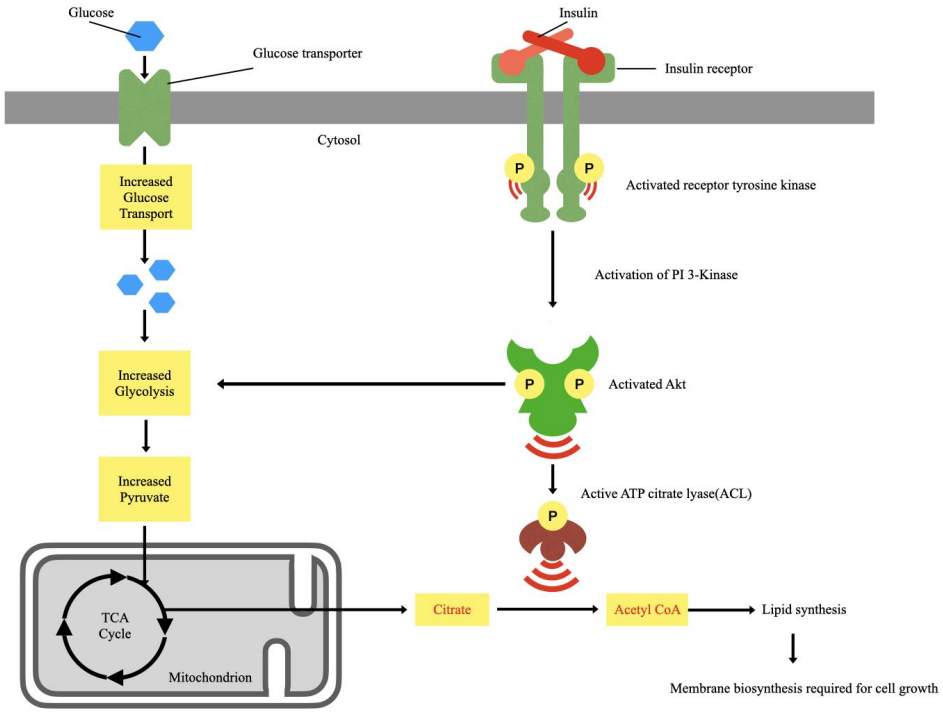


Figure 3 PI3K/Akt pathway mediating glucose uptake and metabolism for cell growth. (This diagram illustrates the PI3K/Akt signaling pathway's role in regulating glucose uptake and metabolism, which supports cell growth. Upon growth factor binding, receptor tyrosine kinases on the cell membrane become activated by insulin, leading to the phosphorylation of PI3K. Activated PI3K then triggers the phosphorylation and activation of Akt, a key downstream signaling protein. Akt activation increases glucose transporter activity on the cell membrane, facilitating glucose entry into the cell. Inside the cell, glucose is metabolized through glycolysis to produce pyruvate, which enters the mitochondria to fuel the TCA cycle, generating citrate. Citrate is then exported to the cytosol, where it is converted to acetyl-CoA, supporting lipid biosynthesis and membrane formation essential for cellular growth and proliferation.)

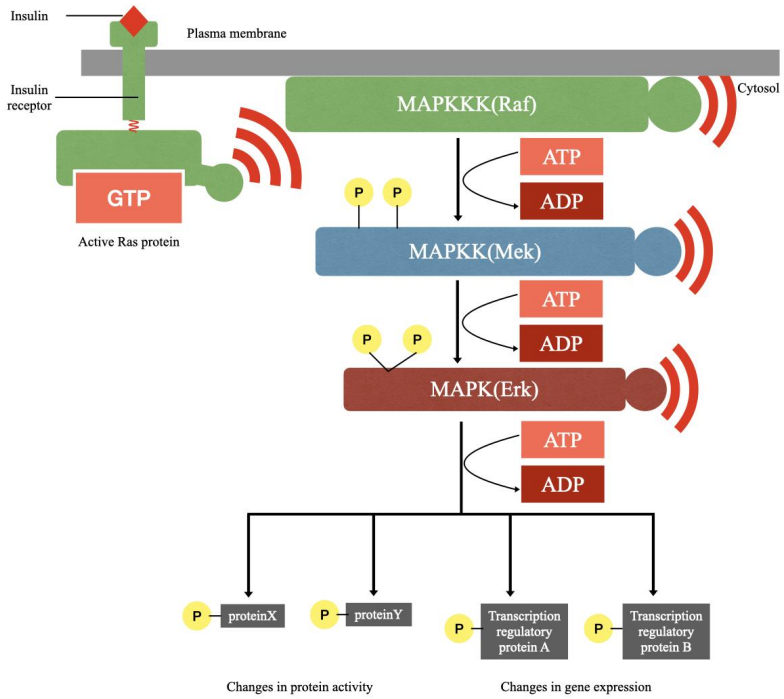


Figure 4 The Ras/MAPK signaling pathway initiated by insulin. (This diagram illustrates the Ras/MAPK signaling pathway from insulin binding with IR, starting with the activation of Ras protein at the plasma membrane through GTP binding. The active Ras protein stimulates Raf, initiating a phosphorylation cascade. Raf phosphorylates Mekusing ATP, converting it to ADP, which then phosphorylates Erk in a similar ATP-dependent manner. Activated Erk can phosphorylate various target proteins, including protein X and protein Y, altering their activity. It also phosphorylates transcription regulatory proteins A and B, leading to changes in gene expression. This pathway mediates cellular responses to extracellular signals, including proliferation, differentiation, and survival.)

Additional functions of insulin and its potential applications in treating multiple diseases

In addition to the well-known function in regulating glucose levels, insulin is also pivotal in maintaining metabolic and physiological homeostasis (Figure 5). A greater understanding of insulin may unlock its innovative applications in treating multiple diseases. This section will review the additional functions of insulin as well as the potential meaning in clinical practice.

Regulation of electrolyte homeostasis

Insulin can also improve the transport efficiency of Na^+/K^+-ATPase. Short-acting regulation is the main form of insulin regulation of Na^+/K^+-ATPase, which is manifested by facilitating the transport of the α1 subunit of Na^+/K^+-ATPase to the plasma membrane and its subsequent internalization into the cytoplasm and alteration of its molecular conformation [34]. This process alters the efficiency of its transport. In addition, Na^+/K^+-ATPase activity may also be regulated by insulin in the long term, i.e., insulin may promote transcription and translation of the α1 subunit of Na^+/K^+-ATPase [34]. Clinical treatment of severe hyperkalemia can utilize insulin mixed with glucose drops to promote the transport of extracellular potassium ions into the intracellular, in order to reduce the concentration of potassium ions.

Cardiovascular protection

The vasculature system includes arteries, veins, capillaries, and the heart muscle. Its primary mission is to distribute nutrients, hormones, cytokines and other signaling molecules between tissues and to remove metabolites. Equally important, the vascular system is capable of producing many of these signaling metabolites, hormones, and cytokines and is profoundly important for many physiological processes through autocrine, paracrine, and hormonal actions [35–39]. A blood vessel consists of endothelial cells (EC), vascular smooth muscle cells (VSMC) and pericytes. The role of insulin in EC and VSMC is focused on metabolism and is vascular-specific. IR signaling in vascular cells occurs mainly through IRS1/2, IRS/PI3K/Akt and Src/ MAPK cascades. Its roles are different in EC and VSMC. Insulin signaling in ECs mainly involves the IRS1/2-PI3K-Akt pathway and the Src-MAPK pathway and upregulates heme oxygenase-1 (HO-1), vascular endothelial growth factor (VEGF), and vascular adhesion molecule-1 (VAMM-1). VCAM-1 and VEGF help enhance the function and stability of ECs, thereby promoting EC proliferation and repair of damaged areas. In VSMC, the PI3K/Akt pathway and the MAPK pathway ultimately lead to NO activation and muscle relaxation, whereas the MAPK/Erk pathway leads to endothelin-1 (ET-1) activation, which results in muscle contraction as well as cell proliferation and migration [40].

Insulin also has anti-atherosclerotic effects, a process that this signal regulates the endothelial nitric oxide synthase (eNOS)/NO signaling pathway in vascular endothelium and cardiac tissues, controlling cardiomyocyte growth, cardiac hypertrophy and heart failure [40, 41]. Activated AKT phosphorylates eNOS to produce NO, which maintains and improves local coronary blood flow and exerts a cardioprotective effect. p-eNOS protein expression increase can stimulate NO cardioprotective effect and reduce myocardial oxidative stress injury [42, 43].

Anti-inflammatory effect

Insulin can inhibit the release of inflammatory cells as well as inflammatory factors, reduce the damage of the vascular wall, and favor the subsequent repair process during inflammation. Insulin demonstrates its anti-inflammatory effect through multiple mechanisms. Firstly, it inhibits inflammatory mediators released by inflammatory cells, such as tumor necrosis factor-alpha (TNF-α), interliukin-1β (IL-1β), and interleukin-6 (IL-6). This inhibition helps reduce the intensity and duration of the inflammatory response [44]. Secondly, insulin further inhibits the progression of the inflammatory response by inhibiting the activation of the NF-κB pathway and decreasing the expression levels of inflammation-related genes (e.g., inflammatory mediators and adhesion molecules) [45–48]. In addition, insulin enhances intracellular antioxidant capacity, promotes the activity of antioxidant enzymes (e.g., superoxide dismutase and glutathione peroxidase), and reduces free radical production and oxidative stress, which contributes to attenuating inflammation-induced cellular damage. Experimental animals infused with TNF-α inhibited insulin-induced forearm glucose uptake and endothelium-dependent vasodilatation, but not sodium nitroprusside-induced blood flow, suggesting that inflammatory cytokines can inhibit insulin-induced NO production and vasodilatation, thereby causing endothelial dysfunction [49–51]. Insulin improves hemodynamics and promotes blood supply and repair of damaged areas by enhancing vasodilatation, regulating NO release and production of vasodilating factors [38].

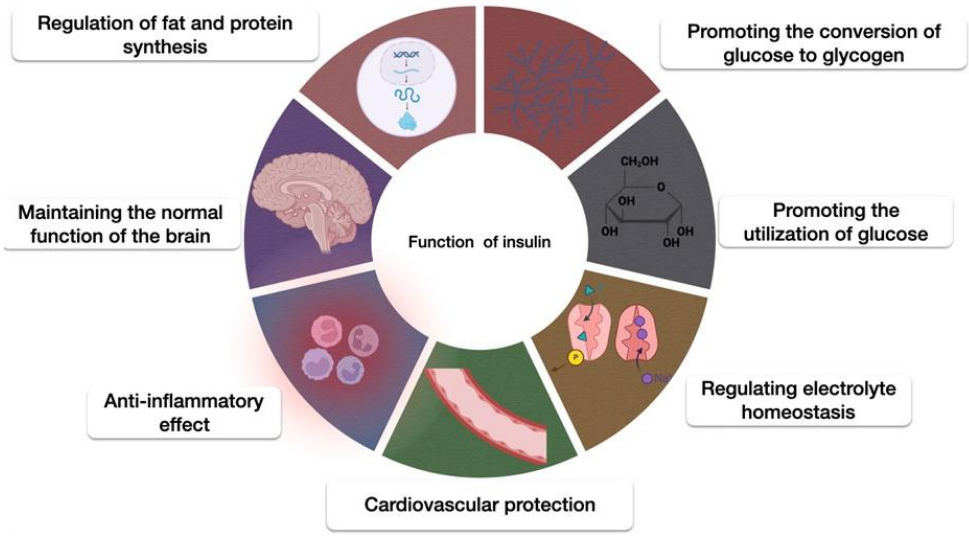


Figure 5 Functions of insulin in maintaining metabolic and physiological homeostasis. (This diagram illustrates the diverse roles of insulin in the body, including promoting the conversion of glucose to glycogen, enhancing glucose utilization, regulating electrolyte homeostasis, providing cardiovascular protection, exerting anti-inflammatory effects, maintaining normal brain function, and regulating fat and protein synthesis. These functions underscore insulin's critical role in energy metabolism and overall health.)

Maintaining the normal function of the brain

Insulin levels in the brain are 10–100 times higher than in plasma. Insulin crosses the blood-brain barrier via the IR as a transporter [52]. Local synthesis by neuronal brain cells in the hippocampus, prefrontal cortex, enteric cortex, hydrosphere, pyramidal neurons of the hypothalamus and amygdala is the second source of insulin in the CNS [53]. A large number of insulin receptors are present in the central temporal lobe, hippocampus, and prefrontal cortex, so that in addition to regulating energy metabolism and energy reserves, it also affects cognition, especially long-term and working memory. The hippocampus has the highest concentration of insulin receptors, glucagon and glutamate [54]. IR is highly expressed in the arcuate nucleus of the hypothalamus, a brain region associated with the regulation of cognition and feeding behavior [55].

The IR signaling pathway improves glucose metabolism and axonal proliferation and differentiation or neuromodulation in adult neurons by increasing mitochondrial membrane potential and ATP inputs and decreasing NAPDH and hexokinase activity [56]. This pathway is involved in the regeneration of small myelinated fibers (neurotropism) as well as the improvement of synaptic plasticity in the brain and the formation of new brain circuits, which are essential for learning, memory formation, consolidation, storage and retrieval. The insulin hormone promotes the release of epinephrine and norepinephrine from adrenergic terminals, which inhibits the synaptic reuptake of norepinephrine, alters catecholamine kinetics, and stimulates neuronal uptake of serotonin [57]. In addition, it modulates the expression of N-methyl-D-aspartate (NMDA) receptors and increases neuronal Ca^{2+} efflux, thereby enhancing synaptic communication between neurons [54].

Regulation of fat and protein synthesis

Insulin promotes adipose synthesis by upregulating PPARγ and CCAAT/enhancer binding protein α (C/EBPα), two major adipogenic transcription factors, via PI3k/Akt/mTORC1. In mature adipocytes, insulin enhances glucose uptake by glucose transporter type 4 (GLUT4) membrane translocation, lipid biosynthesis and lipid degradation is reduced [58, 59]. Inhibition of gluconeogenesis and glycogenolysis by insulin in the liver stimulates lipogenesis and glycogen synthesis [60]. AKT regulates DNL in the liver through activation of SREBP-1c, through mTORC1-dependent and -independent pathways, and inhibition of MAPK. mTORC1 is key to adaptive switching between catabolic and anabolic processes, and its Strict and reciprocal regulation by MAPK and AKT counteracts the Non-Alcoholic Fatty Liver Disease (NAFLD) that accompanies obesity [61].

In skeletal muscle, insulin not only facilitates glucose uptake, but also promotes protein synthesis in concert with amino acids [62, 63]. Insulin-mediated vasodilation can help insulin to access receptors on the surface of muscle cells [64]. Insulin provides the energy and raw materials required for protein synthesis by enhancing the activity of glycogen synthase and fatty acid synthase [65]. In addition, insulin accelerates the rate of protein synthesis by directly affecting the function of ribosomes [66]. Taken together, insulin plays a critical role in cellular function, tissue repair, and overall health by regulating multiple key steps in protein synthesis through several complex intracellular pathways of action.

Insulin deficiency and resistance

Inadequate insulin secretion is a contributing factor to the development of both type 1 and type 2 diabetes. Type 1 diabetes is an autoimmune disease that results in the destruction of insulin-producing pancreatic β-cells [11]. Type 2 diabetes is characterized by the presence of peripheral insulin resistance in tissues such as skeletal muscle, adipose tissue, and the liver. Furthermore, maternal or fetal insulin deficiency exerts a substantial influence on fetal growth and development [67]. In human subjects, maternal insulin deficiency or diabetes has been demonstrated to result in increased fetal macrosomia and obesity. Concurrently, specific fetal hypoinsulinemia has been shown to be associated with intrauterine growth retardation [67].

From a physiological perspective, insulin resistance is characterized by the impaired response of specific tissues to physiological levels of insulin, necessitating higher than normal insulin dosages to maintain optimal function. Consequently, two categories of pharmaceutical agents are currently employed to treat type 2 diabetes: those that stimulate insulin secretion and those that enhance insulin sensitivity.

Skeletal muscle is the primary site of insulin-stimulated glucose consumption, and thus insulin resistance in skeletal muscle has important implications for systemic metabolism [68]. Insulin-stimulated glucose uptake efficiency is decreased due to impaired GLUT4 translocation in skeletal muscle, and the activity of signaling molecules such as IRTK (insulin receptor tyrosine kinase), IRS1, and PI3K/AKT is decreased. Consequently, this may exacerbate systemic fat accumulation and augment the risk of fatty liver disease and obesity [69–71]. Furthermore, the de-repression of FOXO1 activity and enhanced lipolysis contribute to hepatic insulin resistance, leading to inadequate inhibition of hepatic gluconeogenesis and elevated fasting glucose levels [72]. Additionally, this results in diminished stimulation of hepatic glycogen synthesis by insulin.Adipose tissue insulin resistance increases fatty acid influx into the liver, thereby exacerbating insulin signaling disturbances [73, 74].

In the context of insulin resistance, certain insulin signaling pathways maintain sensitivity while others become unresponsive, a phenomenon referred to as selective insulin resistance or pathway-selective insulin response [75]. This condition is marked by a reduction in the inhibitory effect of insulin on hepatic gluconeogenesis, leading to elevated blood glucose levels. The stimulatory effect of insulin on lipogenesis is enhanced, leading to hyperlipidemia and fatty liver [75]. It has been hypothesized that gluconeogenesis requires phosphorylation of the Ser473 site of Akt to activate substrates such as FOXO, but this activation may be impaired in cases of insulin resistance [76]. In contrast, lipogenesis requires phosphorylation of the Thr308 site of Akt, which is less affected by insulin resistance and therefore maintains function [77, 78]. An alternative hypothesis posits that gluconeogenesis inhibition and adipogenesis exhibit divergent insulin sensitivities: gluconeogenesis inhibition necessitates elevated insulin levels, while adipogenesis requires diminished insulin levels [76, 79, 80].

Side effects of insulin

Abnormal cellular proliferation triggered by long-term use of insulin

Insulin has been shown to stimulate abnormal protein synthesis when used in excess or for a prolonged duration [81]. This stimulation can potentially lead to intracellular stress or abnormal tissue proliferation, particularly in cases of insulin resistance, where the effects of insulin may become imbalanced or disturbed [81, 82]. The sustained stimulation of lipogenesis in the liver and adipose tissue by elevated insulin levels has been shown to result in hepatic steatosis, as well as to inhibit the activity of hormone-sensitive lipase (HSL), which in turn impedes lipolysis and contributes to the development of obesity [83]. In cases of selective insulin resistance, the adipogenic pathway exhibits heightened sensitivity to insulin, a phenomenon that can further exacerbate disturbances in fat metabolism. Chronically elevated insulin levels have been associated with an increased risk of tumor growth and a higher incidence of certain cancers. Insulin also inhibits cellular autophagy, which can lead to decreased cellular function and metabolic disorders [82]. Insulin inhibits cellular antioxidant response mechanisms and may increase the accumulation of reactive oxygen species (ROS), leading to elevated oxidative stress, which can impair cellular function and induce inflammation and metabolic disorders [84, 85].

In summary, the long-term use of insulin, whether in high or low doses, can enhance its anabolic effects but may also hinder lipolysis,

autophagy, and antioxidant defense mechanisms, thereby triggering metabolic diseases as well as inflammation and cellular dysfunction.

Insulin overdose and hypoglycemic brain injury

An overdose of insulin can be life-threatening and, in severe cases, can indeed result in death [86]. The first reported case of homicide using insulin as the murder weapon took place in 1958, as detailed in the study by Birkinshaw et al Insulin and Oral Hypoglycemic Drug Overdose in Post-Mortem Investigations: A Literature Review. In this case, the deliberate administration of insulin to cause hypoglycemia, leading to death, was recognized as a potential method of homicide [87]. This case marked the beginning of forensic awareness regarding insulin's misuse as a tool for murder due to the challenges in detection and the difficulty in identifying insulin overdose through postmortem analysis. Homicide by insulin overdose is exceedingly rare, primarily due to the requirement for access to insulin and knowledge of its effects. Most cases involve individuals with medical backgrounds or close relationships with victims, such as healthcare workers or relatives [88]. In recent years, the incidence of reported cases has slightly increased, possibly due to better awareness and forensic techniques, though it remains uncommon. In the field of forensic science, insulin has been regarded as a potential instrument of murder due to its elusive toxicological and pathological effects. Insulin overdose induces severe hypoglycemia, which can be fatal, but is challenging to detect in forensic analysis. The quick degradation of insulin and the need for specific immunohistochemical techniques make it difficult to confirm insulin-related deaths postmortem. Additionally, hypoglycemia symptoms mimic natural medical conditions, making insulin-related homicide cases particularly challenging to investigate.

The primary cause of death from an insulin overdose is hypoglycemia, a critical drop in blood glucose levels [89]. Severe hypoglycemia deprives the brain of its main energy source, leading to neurological symptoms such as confusion, seizures, and eventually, coma or death. This condition, termed as 'hypoglycemic encephalopathy', can cause irreversible damage to the central nervous system, particularly in regions with high glucose demand.

The normal value of human fasting blood glucose is 3.9–6.1 mmol/L, when blood glucose is lower than 2.2 mmol/L, the glucose level in the brain drops to 0 . If not corrected in time, hypoglycemic brain injury will occur. Hypoglycemic brain injury include a series of clinical manifestations, such as unresponsiveness, speech disorders, delirium, loss of consciousness, and even epilepsy [90]. Additionally, hypoglycemic brain injury also involves changes in neurotransmitters, particularly increased glutamate levels, leading to hyperexcitability and excitatory neurotoxicity, a process that is particularly pronounced in the hippocampal region. The combination of oxaloacetate with glutamate generates aspartate, which further exacerbates neurotoxicity. In vitro experiments have shown that glutamate levels rise under hypoglycemic conditions and oxidative stress increases, whereas GABA may have a protective effect. The use of NMDA receptor antagonists attenuated hypoglycemia-induced neuronal necrosis, suggesting that excitatory neurotoxicity plays a key role in hypoglycemic brain injury [91]. The main pathological manifestations of hypoglycemic brain injury are neuronal necrosis in the dentate gyrus (DG) of the hippocampus, CA1 and CA3 regions, as well as astrocyte reaction and severe brain edema [91, 92]. Hypoglycemia-induced water diffusion abnormalities in the brain can be observed by diffusion-weighted imaging, and these abnormalities are reversible after treatment. Therefore, great care should be taken to guard against hypoglycemic brain damage for insulin application in clinical practice to avoid irreversible consequences93. The consequences of hypoglycemia are demonstrated in Figure 6.

Conclusion

The literature review discusses insulin's multifaceted roles beyond glucose control, highlighting its importance in managing diabetes, its molecular evolution, and its physiological effects. Historically, insulin has transformed from animal-derived preparations to advanced synthetic analogs, significantly improving diabetes care. The review emphasizes two major insulin signaling pathways—PI3K/Akt and Ras/MAPK—that regulate metabolism, cellular growth, and differentiation, impacting not only glucose levels but also the cardiovascular, nervous, and immune systems.

Insulin analog development, involving molecular engineering to enhance insulin's stability and physiological compatibility, has enabled more tailored therapeutic solutions. This allows for individualization in diabetes management, particularly in diverse types of diabetes where insulin efficacy and duration are critical. Additionally, insulin's capacity to impact cardiovascular health, electrolyte balance, and brain function reveals its extensive systemic importance.

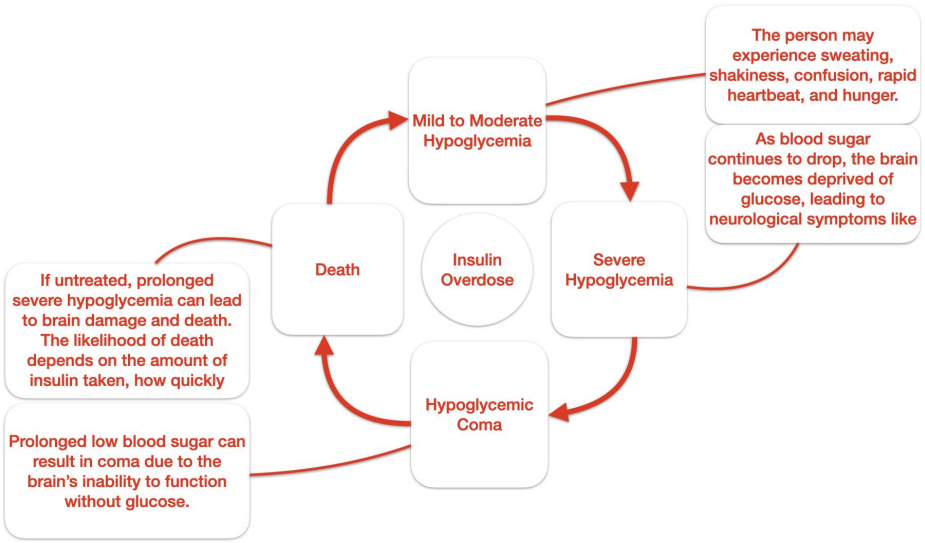


Figure 6 Pathophysiological effects of hypoglycemia induced by insulin overdose. (This flowchart illustrates the progression of hypoglycemic conditions resulting from an insulin overdose. Starting with insulin overdose, individuals may experience mild to moderate hypoglycemia, leading to symptoms like sweating, shakiness, confusion, rapid heartbeat, and hunger. If untreated, hypoglycemia can progress to severe hypoglycemia, manifesting as neurological symptoms such as confusion, seizures, unconsciousness, and potentially coma. Prolonged and untreated severe hypoglycemia can result in hypoglycemic coma, and if left unmanaged, it may ultimately lead to death due to the brain's inability to function without adequate glucose.)

Great attention should be paid to the side effects from insulin. Long-term use of insulin may lead to abnormal cellular proliferation triggering metabolic diseases, inflammation and cellular dysfunction. Hypoglycemia is a dangerous consequence from insulin overdose, resulting in brain injury with serious sequelae and even death.

Future research could focus on refining insulin analogs for greater therapeutic precision, minimizing side effects, and extending benefits beyond glycemic control. Exploring insulin's additional effects may unlock potential applications in treating multiple diseases.

References

1. Deepa Maheshvare M, Raha S, König M, Pal D. A pathway model of glucose-stimulated insulin secretion in the pancreatic β-cell. *Front Endocrinol.* 2023;14:1185656. Available at: http://doi.org/10.3389/fendo.2023.1185656
2. Hulse RE, Ralat LA, Wei-Jen T. Chapter 22 Structure, Function, and Regulation of Insulin-Degrading Enzyme. *Vitamins Hormones.* 2009:635–648. Available at: http://doi.org/10.1016/S0083-6729(08)00622-5
3. Molina-Fernández R, Picón-Pagès P, Barranco-Almohalla A, et al. Differential regulation of insulin signalling by monomeric and oligomeric amyloid beta-peptide. *Brain Commun.* 2022;4(5):fcac243. Available at: http://doi.org/10.1093/braincomms/fcac243
4. Amata O, Marino T, Russo N, Toscano M. Human Insulin-Degrading Enzyme Working Mechanism. *J Am Chem Soc.* 2009;131(41):14804–14811. Available at: http://doi.org/10.1021/ja9037142
5. Santo-Domingo J, Galindo AN, Cominetti O, et al. Glucose-dependent phosphorylation signaling pathways and crosstalk to mitochondrial respiration in insulin secreting cells. *Cell Commun Signal.* 2019;17(1):14. Available at: http://doi.org/10.1186/s12964-019-0326-6
6. Patino SC, Orrick JA. Biochemistry - Glycogenolysis. StatPearls. *StatPearls.* US: National Library of Medicine; 2024. Available at: http://www.ncbi.nlm.nih.gov/books/NBK549820/
7. Granchi C, Bertini S, Macchia M, Minutolo F. Inhibitors of Lactate Dehydrogenase Isoforms and their Therapeutic Potentials. *Curr Med Chem.* 2010;17(7):672–697. Available at: http://doi.org/10.2174/092986710790416263
8. Akram M. Mini-review on Glycolysis and Cancer. *J Cancer Educ.* 2013;28(3):454–457. Available at: http://doi.org/10.1007/s13187-013-0486-9
9. Chandel NS. Glycolysis. *Cold Spring Harb Perspect Biol.* 2021;13(5):a040535. Available at: http://doi.org/10.1101/cshperspect.a040535
10. Syed FZ. Type 1 Diabetes Mellitus. *Ann Intern Med.* 2022;175(3):ITC33–ITC48. Available at: http://doi.org/10.7326/AITC202203150
11. Heller SR, Peyrot M, Oates SK, Taylor AD. Hypoglycemia in patient with type 2 diabetes treated with insulin: it can happen. *BMJ Open Diab Res Care.* 2020;8(1):e001194. Available at: http://doi.org/10.1136/bmjdrc-2020-001194
12. Dhatariya KK, Glaser NS, Codner E, Umpierrez GE. Diabetic ketoacidosis. *Nat Rev Dis Primers.* 2020;6(1):40. Available at: http://doi.org/10.1038/s41572-020-0165-1
13. Sotiriadis A, Chatzakis C, Cavoretto P. Gestational Diabetes Mellitus Pharmacological Prevention and Treatment. *Curr Pharm Des.* 2021;27(36):3833–3840. Available at: http://doi.org/10.2174/1381612827666210125155428
14. Eugene LO. The Relation OE Diabetes Mellitus To Lesions Of The Pancreas. Hyaline Degeneration Of The Islands OE Langerhans. *J Exp Med.* 1901;5(5):527–540. Available at: http://doi.org/10.1084/jem.5.5.527
15. Baker EN, Blundell TL, Cutfield JF. The structure of 2Zn pig insulin crystals at 1. 5 A resolution. *Philos Trans R Soc Lond B Biol Sci.* 1988;319(1195):369–456. Available at: http://doi.org/10.1098/rstb.1988.0058
16. Silver B, Ramaiya K, Andrew SB, et al. EADSG Guidelines: Insulin Therapy in Diabetes. *Diabetes Ther.* 2018;9(2):449–492. Available at: http://doi.org/10.1007/s13300-018-0384-6
17. Templer S. Closed-Loop Insulin Delivery Systems: Past, Present, and Future Directions. *Front Endocrinol.* 2022;13:919942. Available at: http://doi.org/10.3389/fendo.2022.919942
18. Avari P, Lumb A, Flanagan D, et al. Insulin Pumps and Hybrid Close Loop Systems Within Hospital: A Scoping Review and Practical Guidance From the Joint British Diabetes Societies for Inpatient Care. *J Diabetes Sci Technol.* 2022;17(3):625–634. Available at: http://doi.org/10.1177/19322968221137335
19. Rodríguez-Sarmiento DL, León-Vargas F, García-Jaramillo M. Artificial pancreas systems: experiences from concept to commercialisation. *Expert Rev Med Devices.* 2022;19(11):877–894. Available at: http://doi.org/10.1080/17434440.2022.2150546
20. Nwokolo M, Hovorka R. The Artificial Pancreas and Type 1 Diabetes. *JCEM.* 2023;108(7):1614–1623. Available at: http://doi.org/10.1210/clinem/dgad068
21. Saltiel AR, Kahn CR. Insulin signalling and the regulation of glucose and lipid metabolism. *Nature.* 2001;414(6865):799–806. Available at: http://doi.org/10.1038/414799a
22. Boucher J, Kleinridders A, Kahn CR. Insulin Receptor Signaling in Normal and Insulin-Resistant States. *Cold Spring Harb Perspect Biol.* 2014;6(1):a009191. Available at: http://doi.org/10.1101/cshperspect.a009191
23. Ghasemi R, Haeri A, Dargahi L, Mohamed Z, Ahmadiani A. Insulin in the Brain: Sources, Localization and Functions. *Mol Neurobiol.* 2012;47(1):145–171. Available at: http://doi.org/10.1007/s12035-012-8339-9
24. Saltiel AR. Insulin signaling in health and disease. *J Clin Invest.* 2021;131(1):e142241. Available at: http://doi.org/10.1172/JCI142241
25. Le TKC, Dao XD, Nguyen DV, et al. Insulin signaling and its application. *Front Endocrinol.* 2023;14:1226655. Available at: http://doi.org/10.3389/fendo.2023.1226655
26. Brüning JC, Michael MD, Winnay JN, et al. A Muscle-Specific Insulin Receptor Knockout Exhibits Features of the Metabolic Syndrome of NIDDM without Altering Glucose Tolerance. *Mol Cell.* 1998;2(5):559–569. Available at: http://doi.org/10.1016/S1097-2765(00)80155-0
27. Araki E, Lipes MA, Patti M-E, et al. Alternative pathway of insulin signalling in mice with targeted disruption of the IRS-1 gene. *Nature.* 1994;372(6502):186–190. Available at: http://doi.org/10.1038/372186a0
28. Dong XC, Copps KD, Guo S, et al. Inactivation of Hepatic Foxo1 by Insulin Signaling Is Required for Adaptive Nutrient Homeostasis and Endocrine Growth Regulation. *Cell Metab.* 2008;8(1):65–76. Available at: http://doi.org/10.1016/j.cmet.2008.06.006
29. Petersen MC, Shulman GI. Mechanisms of Insulin Action and Insulin Resistance. *Physiol Rev.* 2018;98(4):2133–2223. Available at: http://doi.org/10.1152/physrev.00063.2017
30. Withers DJ, Gutierrez JS, Towery H, et al. Disruption of IRS-2 causes type 2 diabetes in mice. *Nature.* 1998;391(6670):900–904. Available at: http://doi.org/10.1038/36116
31. Schultze SM, Hemmings BA, Niessen M, Tschopp O. PI3K/AKT, MAPK and AMPK signalling: protein kinases in glucose homeostasis. *Expert Rev Mol Med.* 2012;14:e1. Available at: http://doi.org/10.1017/S1462399411002109
32. ZHANG W, LIU HT. MAPK signal pathways in the regulation of cell proliferation in mammalian cells. *Cell Res.* 2002;12(1):9–18. Available at:

http://doi.org/10.1038/sj.cr.7290105
33. Sarg NH, Zaher DM, Abu Jayab NN, Mostafa SH, Ismail HH, Omar HA. The interplay of p38 MAPK signaling and mitochondrial metabolism, a dynamic target in cancer and pathological contexts. *Biochem Pharmacol.* 2024;225:116307. Available at: http://doi.org/10.1016/j.bcp.2024.116307
34. Wen XP, Wan QQ. Regulatory effect of insulin on the structure, function and metabolism of Na^+/K^+-ATPase (Review). *Exp Ther Med.* 2021;22(5):1243. Available at: http://doi.org/10.3892/etm.2021.10678
35. Mather KJ, Steinberg HO, Baron AD. Insulin resistance in the vasculature. *J Clin Invest.* 2013;123(3):1003–1004. Available at: http://doi.org/10.1172/JCI67166
36. King GL, Park K, Li Q. Selective Insulin Resistance and the Development of Cardiovascular Diseases in Diabetes: The 2015 Edwin Bierman Award Lecture. *Diabetes.* 2016;65(6):1462–1471. Available at: http://doi.org/10.2337/db16-0152
37. King GL. The Role of Hyperglycaemia and Hyperinsulinaemia in Causing Vascular Dysfunction in Diabetes. *Ann Med.* 1996;28(5):427–432. Available at: http://doi.org/10.3109/07853899608999103
38. Muniyappa R, Montagnani M, Koh KK, Quon MJ. Cardiovascular Actions of Insulin. *Endocr Rev.* 2007;28(5):463–491. Available at: http://doi.org/10.1210/er.2007-0006
39. Muniyappa R, Quon MJ. Insulin action and insulin resistance in vascular endothelium. *Curr Opin Clin Nutr Metab Care.* 2007;10(4):523–530. Available at: http://doi.org/10.1097/MCO.0b013e32819f8ecd
40. Fu J, Yu MG, Li Q, Park K, King GL. Insulin's actions on vascular tissues: Physiological effects and pathophysiological contributions to vascular complications of diabetes. *Mol Metab.* 2021;52:101236. Available at: http://doi.org/10.1016/j.molmet.2021.101236
41. Ye F, He J, Wu X, et al. The regulatory mechanisms of Yulangsan MHBFC reversing cardiac remodeling in rats based on eNOS-NO signaling pathway. *Biomed Pharmacother.* 2019;117:109141. Available at: http://doi.org/10.1016/j.biopha.2019.109141
42. Taguchi K, Tano I, Kaneko N, Matsumoto T, Kobayashi T. Plant polyphenols Morin and Quercetin rescue nitric oxide production in diabetic mouse aorta through distinct pathways. *Biomed Pharmacother.* 2020;129:110463. Available at: http://doi.org/10.1016/j.biopha.2020.110463
43. Kim TK, Jeon S, Park S, et al. 2′–5′ oligoadenylate synthetase-like 1 (OASL1) protects against atherosclerosis by maintaining endothelial nitric oxide synthase mRNA stability. *Nat Commun.* 2022;13(1):6647. Available at: http://doi.org/10.1038/s41467-022-34433-z
44. Yu T, Gao M, Yang P, et al. Insulin promotes macrophage phenotype transition through PI3K/Akt and PPAR-γ signaling during diabetic wound healing. *J Cell Physiol.* 2018;234(4):4217–4231. Available at: http://doi.org/10.1002/jcp.27185
45. Dandona P, Chaudhuri A, Ghanim H, Mohanty P. Insulin as an Anti-Inflammatory and Antiatherogenic Modulator. *J Am Coll Cardiol.* 2009;53(5):S14–S20. Available at: http://doi.org/10.1016/j.jacc.2008.10.038
46. Chen Q, Yu W, Shi J, et al. Insulin alleviates the inflammatory response and oxidative stress injury in cerebral tissues in septic rats. *J Inflamm.* 2014;11(1):18. Available at: http://doi.org/10.1186/1476-9255-11-18
47. Huang C-T, Lue J-H, Cheng T-H, Tsai Y-J. Glycemic control with insulin attenuates sepsis-associated encephalopathy by inhibiting glial activation via the suppression of the nuclear factor kappa B and mitogen-activated protein kinase signaling pathways in septic rats. *Brain Res.* 2020;1738:146822. Available at: http://doi.org/10.1016/j.brainres.2020.146822
48. Aljada A, Ghanim H, Saadeh R, Dandona P. Insulin Inhibits NF κ B and MCP-1 Expression in Human Aortic Endothelial Cells. *J Clin Endocrinol Metab.* 2001;86(1):450–453. Available at: http://doi.org/10.1210/jcem.86.1.7278
49. Rask-Madsen C, Domínguez H, Ihlemann N, Hermann T, Køber L, Torp-Pedersen C. Tumor Necrosis Factor-α Inhibits Insulin's Stimulating Effect on Glucose Uptake and Endothelium-Dependent Vasodilation in Humans. *Circulation.* 2003;108(15):1815–1821. Available at: http://doi.org/10.1161/01.CIR.0000091406.72832.11
50. Carmeliet P, Jain RK. Molecular mechanisms and clinical applications of angiogenesis. *Nature.* 2011;473(7347):298–307. Available at: http://doi.org/10.1038/nature10144
51. Eelen G, de Zeeuw P, Treps L, Harjes U, Wong BW, Carmeliet P. Endothelial Cell Metabolism. *Physiol Rev.* 2018;98(1):3–58. Available at: http://doi.org/10.1152/physrev.00001.2017
52. Quintana-Pajaro L, Padilla-Zambrano HS, Ramos-Villegas Y, et al. Cerebral traumatic injury and glucose metabolism: a scoping review. *Egypt J Neurosurg.* 2023;38(1):62. Available at: http://doi.org/10.1186/s41984-023-00255-4
53. BlÃizquez E, VelÃizquez E, Hurtado-Carneiro V, Ruiz-Albusac JM. Insulin in the brain: its pathophysiological implications for States related with central insulin resistance, type 2 diabetes and Alzheimer's disease. *Front Endocrinol.* 2014;5:161. Available at: http://doi.org/10.3389/fendo.2014.00161
54. Hamed SA. Brain injury with diabetes mellitus: evidence, mechanisms and treatment implications. *Expert Rev Clin Pharmacol.* 2017;10(4):409–428. Available at: http://doi.org/10.1080/17512433.2017.1293521
55. Milstein JL, Ferris HA. The brain as an insulin-sensitive metabolic organ. *Mol Metab.* 2021;52:101234. Available at: http://doi.org/10.1016/j.molmet.2021.101234
56. Rhea EM, Leclerc M, Yassine HN, et al. State of the Science on Brain Insulin Resistance and Cognitive Decline Due to Alzheimer's Disease. *Aging dis.* 2024;15(4):1688–1725. Available at: http://doi.org/10.14336/AD.2023.0814
57. Duarte AI, Moreira PI, Oliveira CR. Insulin in Central Nervous System: More than Just a Peripheral Hormone. *J Aging Res.* 2012;2012:1–21. Available at: http://doi.org/10.1155/2012/384017
58. Homan EP, Brandão BB, Softic S, et al. Differential roles of FOXO transcription factors on insulin action in brown and white adipose tissue. *J Clin Invest.* 2021;131(19):e143328. Available at: http://doi.org/10.1172/JCI143328
59. Xia W, Pessentheiner AR, Hofer DC, et al. Loss of ABHD15 Impairs the Anti-lipolytic Action of Insulin by Altering PDE3B Stability and Contributes to Insulin Resistance. *Cell Rep.* 2018;23(7):1948–1961. Available at: http://doi.org/10.1016/j.celrep.2018.04.055
60. Molinaro A, Becattini B, Mazzoli A, et al. Insulin-Driven PI3K-AKT Signaling in the Hepatocyte Is Mediated by Redundant PI3Kα and PI3Kβ Activities and Is Promoted by RAS. Cell Metabolism. 2019;29(6):1400–1409. Available at: http://doi.org/10.1016/j.cmet.2019.03.010
61. Chen X, Yu C, Liu X, et al. Intracellular galectin-3 is a lipopolysaccharide sensor that promotes glycolysis through mTORC1 activation. *Nat Commun.* 2022;13(1):7578. Available at: http://doi.org/10.1038/s41467-022-35334-x
62. Mok J, Park TS, Kim S, Kim D, Choi CS, Park J. Prokineticin

receptor 1 ameliorates insulin resistance in skeletal muscle. *FASEB j.* 2020;35(2):e21179. Available at: http://doi.org/10.1096/fj.202001641R
63. Tang D, Chen Q-B, Xin X-L, Aisa H-A. Anti-diabetic effect of three new norditerpenoid alkaloids in vitro and potential mechanism via PI3K/Akt signaling pathway. *Biomed Pharmacother.* 2017;87:145–152. Available at: http://doi.org/10.1016/j.biopha.2016.12.058
64. Sylow L, Tokarz VL, Richter EA, Klip A. The many actions of insulin in skeletal muscle, the paramount tissue determining glycemia. *Cell Metab.* 2021;33(4):758–780. Available at: http://doi.org/10.1016/j.cmet.2021.03.020
65. Bouskila M, Hirshman MF, Jensen J, Goodyear LJ, Sakamoto K. Insulin promotes glycogen synthesis in the absence of GSK3 phosphorylation in skeletal muscle. *Am J Physiol Endocrinol Metab.* 2008;294(1):E28–E35. Available at: http://doi.org/10.1152/ajpendo.00481.2007
66. Proud CG. Regulation of protein synthesis by insulin. *Biochem Soc Trans.* 2006;34(2):213–216. Available at: http://doi.org/10.1042/BST0340213
67. FOWDEN AL. Insulin deficiency: Effects on fetal growth and development. *J Paediatr Child Health.* 1993;29(1):6–11. Available at: http://doi.org/10.1111/j.1440-1754.1993.tb00428.x
68. DeFronzo RA, Tripathy D. Skeletal Muscle Insulin Resistance Is the Primary Defect in Type 2 Diabetes. *Diabetes Care.* 2009;32(suppl_2):S157–163. Available at: http://doi.org/10.2337/dc09-S302
69. Kim JK, Michael MD, Previs SF, et al. Redistribution of substrates to adipose tissue promotes obesity in mice with selective insulin resistance in muscle. *J Clin Invest.* 2000;105(12):1791–1797. Available at: http://doi.org/10.1172/JCI8305
70. Kim JK, Zisman A, Fillmore JJ, et al. Glucose toxicity and the development of diabetes in mice with muscle-specific inactivation of GLUT4. *J Clin Invest.* 2001;108(1):153–160. Available at: http://doi.org/10.1172/JCI10294
71. Le Marchand-Brustel Y, Grémeaux T, Ballotti R, Van Obberghen E. Insulin receptor tyrosine kinase is defective in skeletal muscle of insulin-resistant obese mice. *Nature.* 1985;315(6021):676–679. Available at: http://doi.org/10.1038/315676a0
72. Lewis GF, Carpentier AC, Pereira S, Hahn M, Giacca A. Direct and indirect control of hepatic glucose production by insulin. *Cell Metab.* 2021;33(4):709–720. Available at: http://doi.org/10.1016/j.cmet.2021.03.007
73. Krssak M, Brehm A, Bernroider E, et al. Alterations in Postprandial Hepatic Glycogen Metabolism in Type 2 Diabetes. *Diabetes.* 2004;53(12):3048–3056. Available at: http://doi.org/10.2337/diabetes.53.12.3048
74. Magnusson I, Rothman DL, Katz LD, Shulman RG, Shulman GI. Increased rate of gluconeogenesis in type II diabetes mellitus. A 13C nuclear magnetic resonance study. *J Clin Invest.* 1992;90(4):1323–1327. Available at: http://doi.org/10.1172/JCI115997
75. Wu X, Chen K, Williams KJ. The role of pathway-selective insulin resistance and responsiveness in diabetic dyslipoproteinemia. *Curr Opin Lipidol.* 2012;23(4):334–344. Available at: http://doi.org/10.1097/MOL.0b013e3283544424
76. Li S, Brown MS, Goldstein JL. Bifurcation of insulin signaling pathway in rat liver: mTORC1 required for stimulation of lipogenesis, but not inhibition of gluconeogenesis. *Proc Natl Acad Sci USA.* 2010;107(8):3441–3446. Available at: http://doi.org/10.1073/pnas.0914798107
77. Guertin DA, Stevens DM, Thoreen CC, et al. Ablation in Mice of the mTORC Components raptor, rictor, or mLST8 Reveals that mTORC2 Is Required for Signaling to Akt-FOXO and PKCα, but Not S6K1. *Dev Cell.* 2006;11(6):859–871. Available at: http://doi.org/10.1016/j.devcel.2006.10.007
78. Jacinto E, Facchinetti V, Liu D, et al. SIN1/MIP1 Maintains rictor-mTOR Complex Integrity and Regulates Akt Phosphorylation and Substrate Specificity. *Cell.* 2006;127(1):125–137. Available at: http://doi.org/10.1016/j.cell.2006.08.033
79. Kim K, Qiang L, Hayden MS, Sparling DP, Purcell NH, Pajvani UB. mTORC1-independent Raptor prevents hepatic steatosis by stabilizing PHLPP2. *Nat Commun.* 2016;7(1):10255. Available at: http://doi.org/10.1038/ncomms10255
80. Kim K, Ryu D, Dongiovanni P, et al. Degradation of PHLPP2 by KCTD17, via a Glucagon-Dependent Pathway, Promotes Hepatic Steatosis. *Gastroenterology.* 2017;153(6):1568–1580.e10. Available at: http://doi.org/10.1053/j.gastro.2017.08.039
81. Boden G, Cheung P, Salehi S, et al. Insulin Regulates the Unfolded Protein Response in Human Adipose Tissue. *Diabetes.* 2014;63(3):912–922. Available at: http://doi.org/10.2337/db13-0906
82. Kolb H, Kempf K, Röhling M, Martin S. Insulin: too much of a good thing is bad. *BMC Med.* 2020;18(1):224. Available at: http://doi.org/10.1186/s12916-020-01688-6
83. Kolb H, Stumvoll M, Kramer W, Kempf K, Martin S. Insulin translates unfavourable lifestyle into obesity. *BMC Med.* 2018;16(1):232. Available at: http://doi.org/10.1186/s12916-018-1225-1
84. Yagihashi S. Glucotoxic Mechanisms and Related Therapeutic Approaches. *Int Rev Neurobiol.* 2016:121–149. Available at: http://doi.org/10.1016/bs.irn.2016.03.006
85. Roma LP, Jonas J-C. Nutrient Metabolism, Subcellular Redox State, and Oxidative Stress in Pancreatic Islets and β-Cells. *J Mol Biol.* 2020;432(5):1461–1493. Available at: http://doi.org/10.1016/j.jmb.2019.10.012
86. Tong F, Wu R, Huang W, et al. Forensic aspects of homicides by insulin overdose. *Forensic Sci Int.* 2017;278:9–15. Available at: http://doi.org/10.1016/j.forsciint.2017.06.015
87. Manetti AC, Visi G, Spina F, et al. Insulin and Oral Hypoglycemic Drug Overdose in Post-Mortem Investigations: A Literature Review. *Biomedicines.* 2022;10(11):2823. Available at: http://doi.org/10.3390/biomedicines10112823
88. Rzepczyk S, Dolińska-Kaczmarek K, Uruska A, Żaba C. The Other Face of Insulin—Overdose and Its Effects. *Toxics.* 2022;10(3):123. Available at: http://doi.org/10.3390/toxics10030123
89. Hasebe M, Shibue K, Honjo S, Hamasaki A. Hypoglycemic encephalopathy. *QJM.* 2022;115(7):478–479. Available at: http://doi.org/10.1093/qjmed/hcac125
90. Tong F, Yang Y, Liang Y, et al. Advances in Neuropathologic Research of Hypoglycemic Brain Damage Caused by Insulin Overdose. *Fa Yi Xue Za Zhi.* 2020;36(1):99–103. Available at: http://doi.org/10.12116/j.issn.1004-5619.2020.01.019
91. Tong F, Liang Y, Shi Q, Zhang L, L WH, Zhou YW. Advance of Forensic Research in Insulin Poisoning. *Fa Yi Xue Za Zhi.* 2017;33(1):48–51. Available at: http://doi.org/10.3969/j.issn.1004-5619.2017.01.012
92. Tong F, Zou Y, Liang Y, et al. The Water Diffusion of Brain Following Hypoglycemia in Rats – A Study with Diffusion Weighted Imaging and Neuropathologic Analysis. *Neuroscience.* 2019;409:58–68. Available at: http://doi.org/10.1016/j.neuroscience.2019.04.036