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CHEMISTRY OF PANCREATIC POLYPEPTIDE HORMONE WITH OFFICIAL PREPARATION

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ABSTRACT

Insulin which is a peptide hormone is produced by β–cells of the pancreatic islets and it is considered to be the main anabolic hormone of the body. It further regulates the metabolism of carbohydrates, fats and protein by promoting the absorption of glucose from the blood into liver, fat and skeletal muscle cells. In these tissues the absorbed glucose from the blood is thus converted into either glycogen via glycogenesis or fats (triglycerides) via lipogenesis, or, in the case of the liver both glycogenesis and lipogenesis. Glucose production and secretion by the liver is strongly supported by high concentrations of insulin in the blood. Circulating insulin on the other hand also affects the synthesis of proteins in a wide variety of tissues. It is thus an anabolic hormone which promotes the conversion of small molecules in the circulating blood into large molecules inside the cells. Conversely low insulin levels in the blood have the opposite effect that promotes widespread catabolism, especially of reserve body fat. β–cells of the pancreatic islets are specifically sensitive to blood sugar levels thus they secrete insulin into the circulating blood in response to high level of glucose; and conversely inhibits the secretion of insulin when blood glucose levels are low. Insulin is responsible for glucose uptake and metabolism in the cells, therefore reducing the blood sugar level. The α– cells which are present near the β–cells of the pancreatic islets, takes their cues from the β–cells and secretes glucagon into the blood in just the opposite manner i.e. increased glucagon secretion when blood glucose is low, and decreased glucagon secretion when glucose concentrations are high. Glucagon thus increases blood glucose level by stimulating glycogenolysis and gluconeogenesis in the liver. The secretion of insulin and glucagon into the circulating blood in respective response to the glucose concentrations in the blood is termed as glucose homeostasis. Insulin was the first peptide hormone discovered. Frederick Banting and Charles Herbert Best, working in the laboratory of J.J.R. Macleod at the University of Toronto, were the first to isolate insulin from dog pancreas in 1921. Frederick Sanger sequenced the amino acid structure in 1951, which made insulin the first protein to be fully sequenced. The crystal structure of insulin in the solid state was determined by Dorothy Hodgkin in 1969. Insulin is also the first protein to be chemically synthesized and produced by DNA recombinant technology. It is on the WHO Model List of Essential Medicines, the most important medications needed in a basic health system.

KEYWORDS: α-cells, β-cells, Islets of Langerhans, Insulin, Glucagon, Polypeptide, Disulfide bonds, Lente Insulin, Semi Lente Insulin, Glucogenesis, Glycogenesis, Type-1 diabetes, Type-2 diabetes.

INTRODUCTION

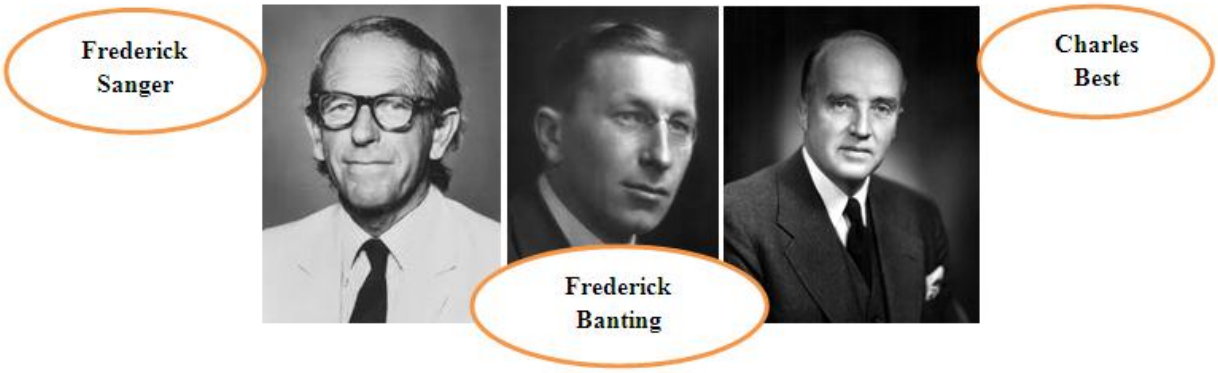
Physiological insulin consists of two constituents they are:

1. Basal (a relatively constant background level of insulin during the fasting and post absorptive period) and
2. Bolus (prandial spikes of insulin after eating)

These two are the basis of administration of two different types of commercially available insulins. The main objective of insulin therapy in diabetes is to accomplish permanent blood glucose management by simulating normal insulin secretion from the pancreas.

Long–acting insulins supply basal insulin, on the other hand short–acting ones provide postprandial requirements. Elevation of postprandial blood glucose

level results to notably elevation of glycated hemoglobin (HbA1C) values and resulting long term complications of diabetes. Therefore, postprandial regulation of blood glucose is indispensable for optimum diabetes disease management. Test results vary by age and are usually measured in milligrams per deciliter (mg/dL). Normal results for the two–hour postprandial test based on age are: For those who do not have diabetes: less than 140 mg/dL. For those who have diabetes: less than 180 mg/dL.[1]



Figure–1: Frederick Sanger (Nobel Laureate), Frederick Banting (Nobel Laureate) and Charles Best (Medical Scientist); the scientists in Insulin discovery.

In 1921, insulin was first given to a diabetic dog, and thus was paving the way for human insulin therapy. All insulin preparations are currently generated by recombinant DNA technology. Doses and concentration levels of insulin preparations used in clinics are indicated by international units. Indeed, one international unit of insulin is defined as the bioequivalent of 34.7µg of crystalline insulin. Insulin preparations consist of the amino acid sequence of human insulin or variations thereof (insulin analogues). Eli Lilly and Sanofi used a non–pathogenic strain of *Escherichia coli* to synthesize insulin; whereas Novo Nordisk uses *Saccharomyces cerevisiae*, or bakers' yeast.[2]

Figure–2: Insulin I.P.

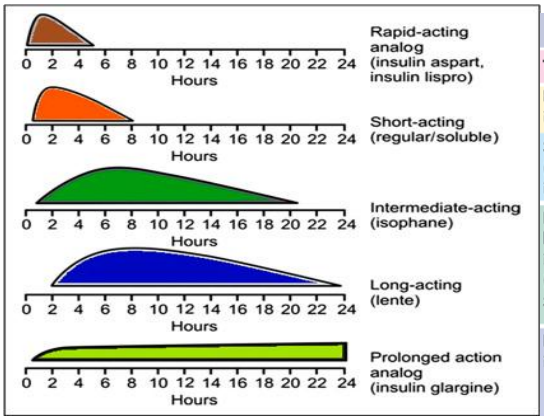
Official Preparation: The human insulin protein is made up of 51 amino acids, with a molecular mass of 5808 Da. It is a heterodimer of two chains i.e. an A–chain and a B–chain, which are linked together by disulfide bonds. Insulin's structure varies slightly between different species of animals. Insulin from animal sources differs somewhat in effectiveness (in carbohydrate metabolism effects) from human insulin because of these variations. Porcine insulin is especially somewhat close to the human version, and was widely used to treat type–1 diabetic before human insulin could be produced in large quantities by recombinant DNA technologies.

Short–acting Insulin Preparations: The regular human insulin and the rapidly acting insulin analogues are considered as short–acting formulations. Usually they are clear solutions at neutral pH and have minor amounts of zinc (for greater stability and shelf life). The insulin molecules usually exist as dimers which joins together to form hexamers. Thus, when two zinc ions are present in the formulation and the formed hexamers further typically exhibit elevated stability in the presence of phenolic compounds such as phenol and meta–cresol.

Pharmacokinetic properties of different types of short–acting insulins are summarized in **Table–1**. Various clinical studies have indicated that the right time for pre–prandial administration of regular human insulin and rapid–acting insulin are 45 and 15 min before food, respectively. In general, the quick onset of action is considered ideal as the patient can begin his food immediately after administering the dose.[3] For healthy adults, normal pre–prandial glucose levels are 70–99

mg/dl. A pre–prandial glucose reading of 100–125 mg/dl indicates prediabetes.

The storage condition of the short–acting insulin preparations are given in **Table–2**.



Main types of insulin preparations

Type	Onset	Peak	Duration	Comments
Rapid-acting insulin analogue	5-15 min	30-60 min	2-5 hr	Can be injected at the start of a meal
Short-acting (soluble/regular insulin)	30 min	1-3 hr	4-8 hr	Usually injected 15-30 minutes before a meal. Clear solution
Intermediate or long-acting insulin (isophane or zinc insulin)	1-2 hr (NPH, Lente) 2-3 hr (Ultralente)	4-8 hr 4-8 hr	8-12 hr (NPH) 8-24 hr (Ultralente)	Used to control glucose levels between meals. May be combined with short-acting insulin
Long-acting insulin analogue	30-60 min	No peak	16-24 hr	Usually taken once daily

Figure–3: Slow and Rapid action of Insulin.

Long–acting Insulin Preparation NPH: NPH is an equivalent mixture of protamine with native insulin in water for injection adjusted to pH 7.1–7.4 with phosphate buffer. It is an opaque suspension consisting of rod–shaped insulin crystals with particle size <30µm. The preparation should be free of large aggregates followed by moderate shaking for consistent absorption. The expiry date of the formulation should not be more than 24–36months from the date of manufacturing. In general, the absorption of NPH insulin is quick from abdominal fat, relatively slow from posterior upper arms and lateral thigh area, and slowest from superior buttocks area. Patients should be cautious for initial signs of frosting or clumping of this insulin, as it shows a noticeable loss of potency. This form of insulin must not be administered through intravenous route. Since the active ingredient of this insulin is in the precipitate state, vial should be gently agitated and mixed thoroughly to assure a uniform mixture for accurate measurement of each dose. This is usually done by slowly swirling or carefully shaking or inverting the vial several times before withdrawal of each insulin dose. Vigorous shaking of the insulin should be avoided because this results in formation of foam, which disrupts the accurate measurement of the dose. To prevent clogging at the tip of the needle, NPH should be injected rapidly (<5sec) subcutaneous.[4] Thawing refrigerated insulin to room temperature prior to use will decrease irritation at the injection site. The storage condition of the long–acting insulin preparations are given in **Table–2**.

Unopened vials of suspensions and prefilled syringes should be refrigerated between 2–8°C and should not be subjected to freezing (<2°C) or exposed to heat (>30°C) or sunlight. Pharmacokinetic properties of long–acting insulins are summarized in **Table–1**. Pharmacokinetic properties of different insulin preparations are summarized in the following Table. There is great variability in the time of onset of action, peak effect, and duration of action of insulin amongst individuals and even with repeated doses in the same individual depending on the dose size, the injection site, the exercise, the desire of circulating anti–insulin antibodies.[4]

Table–1: Pharmacokinetic properties of insulin preparations.

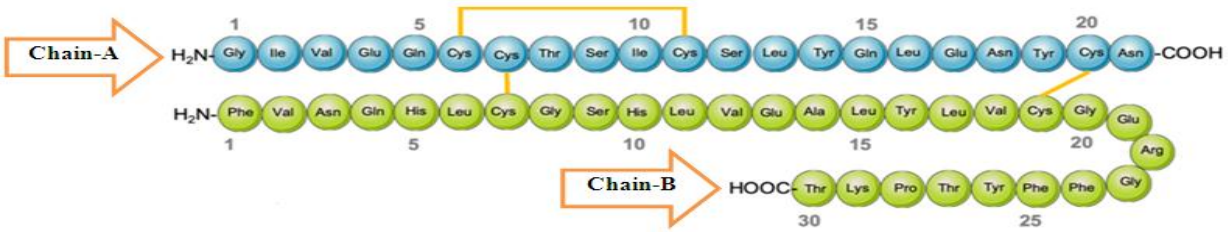
Insulin Preparation	Onset of Action (H)	Peak Effect (H)	Duration of Action (H)
Short–Acting (clear)			
Regular insulin	0.5–1	1–3	6–10
Insulin Lispro	0.25–0.5	0.5–1.0	3.5
Insulin Aspart	0.17–0.33	0.5–1.0	4–5.3
InslinGlulisine	0.25–0.41	0.5–1.0	4–6.3
TechnosphereInslin	<0.25	0.5–1.5	2–3
Long–acting (clear)			
NPH	1–2	6–14	16–24
Insulin Detemir	3–4	6–8	6–24
InslinGlargine	1–2	Flat	24
InslinDegludec	1–2	Flat	42
Mixtures (cloudy)			
Isophane/regular Insulin 70/30,50/50	0.5–1	2–12	8–24
NPL/Lispro mix 75/25	5 min	7–12	1–24

Table–2: Storage condition for insulin preparation.

Insulin preparation	Storage Condition		
	Unopened (RT below 30°C)	Unopened (refrigerated)	Opened (RT below 30°C)
Regular Insulin			
10 mL/3mL vial	42days	Until expiry date	42days
Insulin Lispro			
10 mL/3mL vial	28days	Until expiry date	28days / refrigerated or RT
3 mL cartridge, 3mL kwik pen prefilled (100/200unit/mL)	28days	Until expiry date	28days RT (do not refrigerate)
Insulin Aspart			
10mL	28days	Until expiry date	28days/refrigerated or RT
3mL cartridge, 3mL Novolog®, Flexpen® prefilled, Novolog®penfill®	28days	Until expiry date	28days RT (Do not refrigerate)
Insulin Glulisine			
10 mL vials 3 mL solostart® prefilled pen	28days 28days	Until expiry date Until expiry date	28days/refrigerated or RT 28days RT (Do not refrigerate)
Insulin Glusine			
Technosphere insulin (foil package)	10days	30days	3days
NPH			
10mL/3mL vial	31days	Until expiry date	31days/refrigerated or RT
3mL pen	14days	Until expiry date	14days RT (Do not refrigerate)
NPH and Regular Insulin			
10mL/3mL vial	31days	Until expiry date	31days/refrigerated or RT
3mL pen, 3mL Humulin N pen prefilled–kwik pen	14days	Until expiry date	14days RT (Do not refrigerate)
Insulin Detemir			
10mL/3mL vial	42days	Until expiry date	42days/refrigerated or RT
3mL Flex pen	42days	Until expiry date	42days RT (Do not refrigerate)
Insulin Glargine			
10mL	28days	Until expiry date	28days/refrigerated or RT
3mL prefilled soloSTAR® Pen	28days	Until expiry date	28days RT (Do not refrigerate)
Insulin Degludec			
3mL TRESIBA®, U–100 Flex–Touch 3 Ml pre–filled pen, 3 mL TRESIBA® U–200 Flex–Touch®, 3 mL kwikpen (pre–filled))	31days 31 days 10days	Until expiry date Until expiry date Until expiry date	31days/refrigerated or RT 31days/refrigerated or RT 10days RT (Do not refrigerate)

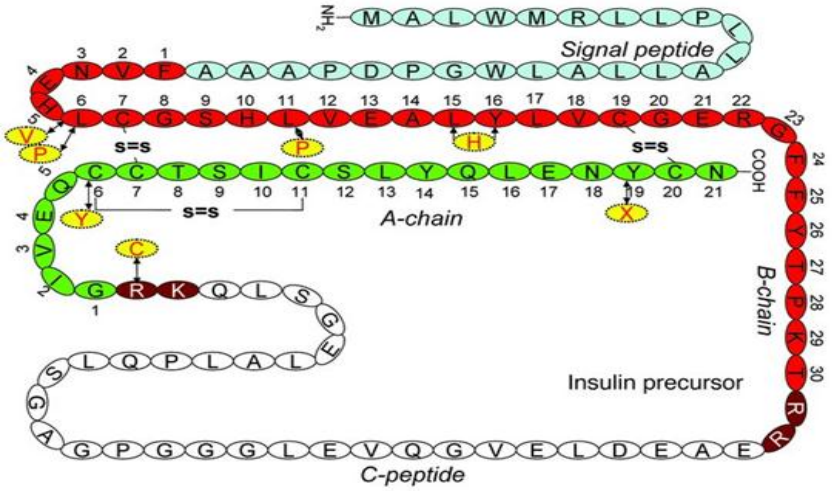
Chemical Nature: Insulin occurs in well–defined crystals seldom exceeding 0.01mm in diameter and falling crystallographically into two distinct groups:

1. First, crystals with well–defined double refraction, of negative character, with several habits, in the rhombohedral class, the habits as a rule differing in the various lots of crystals examined.
2. Secondly, crystals showing a more equant habit, oftentimes with clearly defined crystal edges and no double refraction.



Figure–4: Structure of Insulin.

Contrary to an initial belief that hormones would be generally small chemical molecules, as the first peptide hormone known of its structure, insulin was found to be quite large. A single protein (monomer) of human insulin is composed of 51 amino acids, and has a molecular mass of 5808 Da. The molecular formula of human insulin is $C_{257}H_{383}N_{65}O_{77}S_6$. It is a combination of two peptide chains (dimer) named an A–chain and a B–chain, which are linked together by two disulfide bonds. The A–chain is composed of 21 amino acids, while the B–chain consists of 30 residues. The linking (interchain) disulfide bonds are formed at cysteine residues between the positions A7–B7 and A20–B19. There is an additional (intrachain) disulfide bond within the A–chain between cysteine residues at positions A4 and A11. The A–chain exhibits two α–helical regions at A1–A8 and A12–A19 which are antiparallel; while the B chain has a central α–helix (covering residues B9–B19) flanked by the disulfide bond on either sides and two β–sheets (covering B7–B10 and B20–B23).



Figure–5: Structure of Pro–insulin.

Proinsulin is made up of 81 residues (in cows, 86 in humans), and formed by three distinct chains. The A chain, B chain, and the area connecting the two named the C peptide. The correct structure of proinsulin is crucial for the correct folding of mature insulin, as the placement of the C peptide sets the molecule up to create correctly positioned disulfide bonds in and between the A and B chains. There are three disulfide bonds that are necessary for mature insulin to be the correct structure. Two of these disulfide bonds are between the A and B chains, and one is an intra–A chain bond. The disulfide bonds occur between the seventh residues of the A and B chain, the 19th residue of the A chain and the 20th residue of the B chain, and the 6th and 11th residues of the A chain. The C peptide is between the A and B chains of proinsulin. The connection between the A chain and C peptide is much more stable than the junction between the C peptide and B chain, with alpha helical features being exhibited near the C peptide–A chain connection. The C peptide–A chain junction occurs between residues 64 and 65 of pro–insulin. These are lysine and arginine molecules, respectively. The C peptide–B chain connection is between two arginine residues at positions 31 and 32 of proinsulin. In as much as the investigation of many lots of crystals, consisting mainly of one type or the other, has shown no difference in chemical composition or in physiological activity, the facts submitted suggest that insulin is dimorphous.

Solutions of crystalline insulin are levorotatory, as are those of all proteins, the specific rotation depending to a great extent upon the pH of the solution.[5]

1. The rotation of an approximately 2.5% solution of crystalline insulin in N/10 hydrochloric acid is –29.9ºC.
2. In acetic acid solution the rotation does not change during the course of a day, while in N/10 hydrochloric acid a rise from –29.9ºC to –31.7ºC was observed over a period of one week.
3. Alkaline solutions exhibit stronger levorotation than do acid solutions; the initial value for (cx)~ºC in 1 N ammonia is –49ºC, and for in N/l0 sodium hydroxide –80°C (2.5% solution).
4. In ammonia the rotation slowly rises in the course of 48hours and then remains practically constant.
5. In sodium hydroxide, after a very short rise, it sinks rapidly and then after 30hours reaches a value at which it is unchanged for days.

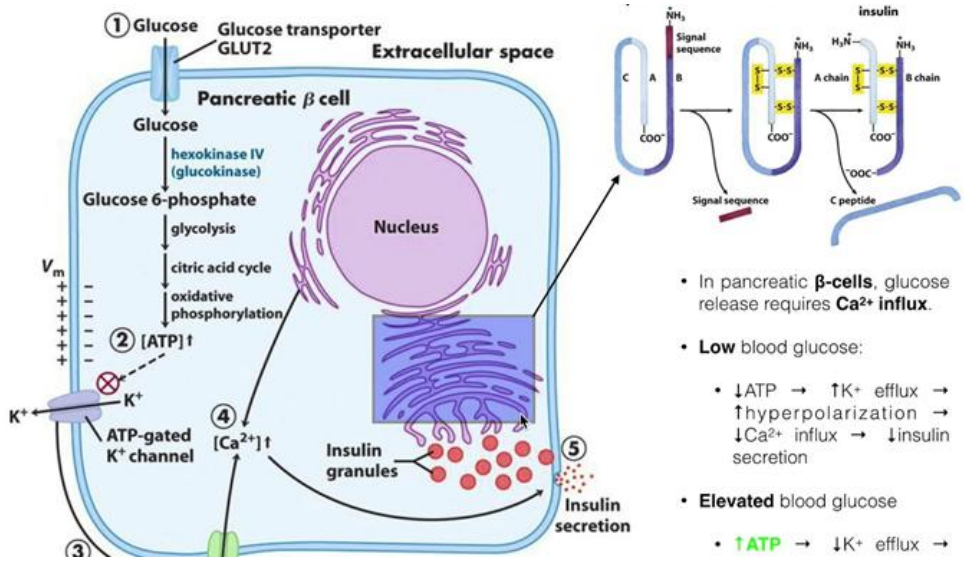
Freudenberg and his co–workers have obtained values similar to these. The optical absorption of insulin in ultra–violet light has been measured up to a frequency of 2000ºA, an absorption band lying around 2800°A being ascribed to the cystine and tyrosine content. From the action of various reagents on the spectrum of insulin, Freudenberg and his co–workers conclude that while insulin can be inactivated without affecting the absorption spectrum, alteration of the absorption spectrum is always associated with inactivation. Ultra–violet light of various wave lengths inactivates the

hormone. On irradiation with long wave lengths corresponding to the tyrosine absorption bands, inactivation follows quickly and almost completely.[6]

The x–ray crystal diffraction patterns of insulin have been investigated by various workers. These studies give little information as to the structure of the molecule, the patterns are similar to those obtained with other crystalline proteins.

If insulin is heated in a melting point tube the crystals begin to brown at 215°C and melt rather sharply at 233°C with decomposition. Insulin exhibits the solubility of a typical protein, dissolving easily in dilute acid and alkali as well as in 90% phenol. It is somewhat soluble in 80% alcohol but not at all in organic solvents which are water free. Quantitative studies have been made of the solubility of the hormone in N/30 acetate buffers over a pH range of 4.8–6.5, and in salt solutions of varying concentration. Estimations of the molecular weight of crystalline insulin have been made in various laboratories. On purely chemical evidence Freudenberg estimates the molecule to have a weight of approximately 20,000, while the ultracentrifuge method of Svedberg places insulin in the group of proteins (egg albumin, Bence Jones protein) having the so–called unit weight of 35,100. Gerlough states, although the data have as yet not been published, that values of 40,000 to 50,000 are obtained by viscosity measurements. With reference to chemical properties, crystalline insulin exhibits the reactions of a typical protein. It is precipitated from solution by the usual protein precipitants and is denatured by strong acid or by boiling.

The following colour reactions are positive: **Biuret, Millon, Pauly, Ninhydrin, Xanthoproteic**. Further, the more specific reactions of Sekiguchi for arginine, of Folin–Looney for the disulphide linkage and tyrosine, and of Sullivan for cystine are positive. The reactions for tryptophan (Voisonet, Hopkins–Cole, and Acree), for sulfhydryl groups (sodium nitroprusside), and the Molisch test for carbohydrate groups are all negative. Moreover, the elementary composition of insulin is similar to that of the average protein, with the exception, perhaps, of the comparatively high sulphur content.



Figure–6: Biosynthesis of Insulin.

Biosynthesis: Insulin is produced in the pancreas and the Brockmann body (in some fish), and released when any of several stimuli are detected. These stimuli include the rise in plasma concentrations of amino acids and glucose resulting from the digestion of food. Carbohydrates can be polymers of simple sugars or the simple sugars themselves. If the carbohydrates include glucose, then that glucose will be absorbed into the bloodstream and blood glucose level will begin to rise. In target cells, insulin initiates a signal transduction, which has the effect of increasing glucose uptake and storage. Finally, insulin is degraded, terminating the response. In mammals, insulin is synthesized in the pancreas within the β cells. One million to three million pancreatic islets form the endocrine part of the pancreas, which is primarily an exocrine gland. The endocrine portion accounts for only 2% of the total mass of the pancreas. Within the pancreatic islets, β cells constitute 65–80% of all the cells.[7]

Insulin consists of two polypeptide chains, the A– and B– chains, linked together by disulfide bonds. It is however first synthesized as a single polypeptide called pre–proinsulin in β–cells. Pre–proinsulin contains a 24–residue signal peptide which directs the nascent polypeptide chain to the rough endoplasmic reticulum (RER). The signal peptide is cleaved as the polypeptide is translocated into lumen of the RER, forming proinsulin. In the RER the proinsulin folds into the correct conformation and 3 disulfide bonds are formed. About 5–10min after its assembly in the endoplasmic reticulum, proinsulin is transported to the trans–Golgi

network (TGN) where immature granules are formed. Transport to the TGN may take about 30 min.

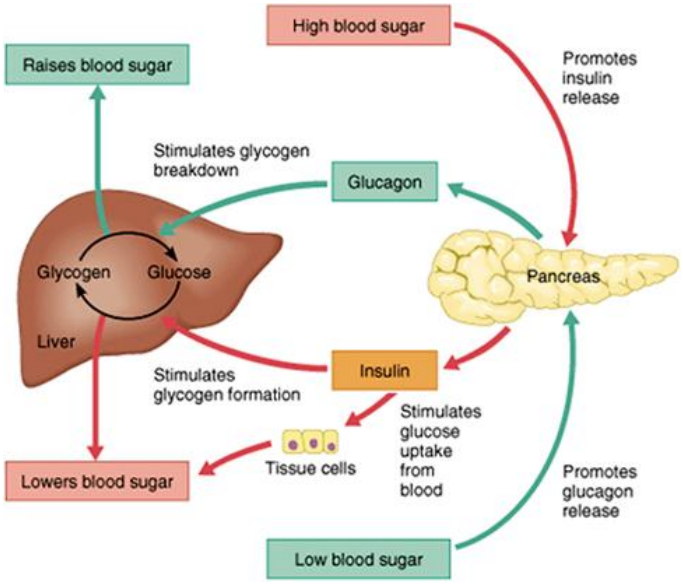
Proinsulin undergoes maturation into active insulin through the action of cellular endopeptidases known as *prohormoneconvertases* (PC1 and PC2), as well as the *exoproteasecarboxypeptidase* E. The endopeptidases cleave at 2 positions, releasing a fragment called the C–peptide, and leaving 2 peptide chains, the B– and A–chains, linked by 2 disulfide bonds. The cleavage sites are each located after a pair of basic residues (lysine–64 and arginine–65, and arginine–31 and −32). After cleavage of the C–peptide, these 2 pairs of basic residues are removed by the carboxypeptidase. The C–peptide is the central portion of proinsulin, and the primary sequence of proinsulin goes in the order "B–C–A" (the B and A chains were identified on the basis of mass and the C–peptide was discovered later).

The resulting mature insulin is packaged inside mature granules waiting for metabolic signals (such as leucine, arginine, glucose and mannose) and vagal nerve stimulation to be exocytosed from the cell into the circulation.

The endogenous production of insulin is regulated in several steps along the synthesis pathway:

- At transcription from the insulin gene
- In mRNA stability
- At the mRNA translation
- In the posttranslational modifications

Insulin and its related proteins have been shown to be produced inside the brain, and reduced levels of these proteins are linked to Alzheimer's disease. Insulin release is stimulated also by β–2 receptor stimulation and inhibited by α–1 receptor stimulation. In addition, cortisol, glucagon and growth hormone antagonize the actions of insulin during times of stress. Insulin also inhibits fatty acid release by hormone sensitive lipase in adipose tissue.



Figure–7: Release of Insulin.

Release: β–cells in the islets of Langerhans release insulin in two phases. The first–phase release is rapidly triggered in response to increased blood glucose levels, and lasts about 10 minutes. The second phase is a sustained, slow release of newly formed vesicles triggered independently of sugar, peaking in 2 to 3 hours. Reduced first–phase insulin release may be the earliest detectable β–cell defect predicting onset of type 2 diabetes. First–phase release and insulin sensitivity are independent predictors of diabetes.

The description of first phase release is as follows:

1. Glucose enters the β–cells through the glucose transporters, GLUT2. These glucose transporters have a relatively low affinity for glucose, ensuring that the rate of glucose entry into the β–cells is proportional to the extracellular glucose concentration (within the physiological range). At low blood sugar levels very, little glucose enters the β–cells; at high blood glucose concentrations large quantities of glucose enter these cells.
2. The glucose that enters the β–cell is phosphorylated to glucose–6–phosphate (G–6–P) by glucokinase (hexokinase IV) which is not inhibited by G–6–P in the way that the hexokinases in other tissues (hexokinase I – III) are affected by this product. This means that the intracellular G–6–P concentration remains proportional to the blood sugar concentration.
3. Glucose–6–phosphate enters glycolytic pathway and then, via the pyruvate dehydrogenase reaction, into the Krebs cycle, where multiple, high–energy ATP molecules are produced by the oxidation of acetyl

CoA (the Krebs cycle substrate), leading to a rise in the ATP:ADP ratio within the cell.[8]

4. An increased intracellular ATP:ADP ratio closes the ATP–sensitive SUR1/Kir6.2 potassium channel. This prevents potassium ions (K^+) from leaving the cell by facilitated diffusion, leading to a buildup of intracellular potassium ions. As a result, the inside of the cell becomes less negative with respect to the outside, leading to the depolarization of the cell surface membrane.
5. Upon depolarization, voltage–gated calcium ion (Ca^{2+}) channels open, allowing calcium ions to move into the cell by facilitated diffusion.
6. The cytosolic calcium ion concentration can also be increased by calcium release from intracellular stores via activation of ryanodine receptors.
7. The calcium ion concentration in the cytosol of the β–cells can also, or additionally, be increased through the activation of phospholipase C resulting from the binding of an extracellular ligand (hormone or neurotransmitter) to a G protein–coupled membrane receptor. Phospholipase C cleaves the membrane phospholipid, phosphatidyl inositol 4,5–bisphosphate, into inositol 1,4,5–trisphosphate and diacylglycerol. Inositol 1,4,5–trisphosphate (IP3) then binds to receptor proteins in the plasma membrane of the endoplasmic reticulum (ER). This allows the release of Ca^{2+} ions from the ER via IP3–gated channels, which raises the cytosolic concentration of calcium ions independently of the effects of a high blood glucose concentration. Parasympathetic stimulation of the pancreatic islets operates via this pathway to increase insulin secretion into the blood.
8. The significantly increased amount of calcium ions in the cells' cytoplasm causes the release into the blood of previously synthesized insulin, which has been stored in intracellular secretory vesicles.

This is the primary mechanism for release of insulin. Other substances known to stimulate insulin release include the amino acids arginine and leucine, parasympathetic release of acetylcholine (acting via the phospholipase C pathway), sulfonylurea, cholecystokinin (CCK, also via phospholipase C), and the gastrointestinally derived incretins, such as glucagon–like peptide–1 (GLP–1) and glucose–dependent insulinotropic peptide (GIP).

Release of insulin is strongly inhibited by norepinephrine (noradrenaline), which leads to increased blood glucose levels during stress. It appears that release of catecholamines by the sympathetic nervous system has conflicting influences on insulin release by β cells, because insulin release is inhibited by α2–adrenergic receptors and stimulated by β2–adrenergic receptors. The net effect of norepinephrine from sympathetic nerves and epinephrine from adrenal glands on insulin release is inhibition due to dominance of the α–adrenergic receptors.[9]

When the glucose level comes down to the usual physiologic value, insulin release from the β–cells slows or stops. If the blood glucose level drops lower than this, especially to dangerously low levels, release of hyperglycemic hormones (most prominently glucagon from islet of Langerhans α–cells) forces release of glucose into the blood from the liver glycogen stores, supplemented by gluconeogenesis if the glycogen stores become depleted. By increasing blood glucose, the hyperglycemic hormones prevent or correct life–threatening hypoglycemia.

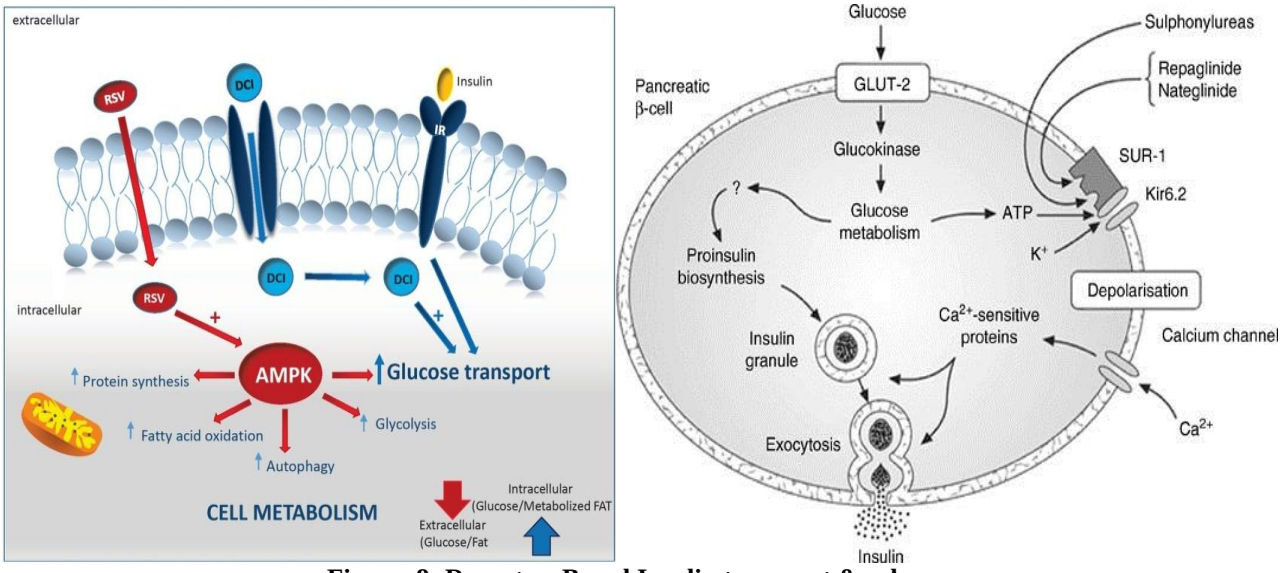
Evidence of impaired first–phase insulin release can be seen in the glucose tolerance test, demonstrated by a substantially elevated blood glucose level at 30minutes after the ingestion of a glucose load (75 or 100 g of glucose), followed by a slow drop over the next 100 minutes, to remain above 120mg/100 ml after two hours after the start of the test. In a normal person the blood glucose level is corrected (and may even be slightly over–corrected) by the end of the test. An insulin spike is a 'first response' to blood glucose increase, this response is individual and dose specific although it was always previously assumed to be food type specific only.

Mechanism of Insulin: Insulin is a key player in the control of intermediary metabolism, and the big picture is that it organizes the use of fuels for either storage or oxidation. Through these activities, insulin has profound effects on both carbohydrate and lipid metabolism, and significant influences on protein and mineral metabolism. Consequently, derangements in insulin signaling have widespread and devastating effects on many organs and tissues.

The Insulin Receptor and Mechanism of Action: Like the receptors for other protein hormones, the receptor for insulin is embedded in the plasma membrane. The insulin receptor is composed of two alpha subunits and two β–subunits linked by di–sulphide bonds. The alpha chains are entirely extracellular and house insulin binding domains, while the linked β chains penetrate through the plasma membrane. The insulin receptor is a tyrosine kinase. In other words, it functions as an enzyme that transfers phosphate groups from ATP to tyrosine residues on intracellular target proteins. Binding of insulin to the alpha subunits causes the β subunits to phosphorylate themselves (autophosphorylation), thus activating the catalytic activity of the receptor. The activated receptor then phosphorylates a number of intracellular proteins, which in turn alters their activity, thereby generating a biological response.[10]

Several intracellular proteins have been identified as phosphorylation substrates for the insulin receptor, the best–studied of which is insulin receptor substrate 1 or IRS1. When IRS–1 is activated by phosphorylation, a lot of things happen. Among other things, IRS–1 serves as a type of docking centre for recruitment and activation of other enzymes that ultimately mediate insulin's effects. A

more detailed look at these processes is presented in the section on Insulin Signal Transduction.



Figure–8: Receptor–Based Insulin transport & release.

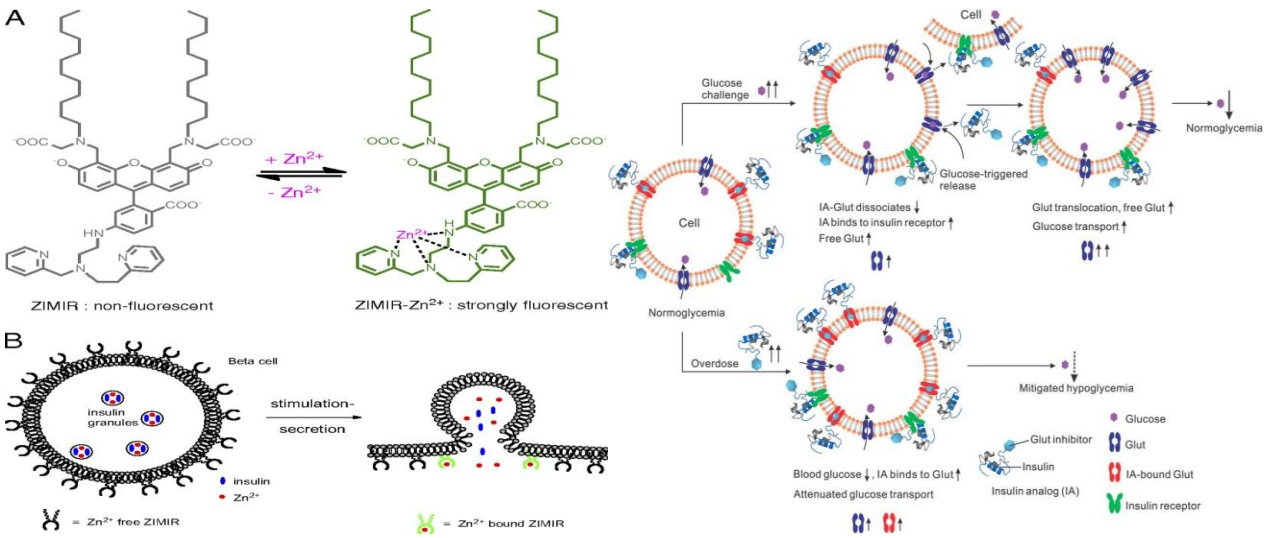
Insulin and Carbohydrate Metabolism: Glucose is liberated from dietary carbohydrate such as starch or sucrose by hydrolysis within the small intestine, and is then absorbed into the blood. Elevated concentrations of glucose in blood stimulate release of insulin, and insulin acts on cells throughout the body to stimulate uptake, utilization and storage of glucose. The effects of insulin on glucose metabolism vary depending on the target tissue. Two important effects are:

1. Insulin facilitates entry of glucose into muscle, adipose and several other tissues. The only mechanism by which cells can take up glucose is by facilitated diffusion through a family of hexose transporters. In many tissues–muscle being a prime example–the major transporter used for uptake of glucose (called GLUT4) is made available in the plasma membrane through the action of insulin.[11]

When insulin concentrations are low, GLUT4 glucose transporters are present in cytoplasmic vesicles, where they are useless for transporting glucose. Binding of insulin to receptors on such cells leads rapidly to fusion of those vesicles with the plasma membrane and insertion of the glucose transporters, thereby giving the cell an ability to efficiently take up glucose. When blood levels of insulin decrease and insulin receptors are no longer occupied, the glucose transporters are recycled back into the cytoplasm. It should be noted here that there are some tissues that do not require insulin for efficient uptake of glucose: important examples are brain and the liver. This is because these cells don't use GLUT4 for importing glucose, but rather, another transporter that is not insulin–dependent.

2. Insulin stimulates the liver to store glucose in the form of glycogen. A large fraction of glucose absorbed from the small intestine is immediately taken up by hepatocytes, which convert it into the storage polymer glycogen. Insulin has several effects in liver which stimulate glycogen synthesis. First, it activates the enzyme hexokinase, which phosphorylates glucose, trapping it within the cell. Coincidently, insulin acts to inhibit the activity of glucose–6–phosphatase. Insulin also activates several of the enzymes that are directly involved in glycogen synthesis, including phosphofructokinase and glycogen synthase. The net effect is clear: when the supply of glucose is abundant, insulin "tells" the liver to bank as much of it as possible for use later.

3. A well–known effect of insulin is to decrease the concentration of glucose in blood, which should make sense considering the mechanisms described above. Another important consideration is that, as blood glucose concentrations fall, insulin secretion ceases. In the absence of insulin, a bulk of the cells in the body become unable to take up glucose, and begin a switch to using alternative fuels like fatty acids for energy. Neurons, however, require a constant supply of glucose, which in the short term, is provided from glycogen reserves. When insulin levels in blood fall, glycogen synthesis in the liver diminishes and enzymes responsible for breakdown of glycogen become active. Glycogen breakdown is stimulated not only by the absence of insulin but by the presence of glucagon, which is secreted when blood glucose levels fall below the normal range.



Figure–9: Insulin Secretion & Binding.

Insulin and Lipid Metabolism: The metabolic pathways for utilization of fats and carbohydrates are deeply and intricately intertwined. Considering insulin's profound effects on carbohydrate metabolism, it stands to reason that insulin also has important effects on lipid metabolism, including the following:

1. Insulin promotes synthesis of fatty acids in the liver. As discussed above, insulin is stimulatory to synthesis of glycogen in the liver. However, as glycogen accumulates to high levels (roughly 5% of liver mass), further synthesis is strongly suppressed. When the liver is saturated with glycogen, any additional glucose taken up by hepatocytes is shunted into pathways leading to synthesis of fatty acids, which are exported from the liver as lipoproteins. The lipoproteins are ripped apart in the circulation, providing free fatty acids for use in other tissues, including adipocytes, which use them to synthesize triglyceride.
2. Insulin inhibits breakdown of fat in adipose tissue by inhibiting the intracellular lipase that hydrolyzes triglycerides to release fatty acids. Insulin facilitates entry of glucose into adipocytes, and within those cells, glucose can be used to synthesize glycerol. This glycerol, along with the fatty acids delivered from the liver, are used to synthesize triglyceride within the adipocyte. By these mechanisms, insulin is involved in further accumulation of triglyceride in fat cells. From a whole–body perspective, insulin has a fat–sparing effect. Not only does it drive most cells to preferentially oxidize carbohydrates instead of fatty acids for energy, insulin indirectly stimulates accumulation of fat in adipose tissue.

Other Notable Effects of Insulin: In addition to insulin's effect on entry of glucose into cells, it also stimulates the uptake of amino acids, again contributing to its overall anabolic effect. When insulin levels are low, as in the fasting state, the balance is pushed toward intracellular protein degradation.

Insulin also increases the permeability of many cells to potassium, magnesium and phosphate ions. The effect on potassium is clinically important. Insulin activates sodium–potassium ATPases in many cells, causing a flux of potassium into cells. Under certain circumstances, injection of insulin can kill patients because of its ability to acutely suppress plasma potassium concentrations.

Physiological Effects: The actions of insulin on the global human metabolism level include:

- Increase of cellular intake of certain substances, most prominently glucose in muscle and adipose tissue (about two–thirds of body cells)
- Increase of DNA replication and protein synthesis via control of amino acid uptake
- Modification of the activity of numerous enzymes.
- The actions of insulin (indirect and direct) on cells include:
- Stimulates the uptake of glucose – Insulin decreases blood glucose concentration by inducing intake of glucose by the cells. This is possible because Insulin causes the insertion of the GLUT4 transporter in the cell membranes of muscle and fat tissues which allows glucose to enter the cell.
- Increased fat synthesis – insulin forces fat cells to take in blood glucose, which is converted into triglycerides; decrease of insulin causes the reverse.
- Increased esterification of fatty acids – forces adipose tissue to make neutral fats (i.e., triglycerides) from fatty acids; decrease of insulin causes the reverse.
- Decreased lipolysis – forces reduction in conversion [of fat cell lipid stores into blood fatty acids and glycerol; decrease of insulin causes the reverse.
- Induce glycogen synthesis – When glucose levels are high, insulin induces the formation of glycogen by the activation of the hexokinase enzyme, which adds a phosphate group in glucose, thus resulting in a molecule that cannot exit the cell. At the same time, insulin inhibits the enzyme glucose–6–phosphatase, which removes the

phosphate group. These two enzymes are key for the formation of glycogen. Also, insulin activates the enzymes phosphofructokinase and glycogen synthase which are responsible for glycogen synthesis.

- Decreased gluconeogenesis and glycogenolysis – decreases production of glucose from noncarbohydrate substrates, primarily in the liver (the vast majority of endogenous insulin arriving at the liver never leaves the liver); decrease of insulin causes glucose production by the liver from assorted substrates.
- Decreased proteolysis – decreasing the breakdown of protein.
- Decreased autophagy – decreased level of degradation of damaged organelles. Postprandial levels inhibit autophagy completely.
- Increased amino acid uptake – forces cells to absorb circulating amino acids; decrease of insulin inhibits absorption.
- Arterial muscle tone – forces arterial wall muscle to relax, increasing blood flow, especially in microarteries; decrease of insulin reduces flow by allowing these muscles to contract.
- Increase in the secretion of hydrochloric acid by parietal cells in the stomach.
- Increased potassium uptake – forces cells synthesizing glycogen (a very spongy, "wet" substance, that increases the content of intracellular water, and its accompanying K^+ ions) to absorb potassium from the extracellular fluids; lack of insulin inhibits absorption. Insulin's increase in cellular potassium uptake lowers potassium levels in blood plasma. This possibly occurs via insulin–induced translocation of the Na^+/K^+–ATPase to the surface of skeletal muscle cells.
- Decreased renal sodium excretion.

Insulin also influences other body functions, such as vascular compliance and cognition. Once insulin enters the human brain, it enhances learning and memory and benefits verbal memory in particular.[12] Enhancing brain insulin signaling by means of intranasal insulin administration also enhances the acute thermoregulatory and glucoregulatory response to food intake, suggesting that central nervous insulin contributes to the co–ordination of a wide variety of homeostatic or regulatory processes in the human body. Insulin also has stimulatory effects on gonadotropin–releasing hormone from the hypothalamus, thus favoring fertility.

In addition to insulin's effect on entry of glucose into cells, it also stimulates the uptake of amino acids, again contributing to its overall anabolic effect. When insulin levels are low, as in the fasting state, the balance is pushed toward intracellular protein degradation. Insulin also increases the permeability of many cells to potassium, magnesium and phosphate ions. The effect on potassium is clinically important. Insulin activates sodium–potassium ATPases in many cells, causing a flux of potassium into cells. Under certain circumstances, injection of insulin can kill patients because of its ability to acutely suppress plasma potassium concentrations.[13]

Figure–10: Type–1 Diabetes and it's Prevention.

Insulin Deficiency and Excess Diseases: Diabetes mellitus, arguably the most important metabolic disease of man, is an insulin deficiency state. It also is a significant cause of disease in dogs and cats. Two principal forms of this disease are recognized:

- Type–1 or insulin–dependent diabetes mellitus is the result of a frank deficiency of insulin. The onset of this disease typically is in childhood. It is due to destruction pancreatic β–cells, most likely the result of autoimmunity to one or more components of those cells. Many of the acute effects of this disease can be controlled by insulin replacement therapy. Maintaining tight control of blood glucose concentrations by monitoring, treatment with insulin and dietary management will minimize the long–term adverse effects of this disorder on blood vessels, nerves and other organ systems, allowing a healthy life.
- Type–2 or non–insulin–dependent diabetes mellitus begins as a syndrome of insulin resistance. That is, target tissues fail to respond appropriately to insulin. Typically, the onset of this disease is in adulthood.

Despite monumental research efforts, the precise nature of the defects leading to type II diabetes have been difficult to ascertain, and the pathogenesis of this condition is plainly multifactorial. Obesity is clearly a major risk factor, but in some cases of extreme obesity in humans and animals, insulin sensitivity is normal. Because there is not, at least initially, an inability to secrete adequate amounts of insulin, insulin injections are not useful for therapy. Rather the disease is controlled through dietary therapy and hypoglycemic agents. However, a substantial number of those with type–2 diabetes progress to requiring insulin.[14]

Hyperinsulinemia or excessive insulin secretion is most commonly a consequence of insulin resistance, associated with type–2 diabetes or the metabolic syndrome. More rarely, hyperinsulinemia results from an insulin–secreting tumor (insulinoma) in the pancreas. Hyperinsulinemia due to accidental or deliberate injection of excessive insulin is dangerous and can be acutely life–threatening because blood levels of glucose drop rapidly and the brain becomes starved for energy (insulin shock).[15]

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CONCLUSION

Decreased or loss of insulin activity results in diabetes mellitus, a condition of high blood sugar level (hyperglycaemia). There are two types of the disease. In type–1 diabetes mellitus, the β–cells are destroyed by an autoimmune reaction so that insulin can no longer be synthesized or be secreted into the blood. In type–2 diabetes mellitus, the destruction of β–cell is less pronounced than in type–1 diabetes, and is not due to an autoimmune process. Instead, there is an accumulation of amyloid in the pancreatic islets, which likely disrupts their anatomy and physiology. The pathogenesis of type–2 diabetes is not well understood but reduced population of islet β–cells, reduced secretory function of islet β–cells that survive, and peripheral tissue insulin resistance are known to be involved. Type–2 diabetes is characterized by increased glucagon secretion which is unaffected by, and unresponsive to the concentration of blood glucose. But insulin is still secreted into the blood in response to the blood glucose. As a result, glucose accumulates in the blood. Diabetic investigations are now transforming from preliminary controlled in–patient settings to crucial real–world outpatient environment. Continued development of novel diabetic technologies must focus on patient–centered needs and improve clinical outcomes for a broad spectrum of diabetic population. Continued improvements in continuous glucose monitoring technology facilitated both direct benefits to the care of type 1 diabetic patients and paved the way toward the development of emerging artificial pancreas systems. In near future, insulin devices may feature intelligent timer, super bolus for better carbohydrate coverage and faster corrections. It may also account for exercise, provide meaningful suggestions, and carry out pattern spotting, analysis and direct communication capabilities.

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