Insulin is a peptide hormone that plays a crucial role in regulating glucose homeostasis in the human body. It is produced by the beta cells of the pancreas and is essential for the uptake and storage of glucose by cells. Insulin lowers blood glucose levels by promoting the uptake of glucose into cells, particularly muscle and adipose (fat) cells, where it is either used for energy or stored as glycogen or fat. Insulin is a protein hormone composed of two peptide chains, A and B, connected by disulfide bonds. The gene for insulin is located in the human genome, and its expression and synthesis involve a series of complex cellular processes. The A chain consists of 21 amino acids, while the B chain consists of 30 amino acids. The arrangement and bonding of these amino acids give insulin its three-dimensional structure. The peptide hormone undergoes post-translational modifications, including the cleavage of a precursor molecule (proinsulin) to form the mature insulin molecule. The disulfide bonds are crucial for maintaining the stability and biological activity of insulin.

Official Preparation of Insulin: The production of insulin for therapeutic use involves recombinant DNA technology or extraction from animal pancreas. Historically, insulin was extracted from the pancreas of pigs and cows, but due to concerns about potential allergic reactions and supply issues, most therapeutic insulin is now produced using genetically engineered bacteria or yeast. Recombinant DNA technology involves inserting the human insulin gene into a suitable host organism, such as Escherichia coli (E. coli) bacteria or Saccharomyces cerevisiae (yeast). The host organism then produces insulin, which can be harvested, purified, and used for therapeutic purposes. The purification process is crucial to ensure the final product is free from contaminants and has consistent potency. Chromatography and other advanced techniques are employed in the purification process. Insulin is available in various forms, including rapid-acting, short-acting, intermediate-acting, and long-acting formulations, providing flexibility in diabetes management. In summary, insulin is a vital hormone in glucose metabolism, and its chemistry involves a complex sequence of genetic, cellular, and molecular processes. The official preparation of therapeutic insulin has evolved over time, with modern methods utilizing recombinant DNA technology for efficient and safe production. The accessibility of insulin is crucial for individuals with diabetes to manage their condition effectively.
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 insulin. 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 insulin 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]\n
![Image of insulin and Banting](image)

**Figure-1: Insulin & Banting**

**Discovery of Insulin:** July 27 marks one of the most important days in diabetes treatment history. On that date in 1921, Dr. Frederick Banting, a Canadian surgeon and Charles Best, a medical student, successfully isolated the hormone insulin for the first time. The breakthrough research took place at the University of Toronto, where Banting and Best successfully isolated insulin from dogs, produced diabetes symptoms in the animals, and then provided insulin injections that produced normal blood glucose levels. Dr. Banting shared his success with Professor John Macleod.
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 there of (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.

**What Is Insulin?** Insulin, hormone that regulates the level of sugar (glucose) in the blood and that is produced by the beta cells of the islets of Langerhans in the pancreas. Insulin is secreted when the level of blood glucose rises—as after a meal. When the level of blood glucose falls, secretion of insulin stops, and the liver releases glucose into the blood. Several factors stimulate insulin secretion, but by far the most important is the concentration of glucose in the arterial (oxygenated) blood that perfuses the islets.\[2\]
When blood glucose concentrations increase (i.e., following a meal), large amounts of glucose are taken up and metabolized by the beta cells, and the secretion of insulin increases. Conversely, as blood glucose concentrations decrease, the secretion of insulin decreases; however, even during fasting, small amounts of insulin are secreted. The secretion of insulin may also be stimulated by certain amino acids, fatty acids, keto acids, and several hormones secreted by the gastrointestinal tract. The secretion of insulin is inhibited by somatostatin and by activation of the sympathetic nervous system (the branch of the autonomic nervous system responsible for the fight-or-flight response).

Insulin acts primarily to stimulate glucose uptake by three tissues—adipose (fat), muscle, and liver—that are important in the metabolism and storage of nutrients. Like other protein hormones, insulin binds to specific receptors on the outer membrane of its target cells, thereby activating metabolic processes within the cells. A key action of insulin in these cells is to stimulate the translocation of glucose transporters (molecules that mediate cell uptake of glucose) from within the cell to the cell membrane. In adipose tissue, insulin stimulates glucose uptake and utilization. The presence of glucose in adipose cells in turn leads to increased uptake of fatty acids from the circulation, increased synthesis of fatty acids in the cells, and increased esterification (when an acid molecule binds to an alcohol) of fatty acids with glycerol to form triglycerides, the storage form of fat. In addition, insulin is a potent inhibitor of the breakdown of triglycerides (lipolysis). This prevents the release of fatty acids and glycerol from fat cells, saving them for when they are needed by the body (e.g., when exercising or fasting). As serum insulin concentrations decrease, lipolysis and fatty acid release increase.\[^{[3]}\]

In muscle tissue, insulin stimulates the transport of glucose and amino acids into muscle cells. The glucose is stored as glycogen, a storage molecule that can be broken down to supply energy for muscle
contraction during exercise and to supply energy during fasting. The amino acids transported into muscle cells in response to insulin stimulation are utilized for the synthesis of protein. In contrast, in the absence of insulin the protein of muscle cells is broken down to supply amino acids to the liver for transformation into glucose. Insulin is not required for the transport of glucose into liver cells, but it has profound effects on glucose metabolism in these cells.

It stimulates the formation of glycogen, and it inhibits the breakdown of glycogen (glycogenolysis) and the synthesis of glucose from amino acids and glycerol (gluconeogenesis). Therefore, the overall effect of insulin is to increase glucose storage and to decrease glucose production and release by the liver. These actions of insulin are opposed by glucagon, another pancreatic hormone produced by cells in the islets of Langerhans. \[^{[4]}\]

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**Figure-4: Proinsulin structure**

Inadequate production of insulin is responsible for the condition called Diabetes mellitus. Severe diabetics require periodic injections of insulin. The first insulin injections utilized hormone extracts from pigs, sheep, and cattle, but by the early 1980s certain strains of bacteria had been genetically modified to produce human insulin. Today the treatment of diabetes mellitus relies primarily on a form of human insulin that is made using recombinant DNA technology. \[^{[5]}\]

**Chemistry of Insulin:**

1. **Structure of Insulin:**
   
   Insulin is a protein hormone composed of two polypeptide chains, A and B, linked by disulfide bonds. The A chain has 21 amino acids, while the B chain has 30 amino acids. The overall structure of insulin is stabilized by intra- and intermolecular disulfide bonds formed between specific cysteine residues. The disulfide linkages contribute to the stability and biological activity of insulin. It has a molecular mass of 5808 Da.

   (1) Proinsulin is the immediate precursor to insulin in the single-chain peptide.

   (2) Proinsulin folds to adopt the ‘correct orientation of the prevailing ‘disulphide bonds: -S-S-’ plus other relevant conformational constraints whatsoever on account of its primary structure exclusively.

   (3) Proinsulin in reality, has a precursor of its own, preproinsulin—a peptide that essentially comprises of hundreds of ‘additional residues’.

   (4) At an emerging critical situation the insulin gets generated from proinsulin due to the ensuing cleavage of proinsulin at the two points indicated. This eventually produces insulin, that comprises of a 21-residue A chain and strategically linked with two disulphide bonds ultimately to a 30-residue B chain. Interestingly, these bondages between the two aforesaid residual chains ‘A’ and ‘B’ are invariably oriented almost perfectly and correctly by virtue of the prompted nature of proinsulin folding. \[^{[6]}\]

   **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 equate habit, oftentimes with clearly defined crystal edges and no double refraction.

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 C₂₅₇H₃₈₀N₆₅O₇₇S₆. 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 (inter chain) disulfide bonds are formed at cysteine residues between the positions A7–B7 and A20–B19. There is an additional (intra chain) 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).[7]

Uses of Insulin: Insulin regular is used with a proper diet and exercise program to control high blood sugar in people with diabetes. Controlling high blood sugar helps prevent kidney damage, blindness, nerve problems, loss of limbs, and sexual function problems. Proper control of diabetes may also lessen your risk of a heart attack or stroke. This man-made insulin product is the same as human insulin. It replaces the insulin that your body would normally make. It is a short-acting insulin. It works by helping blood sugar (glucose) get into cells so your body can use it for energy. This medication is usually used in combination with a medium- or long-acting insulin product. This medication may also be used alone or with other oral diabetes drugs (such as metformin).[8]

How to use Insulin Regular Human Solution: Read the Patient Information Leaflet if available from your pharmacist before you start using this medication and each time you get a refill. If you have any questions, ask your doctor, diabetes educator, or pharmacist. Learn all preparation and usage instructions from your health care professional and the product package. Before using, check this product visually for particles or discoloration. If either is present, do not use the insulin. Insulin regular should be clear and colorless. Before injecting each dose, clean the injection site with rubbing alcohol. Change where you inject each time to lessen the risk of problems or damage under the skin (for example, pits/lumps or thickened skin). Insulin regular may be injected in the stomach area, the thigh, the buttocks, or the back of the upper arm. Do not inject into a vein or muscle because very low blood sugar (hypoglycemia) may occur. Do not rub the area after the injection. Do not inject skin that is red, swollen, itchy, or damaged. Do not inject cold insulin because this can be painful. The insulin container you are currently using can be kept at room temperature. Inject this medication under the skin as directed by your doctor, usually 30 minutes before meals. Because this insulin is fast-acting, not eating right after a dose of this insulin may lead to low blood sugar (hypoglycemia). Giving insulin regular into a vein should only be done by a health care professional. Very low blood sugar may result.[9]

Do not use insulin regular in an insulin pump. This product may be mixed only with certain other insulin products such as NPH insulin. Always draw the insulin regular into the syringe first, then follow with the longer-acting insulin. Never inject a mixture of different insulin into a vein. Consult your health care professional about which products may be mixed, the proper method for mixing insulin, and the proper way to inject mixtures of insulin. Do not change brands or types of insulin without directions on how to do so from your doctor. Do not share your pen device with another person, even if the needle is changed. You may give other people a serious infection, or get a serious infection from them. Learn how to store and discard medical supplies safely.

The dosage is based on your medical condition and response to treatment. Measure each dose very carefully because even small changes in the amount of insulin may have a large effect on your blood sugar. Check your blood sugar regularly as directed by your doctor. Keep track of your results and share them with your doctor. This is very important in order to determine the correct insulin dose. Use this medication regularly to get the most benefit from it. To help you remember, use it at the same times each day. Tell your doctor if your condition does not improve or if it worsens (your blood sugar is too high or too low).[10]
Official Preparation of Insulin:

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**Short acting:**

- **Regular Insulin:** Regular insulin is a medication used in the management of diabetes mellitus and hyperglycemia of a variety of etiologies. It is in the short-acting insulin class of drugs. This activity outlines the mechanism of action, adverse event profile, labeled and off-labeled indications, contraindications, monitoring, and toxicity for regular insulin pertinent for members of the healthcare team in the management of patients with diabetes mellitus and related conditions. Also called as neutral or soluble insulin. Rapid acting with 0.5-1 hr duration of action.

- **Lispro Insulin:** Rapid acting with 6-8 hrs duration of action. In the carboxyl terminal of B-chain, lysine and proline residues are reversed. It is a recombinant human insulin. Insulin Lispro is used in the treatment of diabetes mellitus (Type 1 & Type 2). It is recommended to patients with diabetes mellitus who require insulin to maintain normal sugar levels. It also helps in the initial stabilization of diabetes mellitus. Hypoglycemia (low blood glucose level), Weight gain, Infusion site reaction, Headache, Pain, Nasopharyngitis (inflammation of the throat and nasal passages), Auto-antibody formation are common side effects.[11]

- **Insulin zinc:** Insulin Zinc Suspension is a sterile suspension of Insulin in buffered Water for Injection, modified by the addition of a suitable zinc salt in a manner such that the solid phase of the suspension consists of a mixture of crystalline and amorphous insulin in a ratio of approximately 7 parts of crystals to 3 parts of amorphous material. Its potency, based on the sum of its insulin and des-amido insulin is NLT 95.0% and NMT 105.0% of the potency stated on the label, expressed in USP Insulin Units/mL. Duration 6-8 hrs.

- **Insulin aspart:** Synthetic form of insulin where a single amino acid, proline (B-28) is replaced by aspartic acid. It has 3-5 hr duration of action.
Intermediate acting:

- **Isophane Insulin:** Also called as Neutral Protamine Hagedorn (NPH) insulin. Insulin is a hormone that works by lowering levels of glucose (sugar) in the blood. Insulin isophane is an intermediate-acting insulin that starts to work within 2 to 4 hours after injection, peaks in 4 to 12 hours, and keeps working for 12 to 18 hours. Insulin isophane is used to improve blood sugar control in adults and children with diabetes mellitus. Insulin isophane may also be used for purposes not listed in this medication guide.[12] Insulin isophane may cause serious side effects:
  1. Fluid retention-weight gain, swelling in your hands or feet, feeling short of breath; or
  2. Low potassium-leg cramps, constipation, irregular heartbeats, fluttering in your chest, increased thirst or urination, numbness or tingling, muscle weakness or limp feeling. Common side effects of insulin isophane may include: low blood sugar; weight gain, swelling in your hands or feet; itching, mild skin rash; or thickening or hollowing of the skin where you injected the medicine.

- **Lente Insulin:** Lente insulin (from Italian lento, "slow"; also called insulin zinc suspension) was an intermediate duration insulin that is no longer used in humans. It is consist of acetate buffer, zinc and regular insulin. The onset of lente insulin is one to two hours after the dose is administered, and the peak effect is approximately 8 to 12 hours after administration, with some effects lasting over 24 hours. This was in part because health care providers began to favor more predictable forms of insulin, such as recombinant NPH insulin. It was known by 1950 that the addition of protamine or zinc could alter the duration of action of these insulin products, and in 1952, K. Hallas-Moller at Novo Nordisk produced the first commercial insulin zinc suspension for use in humans. For decades, lente insulin was used as a basal insulin, designed to mimic the body's continual slow release of insulin throughout the day. Compared to NPH insulin, lente insulin has a similar but more protracted loss of action after a dose is administered.[13]

  **Veterinary use:** After the discontinuation of lente insulin for human use, the FDA approved a veterinary porcine-derived lente insulin (Vetsulin®, Merck Animal Health) for daily use in dogs or twice daily use in cats. Insulin analogs used in humans after the discontinuation of lente insulin have not yet been proven to provide the same benefits and predictability as lente insulin in cats and dogs. For this and other reasons, lente insulin is still commonly used in both dogs and cats.[14]

- **Biphasic Insulin aspart:** This is insulin aspart 70/30, is an admixture consisting of 70% intermediate-acting protamine-crystallized insulin aspart (not NPH) and 30% rapid-acting non-protaminated (soluble) insulin aspart. Biphasic insulin aspart 70/30 has a single peak, which comes from its soluble component. Compared to biphasic human insulin 70/30 (NPH/Regular), biphasic insulin aspart 70/30 has a more rapid and higher peak for more effective mealtime coverage. When injected subcutaneously, protaminated insulin aspart crystals exhibit a delayed absorption pattern such that the duration of action of the intermediate component of biphasic insulin aspart is similar to human NPH insulin. The incorporation of protaminated insulin aspart in biphasic insulin aspart 70/30 conveniently eliminates the need for a separate basal insulin injection. When intermediate acting protamine-crystallized insulin aspart and rapid-acting non-protaminated insulin aspart are
combined to form biphasic insulin aspart 70/30, the peak action of the biphasic insulin aspart occurs between one and four hours after injection with a total duration of action measured as long as 24 hours.

Biphasic isophane insulin+insulin human: This is a combination of two medicines, an intermediate-acting and a short-acting type of insulin, primarily used for the treatment of both type 1 and type 2 diabetes mellitus. It maintains blood sugar levels in adults and children. Diabetes mellitus is a condition that affects the way your body processes glucose. In Diabetes mellitus Type 1, the body does not make enough insulin to control blood sugar levels. In Diabetes mellitus type 2, either the body stops producing enough insulin (the hormone which helps to decrease sugar levels in the blood) or there is resistance to the action of insulin. As a result, insulin is produced in large amounts but it is not able to act on the organs of the body.[15]

**Figure-7: Long acting insulin**

**Figure-8: Insulin Detemir, Insulin Degludec, Insulin Glargine**

**Long acting**

- **Protamine zinc**: The addition of zinc to protamine insulin was suggested by Scott and Fisher; zinc further enhances the prolonged action of protamine insulin and renders the mixture stable for a period of at least six months. Protamine zinc insulin is the only protamine insulin combination obtainable today. Protamine zinc insulin in many cases lowers the blood sugar for much more than 24 hours, while protamine insulin acts for only 12 to 14 hours. Basically the product is still regular insulin which has been mixed with protamine (a simple protein) with small quantities of zinc added to the compound. The resulting mixture, a milky suspension, is much less soluble, and is therefore more slowly absorbed in the subcutaneous tissues, than the clear soluble insulin hydrochloride discovered by Banting and Best. Protamine zinc insulin has been used in the treatment of diabetes mellitus for nearly four years.
• **Ultra lente insulin:** It has 65% of lente insulin. This is an extended insulin zinc suspension has a slower onset of action and a prolonged peak effect. It provides a low basal concentration of insulin throughout the day and is often used in combination with other insulin preparations. The desire for insulin preparations without peak effects prompted the development of long-acting insulin analogs including insulin glargine and insulin detemir. They lack a peak effect and have durations of action of 17 to 24 hours. Insulin glargine is produced by the addition of two arginine residues to the C-terminus of the insulin B chain and replacement of a single asparagine residue with a glycine in the A chain. The resulting insulin forms a solution with a pH of 4. The low pH stabilizes the hexamer and delays absorption but also means that glargine cannot be mixed with short-acting insulin preparations that are at neutral pH. Another long-acting preparation, insulin detemir, is synthesized by the addition of a saturated fatty acid to the lysine at position B29 in human insulin. Insulin degludec is formed by deleting the last amino acid from the B chain of human insulin and adding a glutamyl linkage to a hexadecanedioic fatty acid, thus allowing the formation of slowly absorbing soluble multihexamers at the injection site. Its duration of action is more than 40 hours.[16]

• **Insulin glargine:** This is a recombinant human insulin analog that binds to insulin receptors (IR). Insulin is necessary to regulate lipid, glucose, and energy homeostasis. It acts mainly on the skeletal muscle, adipose tissue, and liver. It is obtained by addition of two arginine residues in B-chain carboxy terminal and by replacement of asparagine with glycine in A-21 position of human insulin. Insulin glargine has an onset of action of 1.5 to 2 hours. It has a long duration of action of up to 24 hours.

• **Insulin detemir:** This is a neutral, soluble, long-acting insulin analogue in which threonine is omitted from position B30 of the insulin β-chain and replaced by myristic acid, a C14 fatty acid chain. Duration of action is upto 24 hrs. Insulin detemir is a soluble long-acting human insulin analogue at neutral pH with a unique mechanism of action. Following subcutaneous injection, insulin detemir binds to albumin via fatty acid chain, thereby providing slow absorption and a prolonged metabolic effect. Insulin detemir has a less variable pharmacokinetic profile than insulin suspension isophane or insulin ultralente. The use of insulin detemir can reduce the risk of hypoglycemia (especially nocturnal hypoglycemia) in type 1 and type 2 diabetic patients.

• **Insulin degludec:** This is (INN/USAN) is an ultralong-acting basal insulin analogue that was developed by Novo Nordisk under the brand name Tresiba. It is obtained by addition of hexadecanedioic acid to lysine residue in B-29 position of human insulin. Degludec has an action duration of more than 24 hours. To demonstrate that this multihexamer formation leads to an ultra-long acting profile of IDeg, a multiple dose clinical pharmacology study was conducted in subjects with type 1 diabetes (n = 12). IDeg was found to have a t½ longer than 24 hours and was detectable in circulation for at least 96 hours after injection. It is used in patients with Type I and Type II *Diabetes mellitus*.

**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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