Discovery and history of Insulin

• In Germany, Oskar Minkowski and Joseph von Mering observed that total pancreatectomy in experimental animals leads to the development of severe diabetes mellitus and began the speculation that a mysterious substance produced by the pancreas is responsible for metabolic control.

• Much evidence support the existence of pancreatic internal secretion emanating from the islet cells that in 1907 a Belgian investigator J de Meyer proposed it be named “insulin”.

• In 1920, Frederick Grant Banting a 22 years old orthopedic surgeon came up with an idea “Diabetes Ligate pancreatic ducts of dog. Keep dogs alive till acini degenerate leaving islets. Try to isolate the internal secretion of these to relieve glycosuria”.

• On 11th January 1922, clinicians at Toronto General Hospital injected a 14 year old, severely diabetic boy Leonard Thompson with 15 ml of pancreatic extract made by Banting and Best. This clinical test was a failure. The injection caused only slight reductions of glycemia and glycosuria, had no effect on ketoacidosis or the patient’s subjective presentation, and resulted in the formation of a sterile abscess. On January 23rd, a new series of injections began. Thompson responded immediately. His glycosuria almost disappeared, his ketonuria did disappear, his blood glucose dropped to normal.

• JB Collip the biochemist produced the successful extract. He had developed a method of extraction that involved changing the concentrations of slightly acidic alcohol solutions of chilled beef pancreas.

• The Nobel committee of the Caroline Institute awarded the 1923 Nobel Prize in Physiology or Medicine to Banting and Macleod.

Structure of Insulin

Like most of the other hormones, insulin is a protein comprising of 2 polypeptide chains A (with 21 amino acid residues) and B (with 30 amino acid residues). Chains A and B are linked by disulphide bridges. In addition A-chain contains an intra-chain disulphide bridge linking residue 6 and 11. C-chain, which connects A and B chains is liberated along with insulin after breakdown of proinsulin. Insulin monomers aggregate to form dimers and hexamers. Zn hexamer is composed of three insulin dimmers associated in threefold symmetrical pattern.
Biosynthesis of Insulin

Insulin is synthesized in the beta cells of pancreas in the form of preproinsulin which is the ultimate precursor and gene for the same is located on chromosome 11 close to that for insulin like growth factor-2 (IGF-2). Within a minute after synthesis it is discharged into cisternal space of rough endoplasmic reticulum where it is cleaved into proinsulin by proteolytic enzymes. Proinsulin with a C (connecting) chain linking A and B chains is then transported by microvesicles to the Golgi apparatus. Proinsulin is released in vesicles. Conversion of proinsulin to insulin continues in maturing granules through the action of prohormone convertase 2 and 3 and carboxy peptidase H. Maturing granules are translocated with the help of microtubules and microfilaments.

Insulin secretion

Insulin is secreted from the beta cells in response to various stimuli like glucose, arginine, sulphonylureas though physiologically glucose is the major determinant. Various neural, endocrine and pharmacological agents can also exert stimulatory effect. Glucose is taken up by beta cells through GLUT-2 receptors. After entering the beta cell, glucose is oxidized by glucokinase, which acts as a glucose sensor. Glucose concentration below 90 mg/dl do not cause any insulin release. At such substimulatory glucose concentrations, K+ efflux through open KATP channels keeps the β cell membrane at a negative potential at which voltage-gated Ca2+ channels are closed. As there is increase in plasma glucose, glucose uptake and metabolism by the β cell is enhanced. Rise in ATP concentration result in closure of KATP channels, leading to a membrane depolarization, opening of voltage-gated Ca2+ channels, Ca2+ influx, a rise in intracellular calcium concentration, and ultimately exocytosis of insulin granules.

Structurally, the pancreatic KATP channel consists of two unrelated subunits: a sulfonylurea receptor (the SUR1 isoform) and a potassium channel subunit (Kir6.2) that forms the central ion-conducting pathway. The mature KATP channel exists as an octamer of Kir6.2 and SUR1 subunits in a 4:4 stoichiometry. A sub unit specific site specific to pancreatic KATP channel, confers glimepiride an advantage over the other sulfonylurea secretagogues. Sulfonylurea and non-sulphonylurea drugs act as insulin secretogogues by closing KATP channels bypassing the β cell metabolism. Diazoxide is a K channel opener and inhibits insulin secretion, independent of blood glucose levels.
Pharmacology of Insulin

Human insulin is now produced by recombinant DNA technology. Various companies differ in their methodology but the basic principal is introduction of human insulin or proinsulin gene into organisms like E. coli or Yeast. The organisms keep on multiplying and in turn producing insulin or proinsulin which is converted to insulin by enzymatic cleavage.

Dry human insulin is a microcrystalline powder with a molecular weight of 5808. Insulin precipitates at its isoelectric pH of 5.4, while it is soluble at a pH of 2-3. 1 IU of insulin corresponds to 38.5 μg dry substance. Insulin is available in the market in the strength of 40U and 100U i.e. 40U/ml and 100 U/ ml respectively. Even U500 is available in US and U10 is sometimes formulated individually for use in infants with diluents provided by manufacturer. Half life of injected insulin is about 40 min.

Insulin preparations Porcine insulin has been withdrawn from the market globally and bovine is expected to be extinct very soon. Human insulin is available in the short acting i.e. regular and intermediate acting i.e. Neutral Protamine Hagedorn (NPH) forms. Insulin analogues which are synthetically modified with some changes in the amino acid sequence are also available.

Rapid acting insulin analogues Lispro (Eli Lilly) and Aspart (Novo Nordisk) are already in the market while glulisine (Sanofi-Aventis) is to be launched shortly. Glargine (Sanofi-Aventis) and Detemir (Novo Nordisk) are the long acting analogues available. Ultralente is now withdrawn. Regular insulin is available as a clear solution at neutral pH. 0.4% of zinc is added to allow the insulin molecules to self associate into hexamers. For the prevention of growth of micro-organisms phenol or m-cresol is added. Regular insulin has its onset of action within 15-30 min after subcutaneous injection, maximum activity peaks at 120-150 min while the action lasts for 6-8 hours. In order match the peaks of glucose and insulin, subcutaneous injection is advised to be taken 30-40 min prior to meals.

NPH or isophane insulin
Isophane insulin is known as NPH insulin as it was developed in Denmark at the Hagedorn Laboratory in 1940s. In order to prolong the action of insulin, a positively charged protein protamine is added in a molar ratio of 1:6 to regular insulin. It binds with the negatively charged insulin at neutral pH. Neutral pH value is achieved by use of phosphate buffer. Zn and m-cresol are also added. NPH insulin is slowly absorbed from subcutaneous tissue with peak at 5-7 hours and the action lasts for 12-15 hours. This insulin is most commonly used at bedtime to control fasting blood sugar.

Lente insulin
If zinc is added in excess amount (10 times that added in NPH), at neutral pH and if acetate is used as a buffer in stead of phosphate, it forms insoluble insulin-zinc complexes. This property is exploited for the production of lente insulins. The action profile of these preparations depends upon the physical conditions of insulin. Semilente is amorphous and has biphasic absorption kinetics with short duration of action. Ultralente is long acting crystalline suspension. These insulins cannot be mixed with regular insulin due to their zinc content and are not very popular.

Premixed formulations
Regular and NPH insulin are available in a premixed formulation with 30:70, 50:50, 25:75 proportions. These preparations are very popular as there is no mixing involved. Patients of type 2 diabetes on split mix regime may be shifted to premixed preparation if they are on approximately similar proportion.
**Pharmacology of Insulin analogues**

**Rapid acting Analogues**
Regular insulin when injected is in hexamer form, which slowly releases into monomers and is responsible for delay in the action. Analogues are synthesized by modifying the amino acid sequence so as to keep insulin molecule in monomeric form which has rapid onset of action and peak resembling physiology. After subcutaneous injection the action starts at 30 min, peaks at 60-90 min and is over by 4 hours. This action profile is very efficient in controlling postprandial glycemic excursions without any risk of delayed hypoglycemia.

**Long acting analogues**
Long acting analogues are designed in an attempt to obtain a steady basal insulin level without any peak unlike NPH, which has a risk of late night hypoglycemia. In insulin glargine, the amino acids sequence is altered so as to change isoelectric pH of insulin to 7.4 from 5.4. It is clear and soluble at an acidic pH. After injection in subcutaneous space it gets precipitated and is released slowly, making its action last for even more than 24 hours. Most of the patients require a single dose for basal cover. Insulin detemir has a long action by virtue of a fatty acid chain attached to it.

**Premixed Analogue preparations**
Insulin glargine cannot be mixed with any other insulin by virtue of its acidic pH and high Zn content i.e. 30 µg/ml. Premixed preparations are available with protamine insulin. In these preparations the protamine-insulin part has to be formulated with the same insulin analogue like lispro or aspart. These preparations are available in 25:75, 30:70, 50:50 proportions.

**Insulin analogues**

**Insulin glargine**
Insulin glargine have substitution of glycine for asparagine at A21 and two arginines added to the carboxy terminal of B chain. The arginine amino acids shift the isoelectric point from a pH of 5.4 to 6.7, making the molecule more soluble at an acidic pH, allowing for the subcutaneous injection of a clear solution. The asparagine substitution prevents deamidization of the acid-sensitive glycine at acidic pH. In the neutral subcutaneous space, higher-order aggregates form, resulting in a slow, peakless dissolution and absorption of insulin from the site of injection. It can achieve a peakless level for at least 24 hours.

**Insulin detemir**
It is an insulin analogue in which a fatty acid (myristic acid) is bound to the lysine amino acid at position B29. It is quickly absorbed after which it binds to albumin in the blood through its fatty acid at position B29. It then slowly dissociates from this complex.

**Insulin lispro**
Engineered through recombinant DNA technology, the penultimate lysine and proline residues on the C-terminal end of the B-chain are reversed. This modification does not alter receptor binding, but blocks the formation of insulin dimers and hexamers. This allowed larger amounts of active monomeric insulin to be immediately available for postprandial injections.

**Insulin aspart**
It was created through recombinant DNA technology so that the amino acid, B28, which is normally proline, is substituted with an aspartic acid residue. This analogue has increased charge repulsion, which prevents the formation of hexamers, to create a faster acting insulin.

**Insulin glulisine** is a rapid-acting insulin analogue that differs from human insulin in that the amino acid asparagine at position B3 is replaced by lysine and the lysine in position B29 is replaced by glutamic acid.
Types of insulin (Conventional) [Physical Appearance & Colour Code]

**Conventional**

Regular insulin – it is clear and watery, short acting.

NPH insulin – Cloudy, intermediate acting. Semelente, Lente and Ultralente are insulin formulations with varying concentration of zinc, their actions being short, intermediate and long acting respectively.

Premixed preparations – with regular and NPH insulin mixed in fixed proportions viz. 30/70, 50/50, 25/75 are available. These combinations are not physiological. They should be used if there is doubt about patient’s compliance or feasibility of mixing insulins.

**Insulin Analogues**

Insulin analogues have been synthesized by modifying structure of insulin so the action profile mimics physiology.

Rapid acting analogues – Aspart, Lispro, Glulisine

Long acting analogues – Giargine & Detemir

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**diabetes**

Diabetes mellitus (DM), or simply diabetes, is a group of metabolic diseases in which a person has high blood sugar, either because the pancreas does not produce enough insulin, or because cells do not respond to the insulin that is produced. This high blood sugar produces the classical symptoms of polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger). There are three main types of diabetes mellitus (DM).

- **Type 1 DM** results from the body’s failure to produce insulin, and currently requires the person to inject insulin or wear an insulin pump. This form was previously referred to as “insulin-dependent diabetes mellitus” (IDDM) or “juvenile diabetes”.

- **Type 2 DM** results from insulin resistance, a condition in which cells fail to use insulin properly, sometimes combined with an absolute insulin deficiency. This form was previously referred to as non insulin-dependent diabetes mellitus (NIDDM) or “adult-onset diabetes”. DM type 2 is characterized by high blood glucose in the context of insulin resistance and relative insulin deficiency. This is in contrast to diabetes mellitus type 1, in which there is an absolute insulin deficiency due to destruction of islet cells in the pancreas.

- The third main form, gestational diabetes occurs when pregnant women without a previous diagnosis of diabetes develop a high blood glucose level. It may preceed development of type 2 DM.

Other forms of diabetes mellitus include congenital diabetes, which is due to genetic defects of insulin secretion, cystic fibrosis-related diabetes, steroid diabetes induced by high doses of glucocorticoids, and several forms of monogenic diabetes.

Untreated, diabetes can cause many complications. Acute complications include diabetic ketoacidosis and nonketotic hyperosmolar coma. Serious long-term complications include cardiovascular disease, chronic renal failure, and diabetic retinopathy (retinal damage). Adequate treatment of diabetes is thus important, as well as blood pressure control and lifestyle factors such as stopping smoking and maintaining a healthy body weight.

All forms of diabetes have been treatable since insulin became available in 1921, and type 2 diabetes may be controlled with medications. Insulin and some oral medications can cause hypoglycemia (low blood sugars), which can be dangerous if severe. Both types 1 and 2 are chronic conditions that cannot be cured. Pancreas transplants have been tried with limited success in type 1 DM; gastric bypass surgery has been successful in many with morbid obesity and type 2 DM. Gestational diabetes usually resolves after delivery.
Insulin

- The human insulin protein is composed of 51 amino acids, and has a molecular weight of 5808 Da. It is a dimer of an A-chain and a B-chain, which are linked together by disulfide bonds.
- Within vertebrates, the amino acid sequence of insulin is strongly conserved. Bovine insulin differs from human in only three amino acid residues, and porcine insulin in one. Even insulin from some species of fish is similar enough to human to be clinically effective in humans. Insulin in some invertebrates is quite similar in sequence to human insulin, and has similar physiological effects. The strong homology seen in the insulin sequence of diverse species suggests that it has been conserved across much of animal evolutionary history. The C-peptide of proinsulin (discussed later), however, differs much more among species; it is also a hormone, but a secondary one.
- The primary structure of bovine insulin was first determined by Frederick Sanger in 1951. After that, this polypeptide was synthesized independently by several groups.

Insulin exists primarily as a monomer at low concentrations (~10^{-6} M) and forms dimers at higher concentrations at neutral pH. At high concentrations and in the presence of zinc ions insulin aggregates further to form hexameric complexes.

Insulin and others

Insulin is a peptide hormone, produced by beta cells of the pancreas, and is central to regulating carbohydrate and fat metabolism in the body. Insulin causes cells in the liver, skeletal muscles, and fat tissue to absorb glucose from the blood. In the liver and skeletal muscles, glucose is stored as glycogen, and in fat cells (adipocytes) it is stored as triglycerides.

Insulin stops the use of fat as an energy source by inhibiting the release of glucagon. With the exception of the metabolic disorder diabetes mellitus and metabolic syndrome, insulin is provided within the body in a constant proportion to remove excess glucose from the blood, which otherwise would be toxic. When blood glucose levels fall below a certain level, the body begins to use stored sugar as an energy source through glycogenolysis, which breaks down the glycogen stored in the liver and muscles into glucose, which can then be utilized as an energy source.

As a central metabolic control mechanism, its status is also used as a control signal to other body systems (such as amino acid uptake by body cells). In addition, it has several other anabolic effects throughout the body.

When control of insulin levels fails, diabetes mellitus can result.

As a consequence, insulin is used medically to treat some forms of diabetes mellitus. Patients with type 1 diabetes depend on external insulin (most commonly injected subcutaneously) for their survival because the hormone is no longer produced internally. Patients with type 2 diabetes are often insulin resistant and, because of such resistance, may suffer from a “relative” insulin deficiency. Some patients with type 2 diabetes may eventually require insulin if other medications fail to control blood glucose levels adequately. Over 40% of those with Type 2 diabetes require insulin as part of their diabetes management plan.
Physiological effects of insulin

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

• Control 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:

• Increased glycogen synthesis – insulin forces storage of glucose in liver (and muscle) cells in the form of glycogen; lowered levels of insulin cause liver cells to convert glycogen to glucose and excrete it into the blood. This is the clinical action of insulin, which is directly useful in reducing high blood glucose levels as in diabetes.
• Increased lipid synthesis – insulin forces fat cells to take in blood lipids, which are converted to triglycerides; lack of insulin causes the reverse.
• Increased esterification of fatty acids – forces adipose tissue to make fats (i.e., triglycerides) from fatty acid esters; lack of insulin causes the reverse.
• Decreased proteolysis – decreasing the breakdown of protein
• Decreased lipolysis – forces reduction in conversion of fat cell lipid stores into blood fatty acids; lack of insulin causes the reverse.
• Decreased gluconeogenesis – decreases production of glucose from nonsugar substrates, primarily in the liver (the vast majority of endogenous insulin arriving at the liver never leaves the liver); lack of insulin causes glucose production from assorted substrates in the liver and elsewhere.
• 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; lack of insulin inhibits absorption.
• Increased potassium uptake – forces cells to absorb serum potassium; lack of insulin inhibits absorption. Insulin’s increase in cellular potassium uptake lowers potassium levels in blood. This possibly occurs via insulin-induced translocation of the Na+/K+-ATPase to the surface of skeletal muscle cells.
• Arterial muscle tone – forces arterial wall muscle to relax, increasing blood flow, especially in microarteries; lack of insulin reduces flow by allowing these muscles to contract.
• Increase in the secretion of hydrochloric acid by parietal cells in the stomach
• 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. 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 control of whole-body energy homeostasis in humans.

A chain

The insulin monomer is a compact globular structure with a hydrophobic core. Although the surface residues are primarily polar, there are two hydrophobic surfaces on each side of the molecule which are buried during the formation of dimers and 2-zinc hexamers. In the insulin fold, the A chain is a compact unit around which the B chain is wrapped.

The A chain consists of two anti-parallel stretches of imperfect alpha helices (A2 Ile - A8 Thr and A13 Leu - A19 Tyr) which are joined by a turn from A9 Ser to A12 Ser, stabilized by the A6-A11 disulphide. The A chain lies in a plane in which the N and C terminii are brought to the same side, bringing A2 Ile and A19 Tyr into van der Waals contact.
**B chain**

The B chain consists of an alpha-helix (B9 Ser - B19 Cys) from which both N and C terminii residues extend. The glycine residues at B20 and B23 allow the chain to fold back on itself in an approximate V-shape, and this brings the C terminal residues B24 Phe and B26 Tyr into van der waals contact with B15 Leu and B11 Leu of the alpha-helix.

**AB dimer**

The insulin fold is formed when interchain disulphides at A7 and A20 form interchain disulphides with the B chain cysteines at B7 and B19 respectively. The (A7-B7) disulphide is fully exposed on the surface of the molecule, whereas the (A20-B19) disulphide is part of the hydrophobic core. Burial of the intrachain (A6-A11) disulphide and the non-polar side chains of A16 Leu, B11 Leu, B15 Leu, A2 Ile and B24 Phe provides the hydrophobic interior stabilising the fold. The N termini residues of the B chain are folded across and run anti-parallel to the turn in the A chain, giving rise to hydrogen bonding between A11 Leu and B4 Gln, A7 Cys and B5 His, and between A19 Tyr and B25 Phe. Further stability is also provided by a salt bridge between the polypeptide chains at A11 Cys and B4 Gln, between the A7 carbonyl oxygen and the B5 His side chain, and between the A19 carbonyl oxygen and the B25 back-bone nitrogen. Further stability is also provided by a salt bridge between B29 Lys and A4 Glu and between the positively charged B22 Arg side chain and the negatively charged A21 terminal carboxyl group (in molecule 1 only).
In solution at neutral pH and at physiological concentrations (about 1 ng/ml) insulin exists as a monomer, and it is the monomer which is the active form of the hormone. At higher concentrations at acid or neutral pH (in the absence of zinc) the insulin monomer self-associates to form dimers and (in the presence of zinc) hexamers. Studies of insulin analogues demonstrate how activity depends on the integrity of the insulin fold, and also allow mapping of the interactive residues on the surface of the molecule. The central residues responsible for hexamer formation include B10 His, which binds zinc ions, and B14 Ala, B17 Leu, B20 Gly and A13 Leu, which are involved in close-packed hydrophobic interactions. The ability to form dimers is mediated by hydrophobic interactions involving B8 Gly, B9 Ser, B12 Val, B13 Glu, B16 Tyr, B24 Phe, B25 Phe, B26 Tyr, B27 Thr and B28 Pro. The B chain of one monomer packs against the B chain of the second monomer and further stability is provided by hydrogen bonding between the antiparallel beta-strands B24 - B26 of each molecule. The packing arrangement in the dimer results in perturbations of side-chain and main-chain structure, such that the monomers differ slightly in conformation. The B25 Phe side-chain of molecule 2 is squeezed from its pocket in molecule 1 and appears on the monomer-monomer interaction surfaces, as indicated below.