Diabetes

Insulin

Analyte Information

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Introduction

Insulin plays a key role in the regulation of glucose utilization. Insulin is a polypeptide hormone, produced by Langerhans islets β-cells in pancreas. This hormone is composed of 51 amino acids with molecular weight of 5808 Da. It is a dimer of an A-chain and a B-chain, which are linked together by two disulfidic bridges.

Insulin, also called as storage hormone, is a hormone which plays a key role in the regulation of blood glucose levels. A lack of insulin, or an inability to adequately respond to insulin, can each lead to the development of the symptoms of diabetes. In addition to its role in controlling blood sugar levels, insulin is also involved in the storage of fat.

In the liver, insulin promotes glycogen synthesis from glucose by stimulating glycogen synthetase and inhibiting glycogen phosphorylase. In contrast, insulin induces a rapid uptake of glucose in muscle and fat tissue. As a consequence, muscle converts glucose to glycogen. In adipose tissue, glucose is converted to fatty acids for storage as triglyceride. Insulin also stimulates the uptake of amino acids into muscle.

Despite large fluctuations in the supply and demand of carbohydrates, the concentration of glucose in the blood is normally maintained within a narrow range by hormones that modulate the glucose movement in and out of the circulation. Insulin decreases blood glucose, but on the other hand there are the counter-regulatory hormones (glucagon, epinephrine, cortisol, and growth hormone), which increase blood glucose concentrations. Glucagon, as the main opponent of insulin, is also produced in pancreas. As glucagon is responsible for increase of the blood sugar it is also called as the mobilization hormone.

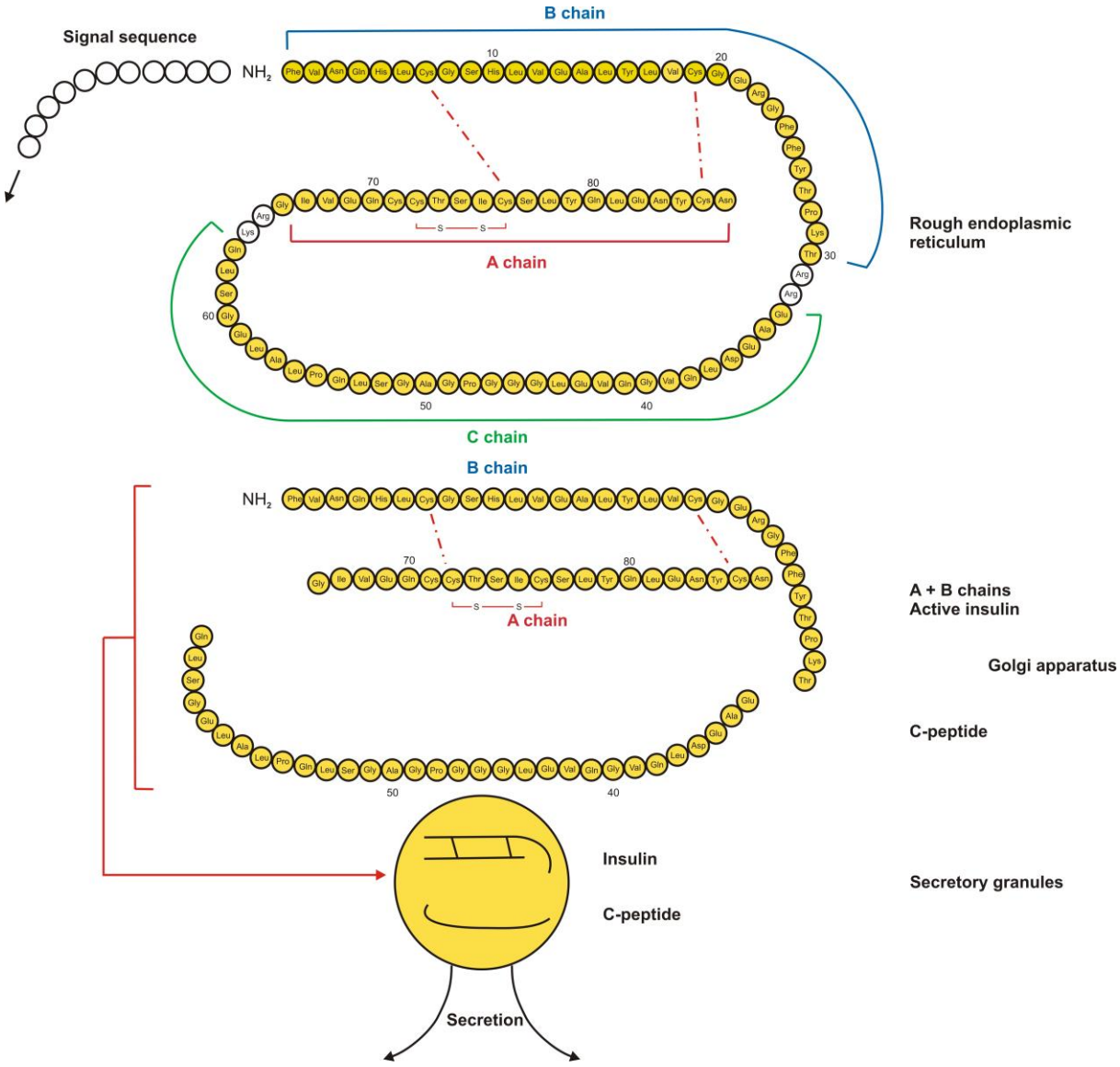
Biosynthesis

Insulin is secreted primarily in response to elevated blood glucose concentrations. Insulin is "in charge" of facilitating glucose entry into cells. Some neural stimuli (e.g. sight and taste of food) and increased blood concentrations of other fuel molecules, including amino acids and fatty acids, also promote insulin secretion.

Human insulin, with its 51 amino acids, is structurally homologous to insulin-like growth factors 1 and 2 (IGF-1 and IGF-2) and also to the ovarian hormone, relaxin. It is synthesized in the β-cells of the pancreatic islets.

Pre-proinsulin is made up of a signal sequence (approximately 23 amino acids that are rapidly cleaved after hormone synthesis), and B chain, connecting (or C) peptide and A chain. After cleavage of signal sequence Proinsulin is created. A and B chains are joined together by two disulfide bonds between common cysteine amino-acid residues. The connecting peptide is essential to the formation of these disulfide bonds and is split off in the Golgi apparatus leaving the active insulin molecule composed of joined A and B chains. The cleaved C-peptide is co-secreted with insulin and both are released into blood stream in equimolar quantity. Previously considered to have no physiological role, C-peptide is now recognized to have G-protein-coupled cellular receptors and it is likely to have some function in regulation of blood flow and renal function.

Fig.1: Insulin and C-peptide synthesis



Metabolism

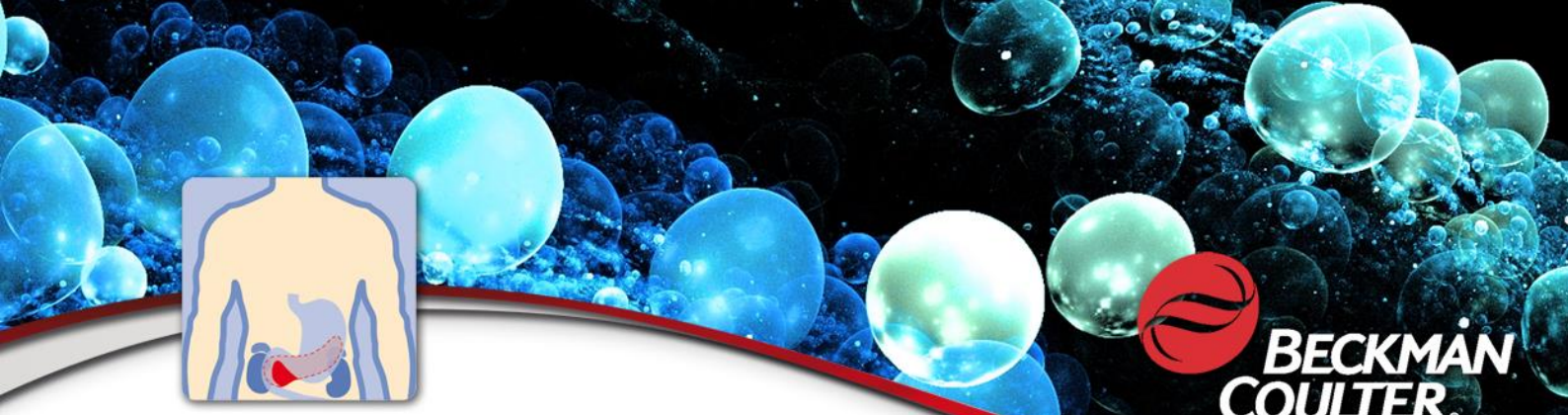
The total insulin production per day is about 20 – 40 IU in healthy individuals, out of which about a half represents basal secretion, and the second half stimulated secretion. **Basal insulin** secretion ranges between 0.25 – 1.5 IU per hour, and this secretion is **independent** from food intake, thus proceeding also at night. Its significance consists above all in gluconeogenesis and other metabolic processes regulation when fasting. Increased basal secretion appears in the morning and in the late afternoon as a reaction to contra-insular hormone circadian rhythms.

Stimulated insulin secretion represents insulin released in the response to food intake and plays a key role in the postprandial glucose level regulation. This secretion consists of two phases – the **early one**, appearing after secretory impulse (glucose, glucagon, arginin) in about first 10 minutes (the peak being between 3 – 6 minutes). Insulin released in the early phase represents endogenous stock of ready insulin stored in β-cells secretory granules. The insulin secretion **late phase** comes slowly after 10 minutes, and endures for at least 60 minutes or for the period of secretory stimulus duration. This secretory phase is a manifestation of a new insulin formation.

Both type I. and type II. diabetic patients exhibit characteristic changes in values of insulin when fasting, as well as in the early and the late phases after secretory stimulus application, or after mixed food intake.

Insulin is transported from Langerhans islets to liver via v. portae. In liver, insulin is being taken in to variable extent - to 60 % in the average – depending on metabolic situation. For this reason, insulin concentration measured in periphery blood is 2.5 – 3 times lower than that in portal blood, and thus does not correspond to actual insulin secretion by β-cells.

Insulin inactivation takes place in liver and kidneys in particular. On the first pass through the portal circulation, approximately 50% of the insulin is extracted by the liver, where it is degraded. In kidneys, insulin is filtrated through the glomeruli, reabsorbed, and degraded in the proximal tubule. The half-time of insulin in the circulation is short, between 4-5 minutes.



Physiological function

Insulin is a key player in the control of intermediary metabolism, 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.

Tab.1: Major effects of Insulin

Liver	Muscle	Adipose tissue
+ glycogen synthesis	**+ glucose uptake**	**+ glucose uptake**
+ glycolysis	**+ amino acid uptake**	**+ free fatty acid uptake**
- glycogenolysis	- proteolysis	- lipolysis
- gluconeogenesis		
- ketogenesis		

+ stimulates - inhibits

Insulin and its role in glucose metabolism:

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. The major transporter used for uptake of glucose (called GLUT4) is made available in the plasma membrane through the action of insulin. Binding of insulin to specific receptors in the plasma membrane leads rapidly to fusion of present cytoplasmic 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.

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 later use.

Insulin and its role in lipid metabolism:

Insulin promotes synthesis of fatty acids in the liver. As mentioned above, insulin stimulates synthesis of glycogen in the liver. However, when glycogen is accumulated to high levels (roughly 5% of liver mass), further synthesis is suppressed. When the liver is saturated with glycogen, any additional glucose taken up by hepatocytes is divert 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.

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, is 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. Insulin indirectly stimulates accumulation of fat in adipose tissue.

Other Notable Effects of Insulin:

Insulin 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.

Levels

Basal plasma insulin is higher in the morning than later in the day, and its response to glucose is also greater in the morning and least about midnight. When a tolerance glucose test is given in the afternoon, higher glucose levels occur than when the test is performed early in the day.

In healthy individuals, insulin is secreted in a pulsatile fashion with glucose and insulin being the main signals in the feedback loop. As mentioned above, glucose elicits the release of insulin from the pancreas in two phases, early and late. Early release of insulin appears 10 minutes after stimuli, late phase follows-up of early one and takes within 60-120 min.

Relative insulin resistance can be found during pregnancy.

Values are consistently higher in plasma than in serum, thus serum is preferred for the detections.

Typical Insulin levels are given in table 2.

For each assay, relevant reference values are given in the appropriate Instructions for Use (IFU).

Tab.2: Typical insulin levels

Immunoreactive insulin (free insulin + insulin bound to anti-insulin antibodies)

Specimen (serum, fasting)	**Reference range** (µU/mL)
Child (2-12)	< 10
Adult	< 35

Free insulin

Specimen (serum, fasting)	**Reference range**(µU/mL)
Infant and pubertal child	< 13
Pubertal child and adult	< 17

Equation for the conversion of units for Insulin: 1 µU/mLx6.945= pmol/L

Free and immunoreactive insulin

Antibodies to insulin develop in almost all patients who are treated with exogenous insulin. Insulin antibodies not only interfere with insulin assays. They also reduce the amount of active insulin present in circulation by binding the exogenous insulin. In insulin-dependent diabetic patients, free (bioactive) insulin levels are usually 10% or less of total insulin. In such case, free insulin is measured after PEG precipitation of insulin antibodies with bound insulin.

The widespread use of human insulin, plus improvement in the purity of porcine and bovine insulin, has led to significant, but not complete, reduction in the production of interfering antibodies.

Diagnostic utility – prospects and possibilities

Increased insulin secretions

Increased insulin secretion is usually found in situations, when insulin effectiveness is decreased from certain reasons (it is called insulin resistance). This situation is most frequently encountered in tissues as a result of age, overweight (obesity), diabetes mellitus (DM) type 2, and possibly also because of the lack of physical activity. Further it is found in organic overproduction of contra insular hormones (Cushing syndrome, acromegaly, pheochromocytoma, and polycystic ovary syndrome - PCOS), or in their artificial increase at certain therapeutical procedures (e.g. glucocorticoid therapy); more rarely it appears at certain receptor disorders.

Hyperinsulinaemia and insulin-resistance is considered to be a significant pathogenic agent at the pathogenesis of several disorders: in addition to already mentioned DM type 2 and obesity, it is found in atherosclerosis, in particular in its precocious manifestation, in metabolic (earlier Reaven's) syndrome, hypertension, in certain forms of hyperlipoproteinemia, in haemocoaggulation, and fibrinolysis disorders, and others. Additionally, some widely used antihypertensive drugs - beta-blockers, thiazide diuretics, niphedipin - may also worsen insulin resistance and lead to hyperinsulinaemia. This fact should be taken into account in diabetic, obese, and metabolic syndrome suffering patients, as well as in all other states with insulin resistance.

Insulin high levels are typical for insulinoma, a tumour formed by β-cells and secreting insulin without any remarkable relation to nutrition stimuli, manifesting itself by deep hypoglycaemia attacks. Insulinoma hyper secretion of insulin is accompanied with higher proinsulin proportion (> 20%), as well as with higher C-peptide values. On the other hand, increased insulin levels at its exogenous application (hypoglycaemia factitia) are not accompanied with increased levels of proinsulin, and also C-peptide levels found in these individuals are very low as a result of inhibited endogenous insulin secretion.

Decreased insulin secretions

Decreased or immeasurable insulin levels are typical for DM type 1, where β-cells are gradually destroyed by autoimmune inflammation. Also in the course of long lasting DM type 2, the secretory capacity of β-cells typically decreases gradually, with subsequent drop in insulin levels in blood. In the early phases, the insulin secretion in both types of DM is impaired primarily in the first stage. The late secretory phase impairment is also typical for the far gone and the final stages in both types of diabetes.

Insulin low levels are also encountered in severe inflammations of pancreas external secretory part, in states after pancreas resections, or in haemochromatosis – these states represents frequent causes of secondary diabetes.

Elevated insulin levels

- insulinoma (pancreatic islet cell tumor)
- DM type 2
- liver disease
- acromegaly
- Cushing´s syndrome
- obesity (may be twice of normal)
- metabolic syndrome
- dystrophia myotinica
- familial fructose and galactose intolerance

Diminished insulin levels

- DM type 1
- late phase of DM type 2
- hypopituitarism

Diagnostic utility – practical applications

From a clinical point of view, the measurement of insulin has little value except in the diagnosis of fasting hypoclycemia. Insulin assays are not included in diagnosis of diabetes mellitus so often.

Insulin levels can be determined in the fasting state, postprandially or during stimulatory or inhibitory tests. For stimulation, either oral or intravenous glucose tolerance test (OGTT or IVGTT) is performed, or glucagon, tolbutamid or arginine are administrated intravenously. Production of insulin can be suppressed by fasting or infusion of somatostatin.

However, insulin measurement during oral glucose tolerance test (OGTT) may help in the diagnosis of early hyperglycemic DM.

Diagnosis of insulinoma

Insulin and glucose response to 72-h fasting test: during prolonged fasting, when the patients glucose is reduced to <40 mg/dL, elevated insulin levels accompanied by elevated levels of C-peptide suggest insulinoma.

Differential diagnosis of some cases of DM type 1 and 2 and LADA (Latent Autoimmune Diabetes of Adults)

Insulin test is done as supplementary one.

Diagnosis and study of conditions associated with insulin resistance and metabolic syndrome

Determination of β-cell secretory reserve after administration of test meal or during OGTT (IVGTT) or hyperglycemic clamp

Insulin measurements during OGTT may help in the diagnosis of early prehyperglycemic DM.

Characterization and follow up of individuals with glucose tolerance disorder

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