Appraisal on causes and treatment of Diabetes Mellitus

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ABSTRACT

Diabetes mellitus is a common metabolic disorder, which is one of the top five global diseases leading to death. Diabetes mellitus is of four types: Type 1 diabetes mellitus, Type 2 diabetes mellitus, gestational diabetes mellitus and other specific types which are mainly caused due to genetic defects or by the usage of drugs like antipsychotics. The pathophysiology involves insulin deficiency, interaction between genetic and environmental factors, insulin resistance, insulin sensitivity coupled with inadequate insulin response. This disease can be treated by synthetic drugs like sulfonylureas, non-sulfonylureas, thiazolidinediones, α-glucosidage inhibitors, biguanides, insulin, inhaled insulin, and natural drugs. Different animal models are used in preclinical studies of diabetes mellitus for the discovery and development of new drugs. It can be induced in experimental animals by different methods like chemical, surgical, and viral methods.

Keywords: Diabetes mellitus, pathophysiology, treatment, animal models, induction methods.
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INTRODUCTION

It is a disease affecting approxmately 150 million people in 2000, which is predicted to rise to 220 million in 2010 worldwide. Diabetes and its associated complications have become a public health problem of considerable magnitude [1] is one of the top five global leading causes of death. In the year 2000, the excess global mortality attributable to diabetes and its late complications was estimated to be 2.9 million deaths, equivalent to 5.2% of all deaths [2]. Individuals with family history of diabetes mellitus are at higher risk of developing the condition and lifestyle modification can help reduce this risk [3].

Diabetes Mellitus is a common metabolic and a multi-system disorder comprising metabolic and vascular abnormalities resulting from insulin deficiency with or without insulin resistance [4, 5]. Classification of Diabetes: Diabetes mellitus is mainly divided into four main types:

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1. Type 1 (type1A, type 1B): ß-cell destruction with little or no endogenous insulin
                                    secretory capacity
                                    Autoimmune (type1A)
                                    Idiopathic (type 1B)
2. Type 2: Ranges from relative insulin deficiency to disorders of insulin secretion and insulin resistance.
3. Gestational diabetes
4. Other specific types: Genetic defects of ß-cell function
    Genetic defects in insulin secretion
    Diseases of the exocrine pancreas
    Endocrinopathies
    Drug-induced or chemical induced Infections (congenital rubella,cytomegalovirus and others)
    Uncommon forms of immune mediated diabetes
    Other genetic syndromes sometimes associated with diabetes [6].

Type I Diabetes Mellitus:
Type-1 diabetes is also known as insulin-dependent diabetes mellitus (IDDM) or juvenile-onset diabetes. In this type of diabetes, the pancreas produces little or no insulin [7].This type of diabetes represents around 10–15% of all cases of diabetes [8]. Type 1 Diabetes mellitus (T1D) develops as a result of the synergistic effects of genetic, environmental and immunologic factors that ultimately destroy the pancreatic β cells. T1D results from autoimmune β cell destruction, which leads to insulin deficiency [9].
Type 1 Diabetes Mellitus is further sub classified into two types:
Type 1A which is associated with the presence of islet cell autoantibodies, and
Type 1B characterized by the absence of such antibodies [10].

Pathophysiology of Type1 Diabetes:
Hyperglycaemia and ketonaemia constitute the most important sequale of insulin deficiency in type I diabetes. Hyperglycaemia is essentially due to varying combination of lack of glucose utilization and over production of glucose through accelerated gluconeogenesis. Ketonaemia is a result of impaired lipogenesis and enhanced lipolysis leading to a release of free fatty acids into the circulation. The synthesis of triglycerides from free fatty acids is regulated by the molar ratio of insulin and glucagon in the liver. There is a secondary hyperglucogonaemia in diabetes mellitus leading to a reduced activity of malonylCoA, thereby affecting triglyceride synthesis. In addition, enhanced activity of carnitine acyl transferase facilitates free fatty acid entry into mitochondria, where through beta oxidation excessive amounts of acetyl CoA are generated, leading to the formation of large amounts of ketone bodies which are utilized only in muscle and other peripheral tissues [11].

Type 2 Diabetes Mellitus:
Type-2 diabetes is also known as non-insulin-dependent diabetes mellitus (NIDDM) or adult onset diabetes [7]. It accounts for 85–90% of all cases of diabetes [3]. The incidence of type 2 diabetes mellitus is increasing worldwide [12]. It is a heterogeneous condition characterized by the presence of both impaired insulin secretion and insulin resistance [13]. It is a complex multifactorial disease involving genetic predisposition and various environmental factors [14]

affecting the length and quality of life of an affected individual [15]. Although the genetic basis of type 2 diabetes has yet to be identified, there is strong evidence that modifiable risk factors such as obesity and physical inactivity are the main nongenetic determinants of the disease [12]. This type of diabetes is characterized by 2 major pathophysiologic defects: insulin resistance, which results in increased hepatic glucose production (HGP) and decreased glucose disposal, and impaired β-cell secretory function (both basal and glucose stimulated) [16]. Type 2 diabetes mellitus is a common disease with substantial associated morbidity and mortality. Up to 80% of patients with type 2 diabetes will develop or die of macrovascular disease [17].

Pathophysiology of Type Ii Diabetes:
Due to complex interaction among multiple susceptibility genes and between genetic and environmental factors, genetic analysis of diabetes is difficult [18].
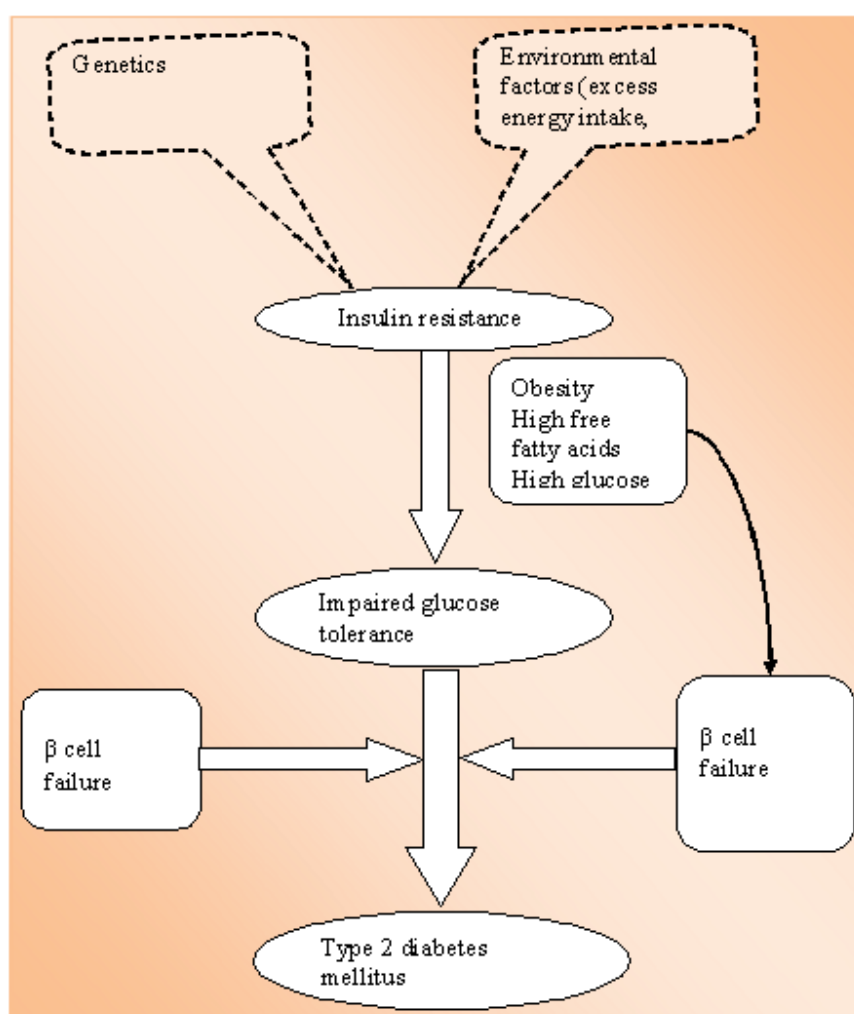

Figure 1
Type 2 diabetes is characterized by 2 major pathophysiologic defects: insulin resistance, which results in increased hepatic glucose production (HGP) and decreased glucose disposal, and impaired β-cell secretory function [16]. Under normal conditions, insulin binds to insulin

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receptors (IRs) on target cells, resulting in cellular cascades that promote intracellular glucose transport and metabolism. Insulin resistance in target tissues (muscle and liver) is observed early in the disease process and is rapidly followed by decreased insulin secretion as a result of progressive pancreatic β-cell dysfunction. This combination leads to overt diabetes with fasting and postprandial hyperglycemia. Insulin resistance is a common defect in type 2 diabetes, with the liver continuing to produce glucose and the uptake of glucose into muscle being impaired [19]. Loss of the acute insulin response to a carbohydrate load, a prototypical defect that occurs early in the natural course of the disease, generally when fasting plasma glucose levels reach 115 mg/dL, leads to postprandial hyperglycemia [16].

Insulin resistance in the hepatocyte and peripheral tissues, particularly skeletal muscle, leads to unrestrained HGP and diminished insulin-stimulated glucose uptake and utilization. The underlying mechanism may be related to defects in insulin receptor binding, decreased numbers of insulin receptors, or post receptor attenuation of insulin action. Hyperglycemia itself further impairs insulin secretion and increases insulin resistance, in part by down regulation of the glucose transport system in β cells and insulin-sensitive tissues. These effects of chronically elevated blood glucose levels are referred to as glucose toxicity. In addition, the high circulating free fatty acid levels associated with diabetes further aggravate insulin resistance and may adversely affect β-cell secretion, a phenomenon known as lipotoxicity [16].

A complex interaction between genetic and environmental factors leads to insulin resistance resulting in impaired glucose tolerance due to obesity, high free fatty acids, high glucose which also acts on β cells causing failure of β cells leading to type 2 diabetes .

Gestational diabetes:
Gestational diabetes mellitus (GDM) is defined as a state of carbohydrate intolerance of variable severity and evolution, which develops or is first detected during pregnancy [20] and is present in approximately 4%–7% of pregnancies [21]. Classically, it is associated with an increase in perinatal morbidity and mortality, as well as a greater frequency of long-term complications in the mother and her offspring [20]. GDM is a very strong risk factor for the development of type 2 diabetes in later life [22]. After pregnancy, 5% to 10% of women with GDM are found to have type 2 diabetes, and women with GDM have a 20% to 50% probability of developing diabetes in the 5 to 10 years following pregnancy [21]. Gestational diabetes is associated with an excess incidence of fetal macrosomia, pre-eclampsia, and cesarean section in the index pregnancy and on long-term follow-up non-insulin-dependent diabetes mellitus develops in approximately one third of women who have ever had gestational diabetes. Gestational diabetes is usually diagnosed on the basis of an oral glucose-tolerance test [22].

Pathophysiology of gestational diabetes:
Pregnancy is normally attended by progressive insulin resistance that begins near mid-pregnancy and progresses through the third trimester to levels that approximate the insulin resistance seen in type 2 diabetes. The insulin resistance of pregnancy may result from a combination of increased maternal adiposity and the insulin-desensitizing effects of hormones made by the placenta [23]. Decreased maternal pregravid insulin sensitivity (insulin resistance) coupled with an inadequate insulin response is the pathophysiological mechanism underlying the development of gestational diabetes. Insulin-regulated carbohydrate, lipid and protein metabolism are all affected to a

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variable degree. Women with GDM had higher insulin resistance, especially those who needed insulin therapy. The lipid profile in GDM was related to the level of insulin resistance. Insulin resistance and beta-cell dysfunction are thought to be major determinants of its development. Its pathophysiological mechanism in many ways resembles that of type 2 diabetes [24].

GDM is a form of hyperglycemia. In general, hyperglycemia results from an insulin supply that is inadequate to meet tissue demands for normal blood glucose regulation. The majority of women with GDM appear to have cell dysfunction that occurs on a background of chronic insulin resistance. Pregnant women with GDM tend to have even greater insulin resistance than normal pregnant women. Differences in whole-body insulin sensitivity tend to be small in the third trimester, owing to the marked effects of pregnancy itself on insulin resistance [25]. GDM results from an endogenous insulin supply that is inadequate to meet tissue insulin demands. Beta cell dysfunction in women diagnosed with GDM may fall into one of three major categories: 1) autoimmune, 2) monogenic, or 3) occurring on a background of insulin resistance. The loss of the first-phase insulin response leads to postprandial hyperglycemia, whereas impaired suppression of hepatic glucose production is responsible for fasting hyperglycemia. Because insulin does not cross the placenta, the fetus is exposed to the maternal hyperglycemia [23]. After delivery, when the acquired insulin resistance of pregnancy abates, women who had GDM end up, on average, with considerably greater insulin resistance than normal women. This finding, which has been consistent across studies in which whole-body insulin sensitivity has been measured directly, indicates that most women who develop GDM have chronic insulin resistance [25]. Autoimmune or monogenic forms of diabetes should be considered in lean patients, who can rapidly develop overt diabetes after pregnancy. These monogenic forms of GDM account for < 10% of GDM cases [23].

Diagnosis of gestational diabetes mellitus:

Glucose challenge test (GCT):
In this test the level of blood sugar was estimated after the intake of 50 gm of glucose dissolved in 200 ml of water. If the blood sugar levels were greater than 140 mg%, the screening test was considered to be positive and then OGTT was performed for the confirmation of gestational diabetes [26].

Oral glucose tolerance test (OGTT):
In this test the blood samples were tested after 10-16 h of fasting and after the intake of 100 gm of glucose dissolved in 200-400 ml of water. The blood samples were tested at 1 h, 2 h and 3 h. The glucose values of fasting 105 mg/dl, 1 hour-190 mg/dl, 2 h-165 mg/dl and 3 h -145 mg/dl were considered normal. If the values were more than the normal values then gestational diabetes is confirmed [26].

4. **Other specific types**
Genetic defects of the β cell:
These conditions are associated with monogenetic defects in β-cell function. They are referred to as maturity-onset diabetes of the young and are characterized by impaired insulin secretion with minimal or no defects in insulin action [27].

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Genetic defects in insulin action:
These are abnormalities associated with mutations of the insulin receptor and may range from hyperinsulinemia and modest hyperglycemia to severe diabetes.

Diseases of the exocrine pancreas:
Any process that diffusely injures the pancreas can cause diabetes. Acquired processes include pancreatitis, trauma, infection, pancreatectomy, and pancreatic carcinoma.

Endocrinopathies:
Acromegaly, Cushing's syndrome, glucagonoma, and pheochromocytoma can all cause diabetes.

Drug- or chemical-induced diabetes:
This form of diabetes occurs with drugs or chemicals that affect insulin secretion, increase insulin resistance or permanently damage pancreatic β cells, as is seen with the administration of high dose of steroids [28].

Corticosteroids have profound effects on carbohydrate metabolism: stimulating liver to form glucose from amino acids and glycerol. In the periphery, corticoids decrease glucose utilization, increase protein breakdown and activate lipolysis, thereby providing amino acids and glycerol for gluconeogenesis. The net result is increase in blood glucose levels [29].

Infections:
Viral infections that may cause β-cell destruction include coxsackie virus B, cytomegalovirus, adenovirus, and mumps.
Genetic syndromes sometimes associated with diabetes are Down's syndrome, Klinefelter's syndrome, Turner's syndrome, and Wolfram syndrome.

Impaired glucose tolerance and impaired fasting glucose:
It was previously recognized that there exists an intermediate group of individuals whose glucose levels, although not meeting the criteria for diabetes, were too high to be considered normal. Members of this group have a condition called pre-diabetes, a term which encompasses both impaired fasting glucose and impaired glucose tolerance [27].

During diabetes, persistent hyperglycemia causes increased production of free radicals especially reactive oxygen species (ROS), for all tissues from glucose auto-oxidation and protein glycosylation. Free radicals are generated as by-products of normal cellular metabolism; however, several conditions are known to disturb the balance between ROS production and cellular defense mechanisms, which causes cell dysfunction and destruction resulting in tissue injury. The increase in the level of ROS in diabetes could be due to their increased production and/ or decreased destruction by nonenzymic and enzymic catalase (CAT), glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD)] antioxidants. The level of these antioxidant enzymes critically influences the susceptibility of various tissues to oxidative stress and is associated with the development of complications in diabetes. Diabetes produces disturbances of lipid profiles, especially an increased susceptibility to lipid peroxidation, which is responsible for increased incidence of atherosclerosis, a major complication of diabetes mellitus [4].

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Beta cells are very sensitive to cytotoxic stress because they express very little of the antioxidant enzymes. Hence, β-cell is at greater risk of oxidative damage than other tissues with higher levels of antioxidant protection. During pathogenesis of diabetes mellitus, oxidative and nitrosative stresses contribute to the destruction of insulin-producing beta cells. Moreover, it is believed that increased oxidative stress is one of the main factors in the etiology and complications of diabetes mellitus [30].

Diabetes mellitus is a disease marked by high levels of blood glucose resulting from a defect in insulin production, insulin action or both, that can lead to macro and micro vascular complications and is a chronic, life threatening condition that depends on medication, diet and life style modification to prevent long term complications [31]. This results primarily in elevated fasting and postprandial blood glucose levels. If this imbalanced homeostasis does not return to normal and continues for a protracted period of time, it leads to hyperglycemia that in due course turns into a syndrome called diabetes mellitus [32].
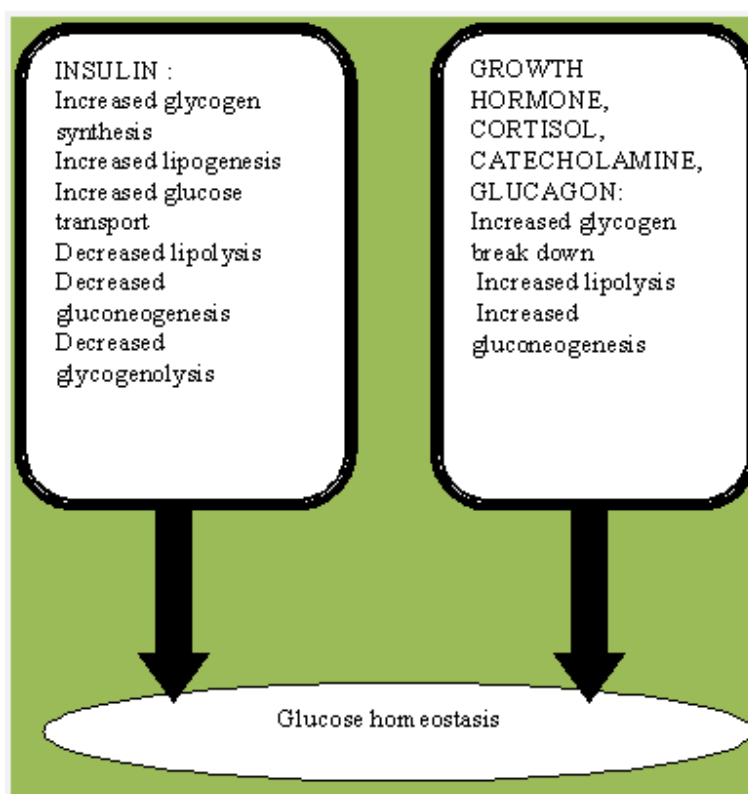

Figure 2

Insulin reduces the blood glucose levels by increasing glycogen synthesis, lipogenesis, glucose transport and decreasing the lipolysis, gluconeogenesis, glycogenolysis. Other hormones that elevates the blood glucose levels comprises of growth hormone, catecholamines, cortisol and glucagons by increasing the glycogen break down, lipolysis, gluconeogenesis

Diabetes mellitus effects on endocrine system:
It is a disorder of carbohydrate, protein and lipid metabolism associated with an absolute or relative insufficiency of insulin secretion accompanied by various degrees of insulin resistance

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[33]. Defects in carbohydrate metabolizing machinery and consistent efforts of the physiological systems to correct the imbalance in carbohydrate metabolism place an overexertion on the endocrine system, which leads to the deterioration of endocrine control. Continuing deterioration of endocrine control exacerbates the metabolic disturbances and leads primarily to hyperglycemia [32].

Insulin resistance:
Insulin resistance is defined as diminished tissue responses to insulin at one or more sites in the complex pathways of the hormone action, which is associated with hyperinsulinemia [34].
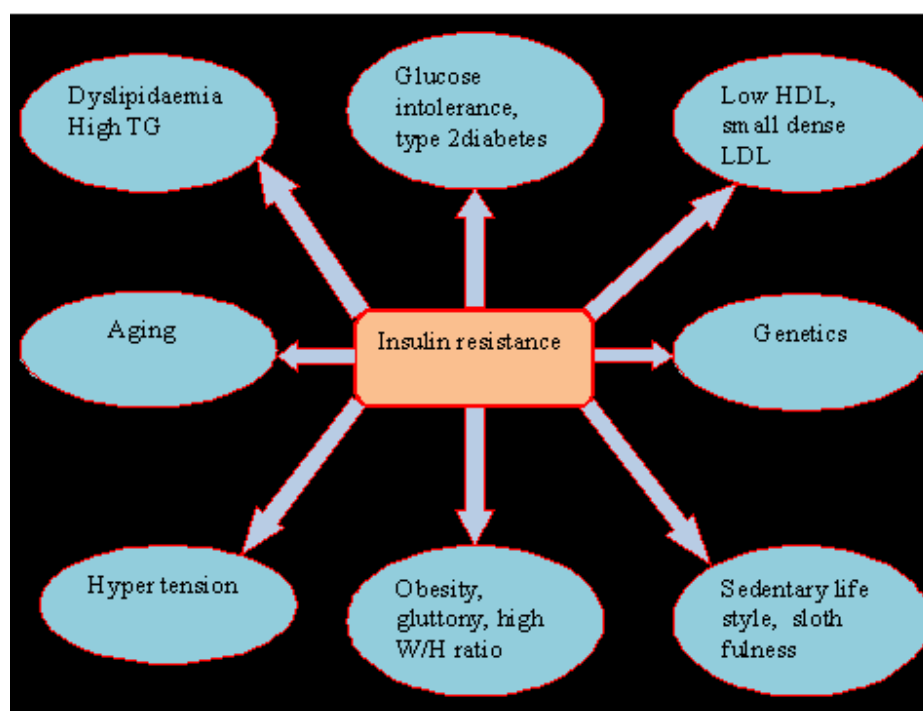

Figure 3: Factors leading to insulin resistance

Symptoms of diabetes mellitus:
Thirst, polyuria, fatigue, general malaise, infections, and blurred vision [32]. It is also characterized by hyperglycaemia, glycosuria, negative nitrogen balance and sometimes ketonaemia [35]. Over time patients with diabetes mellitus also develop symptoms related to major microvascular (i.e. retinopathy, nephropathy, neuropathy, diabetic foot problems) and macrovascular (i.e. cardiovascular disease, cerebro-vascular disease and peripheral vascular disease) complications [36].

Biochemical markers estimated in diabetes:
Glucose, cholesterol, triglycerides, high density lipo protein cholesterol (HDL), low density lipo protein cholesterol levels (LDL) are estimated in the diagnosis of diabetes mellitus [14].

Elevated blood pressure, elevated serum concentrations of glucose, total and low density lipoprotein (LDL) cholesterol and triglycerides are seen in diabetic conditions [14].

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Pharmacological treatment of diabetes mellitus:
Sulfonyl ureas:
These are classified as first and second-generation agents. The second generation agents differ in potency, safety, and pharmacokinetics. The second-generation agents are more potent and have better pharmacokinetic and safety profiles.

The first-generation agents include acetohexamide, chlorpropamide, tolazamide, and tolbutamide.

The second generation agents include glimepiride, glipizide, and glyburide.

Sulfonylureas lower FPG primarily by increasing the release of insulin from functioning pancreatic β cells [37]. The sulfonylureas bind to specific membrane receptors on β cells and inhibit adenosine triphosphate (ATP)-sensitive K^+ channels, resulting in membrane depolarization and Ca^{++} influx; with a release of insulin through exocytosis. The sulfonylureas stimulate early insulin release only, whereas glucose stimulates both early and late insulin secretion through a similar mechanism [38].

Hypoglycemia and weight gain are the most frequent side effects where as hypoglycemia is mainly seen because of the long half-lives of these agents [37].

Non-sulfonylurea secretagogues (meglitinide analogues; benzoic acid derivatives)
The meglitinide analogs, including nateglinide and repaglinide, are nonsulfonylurea secretagogues. These drugs also bind to K^+ ATP channels, albeit at a different site than traditional sulfonylureas. These agents stimulate the release of insulin from pancreatic β cells if glucose is present. In general, meglitinide analogs have much shorter half-lives than do sulfonylureas. Nateglinide is a new meglitinide analogue and a derivative of dphenylalanine. Nateglinide mimics physiologic insulin secretion. Dynamics seen in healthy individuals by increasing early phase insulin secretion into the portal vein and in that way increases hepatic glucose uptake as well as hepatic glucose suppression. In contrast to sulfonylureas and repaglinide, nateglinide is a more potent agent to restore early phase insulin release with less hypoglycemia episodes. Delayed hyperinsulinemia and an increased risk of hypoglycemia are the main side effects [37].

Thiazolidinediones:
Thiazolidinediones (TZDs) which include rosiglitazone and pioglitazone are chemically and functionally unrelated to the other classes of oral antidiabetic agents. A thiazolidine-2,4-dione structure is common to all agents.

Mechanism of action:
Thiazolidinediones are selective agonists for nuclear peroxisome proliferator-activated receptor-gamma (PPAR). The TZDs bind to PPAR, which, in turn, activate insulin-responsive genes that regulate carbohydrate and lipid metabolism. They require insulin to be present for their action. Thiazolidinediones exert their principal action by lowering insulin resistance in peripheral tissue, but an effect to lower glucose production by the liver has also been reported. Thiazolidinediones increase glucose transport into muscle and adipose tissue by enhancing the synthesis and

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translocation of specific forms of the glucose transporter proteins. The thiazolidinediones can also activate genes that regulate free fatty-acid (FFA) metabolism in peripheral tissue, thus lowering triglycerides and non-esterified fatty acid levels and inducing differentiation of adipocytes.

Adverse effects:
Weight gain, fluid retention that may be severe enough to exacerbate or precipitate heart failure. These drugs also cause gastro-intestinal disturbances, anemia, headache, visual disturbances, dizziness, haematuria, impotence; less commonly fatigue, insomnia, vertigo, hypoglycemia and proteinuria [6].

α-glucosidase inhibitors: acarbose
Mechanism of action:
These drugs do not target a specific pathophysiologic aspect of diabetes. This class of oral hypoglycemic agents competitively inhibits enzymes in the small intestinal brush border that are responsible for the breakdown of oligosaccharides and disaccharides into monosaccharides suitable for absorption. It works primarily on α-glucosidase, which is found predominantly in the proximal half of the small intestine. The intestinal absorption of carbohydrates is therefore delayed and shifted to more distal parts of the small intestine and colon. This retards glucose entry into the systemic circulation and lowers postprandial glucose levels. α-Glucosidase inhibitors act locally at the intestinal brush border and are not absorbed. They are excreted in feces [16].

The main side effects of α-glucosidase inhibitors are gastrointestinal disturbances such as, bloating, abdominal discomfort, diarrhea and flatulence occur in about 20% of patients.

α-Glucosidase inhibitors are contraindicated in patients with irritable bowel syndrome or severe kidney or liver dysfunction. Inflammatory bowel disease is a relative contraindication [16].

Biguanides: phenformin, metformin.
The anti-diabetic effect is unlikely to be due to increased insulin secretion for in obese and diabetic subjects, the administration of phenformin reduced the circulating insulin response to a glucose load. Furthermore, in human diabetics and in experimental animals, the administration of a biguanide reduces the quantity of insulin required to control hyperglycaemia. In some way, therefore, the biguanides appear to augment the hypoglycaemic effectiveness of circulating insulin, possibly by enhancing its stimulation of glucose utilization in muscle [39].

The commonest side-effects of the biguanides are upon the gastro-intestinal tract, causing a metallic taste in the mouth, loss of appetite, nausea, diarrhoea and abdominal cramps, anorexia. By starting with small doses and increasing the dose level slowly, the incidence of these side effects can be reduced [39].

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Table 1: oral hypoglycemic agents with mechanism of action and side effects

S. No	Oral antidiabetics	Mechanism of action	Side effects
1.	Sulfonylureas Glimiperide, Glipizide, Glipizide-gits, Glyburide, Glyburide micronized, Tolbutamide, Chlorpropamide, Tolazamide, Acetoheximide	Stimulate first-phase insulin secretion by blocking K+ channel in β-cells.	Late hyperinsulinemia, and hypoglycemia, Weight gain
2.	Meglitinides Repaglinide, Nateglinide	Stimulate first-phase insulin secretion by blocking K+ channel in β-cells.	Hypoglycemia Weight gain
3.	Biguanides Metformin, Metformin-XR	Decrease hepatic glucose production Increase muscle glucose uptake and utilization	Nausea, Diarrhea Anorexia, Lactic acidosis
4.	Thiazolidinediones Rosiglitazone, Pioglitazone	Increase insulin sensitivity via activation of PPAR-g receptors	Fluid retention and weight gain
5.	α-Glucoside Inhibitors Acarbose, Miglitol	Decrease hepatic glucose production Delays glucose absorption	Flatulence Abdominal bloating

Insulin:

Insulin is indicated when treatment goals are not being met with diet, exercise and oral agents. It is stressful to initiate insulin injection for the elderly because of visual impairment, difficulty in drawing and injecting the exact dose of insulin due to decreased manual dexterity [40].

Insulin binds to its receptors, leading to the phosphorylation of tyrosine residues, thus initiating the signal pathways to perform its action. These receptors are glycoprotein molecules that can be found in various tissues, but in greater proportion in adipose tissue, muscle, heart and liver [41].

Impaired insulin secretion leads initially to postprandial hyperglycemia, and as beta cell function declines further, fasting hyperglycemia ensures. Insulin resistance contributes further to and aggravates the fasting and postprandial hyperglycemia. Other abnormalities associated with decreased insulin secretion and insulin resistance (hyperglycemia, hyperinsulinemia, lipolysis, hyperlipidemia, hypertension, and coagulation defects) contribute to the risk of microvascular and macrovascular disease [26].
Physiological action of insulin:

Insulin is secreted by the β cell of the pancreas directly into the portal circulation. Insulin is an anabolic hormone that promotes lipid synthesis and suppresses lipid degradation. Insulin also stimulates lipid synthesis enzymes and inhibits lipolysis in adipose tissue. Insulin is the primary regulator of glucose homeostasis. The hormone has major effects on muscle, adipose tissue, and the liver. Insulin allows glucose from the bloodstream to enter the target tissues where glucose is used for energy. The insulin receptor is a hetero tetrameric protein consisting of two extracellular

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α-subunits and two transmembrane β-subunits [27]. The binding of insulin to one of the extracellular α chains leads to the auto phosphorylation of multiple tyrosine molecules in the intracellular domain of the β chain. The phosphorylated receptor then transfers the message inside the cell by phosphorylating tyrosine residues on insulin receptor substrate-1 (IRS-1). This intracellular protein is considered to play a central role in the intracellular signal cascade that is involved in glucose uptake and glycogen synthesis. The IRS-1 also transfers the growth-promoting and mitogenic signals of insulin to the nucleus, thereby stimulating protein synthesis [42].

Inhaled insulin:
The burden of three to six insulin injections daily may lead to avoidance to self inject, even in the absence of overt needle phobia. Attempts to develop noninvasive routes for insulin administration emerged soon after the introduction of insulin. Degradation by the acidic environment of the stomach or by digestive enzymes in the upper gastrointestinal tract, active mucociliary clearance and presence of proteolytic enzymes in the nasal cavity, and the relative impermeability of the skin have precluded successful delivery by oral, intestinal, intranasal, and transdermal routes. None of these obstacles apply to pulmonary delivery of insulin. On the contrary, the lungs appear perfectly equipped for the absorption of small peptides such as insulin. The efficiency of the inhaler device reflects the percentage of drug emitted from the device by correct inhalation, which is usually 80 to 95% for dry-powder inhalers, but can be as low as 20 to 30% for liquid nebulisers. A good pulmonary function is a prerequisite for inhalation therapy. There is evidence that only 20 to 40% of insulin deposited in the lung reaches the circulation. The remainder undergoes cytosolic biodegradation or exits the lung via the mucociliary escalator. Smoking, both acutely and chronically, enhances the absorption of insulin. Smokers were found to have more than threefold higher peak insulin levels upon inhalation of a standard insulin dose, resulting in hypoglycaemia Absorption of inhaled insulin occurs rapidly. The time to reach maximum insulin concentration and glucose-lowering effect is similar to that of subcutaneous short-acting insulin analogues and shorter than that of subcutaneous regular insulin. The duration of action of inhaled insulin is four to six hours, slightly longer than short-acting analogues and slightly shorter than subcutaneously injected regular insulin. These pharmacokinetic characteristics make inhaled insulin suitable as mealtime insulin [43].
Examples: propellant inhaler, liquid aerosol [43].

Non pharmacological treatment
Exercise:
Exercise has been found to lower fasting glucose and hemoglobin Alc levels. It helps to improve glycemic control by increasing insulin sensitivity, maintaining body weight, reducing cardiovascular risk factors and inducing a sense of well being. Aerobic exercises like walking is more effective than isometric exercises in improving the glycemic status [40].

Diet:
Diet forms the fundamental aspect of therapy. Ideally, 25-30 calories per kilogram body weight should be the aim of a diabetic diet. Out of them, 55 to 60% of calories should be in the form of carbohydrates especially complex carbohydrates like whole grain cereals, pulses, beans, vegetables and salads. Twenty five percent of the calories should be in the form of fats. Protein should be taken at about 0.8 gram per kilogram body weight. 30-40 grams per day of naturally

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occurring dietary fibers have been found to be beneficial. Therefore the entire success of dietary modifications in a diabetic subject depends on the judicious selection of carbohydrates, moderation in protein intake and a determined restriction of total fat intake [40].

Medicinal plants used in the treatment of diabetes mellitus:

Leaves as anti-diabetics:
Mangifera indica, Vernonia amygdalina, Calotropis procera, Cassia goratensis, Moringa oleifera, Senna occidentalis, Gossypium hirsutum, Psidium guajava, Ipomoea batatas, Fiscus thonnigii, Euphorbia convuludiodes, Zizyphus mucronata, Anana senegalensis, Allium sativum, Alluvium cepa, Parkta filicoidea, Vitillarta paradoxa, Anacardium occidentalis, azadiracta indica, citrus medica.

Bark as anti-antidiabetics:
Khaya senegalensis, Angeissus leiocarpus, Cassia arereh, Blighia sapida, Euphorbia convuludiodes, Zizyphus spina, Parkta filicoidea, Vitillarta paradoxa, Balanites aegyptiaca, Vitex gekowskii, Zizyphus mucronata, Bauhinia reticulate, Fiscus thonnigii, Acacia nilotica, Ficus sycomorus. [44].

Chinese herbal medicine used for the treatment of diabetes:
Roots of *Astragalus membranaceus* decreases blood glucose and triglyceride levels.
Roots of *Rehmania glutonosa* stimulates insulin secretion and reduces glycogen content.
Roots of Trichosanthes shows antihypoglycemic action.
Roots of *Pueraria lobata* contains flavonoids as active component which lowers the blood glucose levels.
Roots and rhizomes of *Panax ginseng* lowers hyperglycemia and rises hypoglycemia.
Rhizomes of *Polygonatum sibiricum* Red shows reduced blood glucose levels.
Rhizomes of *Polygonatum odoratum* reduces blood glucose levels.
Pulps of *Cornus officianalis* promotes proliferation of pancreatic islets and increases postprandial secretion of insulin and there fore accelerates glucose transport.
Rhizomes of *Coptis chinensis* contains Bereberine as active component which shows anti hypoglycemic effect.
Fruits and root cortex of *Lycium barbarum* shows antihyperglycemic effect.
Sclerotum of *Poria cocos* shows anti hyperglycemic effect. It is a promising candidate of new type of insulin sensitizing drug.
Rhizomes of *Atractylodes lancea* contains Ataractans A, B, C active component which shows anti hyperglycemic effect.
Root tubers of *Ophiogon japocius* contains active components polysaccharides which lowers the blood glucose levels.
Fruits of *Ligustum lucidium* contains active component oleanolic acid which lowers blood glucose levels.
Fruits of *Schisandra chinensis* contains lignans which are effective as aldose reductase inhibitors which are use full in treatment of diabetes.
Stems and leaves of *Gynostemma pentaphyllum* contains active component saponins which decreases plasma glucose levels and increase plasma triglyceride levels.

Roots and rhizomes of *Salvia mitorrhiza* Bunge are administered to diabetic patients for care of diabetic nephropathy.

Fresh or dried rhizomes of *Phragmites communis* clears away heat and promotes the production of fluid.

Stem tubers *of Alisma orientale* promotes diuresis to eliminate dampness from the lower jiao and expel heat.

Seeds of *Cuscuta chinensis* invigorates the kidney and supplement essence.

Branches and leaves of *Epimedium sagitatum* invigorates kidney and strengthen yang.

Roots and rhizomes of *Clematis chinensis* osbeck expels wind and dampness and dredge the channel.

Roots of *Panax notoginseng* regulates blood glucose two dimensionally. It lowers plasma glucose levels.

Stems of *Dendrobium nobile* shows antihyperglycemic effect [45].

Animal models for diabetes:
NOD (non-obese diabetic) mouse, BB (bio breeding) rat, LETL (Long Evans Tokushima lean) rat, New Zealand white rabbit, Keeshond dog, Chinese hamster, Celebes black ape.

Ob/Ob mouse—monogenic model of obesity (leptin deficient), db/db mouse—monogenic model of obesity (leptin resistant), Zucker (fa/fa) rat—monogenic model of obesity (leptin resistant), Goto Kakizaki rat, KK mouse, NSY mouse, OLETF rat, Israeli sand rat, Fat-fed streptozotocin-treated rat, CBA/Ca mouse, Diabetic Torri rat, New Zealand obese mouse [46].

Some strains like Ob/Ob mouse may maintain euglycemia due to a robust and persistent compensatory pancreatic β-cell response, matching the insulin resistance with hyperinsulinemia. On the other hand, the db/db mouse rapidly develops hyperglycemia since their pancreatic β-cells are unable to maintain the high levels of insulin secretion required throughout life [47].

JCR/LA -cp Rat (James C Russell/LA-corpulent):

This leptin-deficient animal exhibits hyperinsulinemia, obesity, and glucose intolerance due to low insulin sensitivity in peripheral tissues leading to a constant increased insulin demand resulting in pancreatic islet cells hyperplasia [48].

Torri Rat:

This is a new strain of spontaneous diabetic non-obese rat. It was developed by inbreeding of Sprague–Dawley rats [48].

NOD mouse typically presents hyperglycemia for type 1 diabetes between 12 and 30 weeks of age, whereas in BB rats it occurs around 12 weeks of age [47].

Another example is the spontaneously diabetic Goto-Kakizaki rat which is a genetic lean model of type 2 diabetes originating from selective breeding over many generations of glucose-intolerant nondiabetic Wistar rats.

Zucker Diabetic Fatty Rat:
These are a selectively inbred substrain of Zucker fatty rats (ZFR) that develop overt type 2 diabetes mellitus by 7–10 weeks of age [48].

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SHR/N-cp Rat:
The spontaneously hypertensive corpulent (SHR/N-cp) rat is a genetically modified model that exhibits obesity, hyperinsulinemia, hyperglycemia, and hyperlipidemia, which resemble pathological features of type 2 diabetes mellitus [48].

OLETF Rat (Otsuka Long–Evans Tokushima Fatty):
This model is characterized by hereditary late onset (18– 25 weeks old) mild obesity and clinical type 2 diabetes mellitus [48].

Cohen Rat:
Cohen rat is a diet-induced type 2 diabetes mellitus model. It is useful for studying the interaction between nutrition and environmental factors in the development of type 2 diabetes [48].

In embryo transfer experiments, Wistar rats (at low genetic risk of diabetes) are more likely to develop hyperglycaemia as adults if they are reared in the uterus of a Goto Kakizaki (diabetic) mother than a euglycaemic mother. However, transferring Goto Kakizaki embryos into a normal (Wistar) uterus does not seem to reduce their risk of developing diabetes [46].

Substances with a diabetogenic effect in experimental animals:
Alloxan, Chlorothiazide, Chlorozotocin, Cyclosporin, Cyproheptadine, Diazoxide, Dithizone, Furosemide, Hydrochlorothiazide, L-asparaginase, Methylnitrosourea, Oxine, Streptozotocin, Styrylquinoline, Trichlormethiazide, Vacor, Xylazine [49].

S. No.	CHEMICAL	SPECIES	DOSE
1.	ALLOXAN	RAT MICE RABBIT DOG	40-200 (i.v or i.p) 50-200 (i.v or i.p) 100-150 (i.v) 50-75 (i.v)
2.	STREPTOZOTOCIN	RAT MICE HAMSTER DOG PIG PRIMATES	35-65 (i.v or i.p) 100-200 (i.v or i.p) 50 (i.p) 20-30 (i.v) 100-150 (i.v) 50-150 (i.v)

Induction of diabetes by chemical method:
Streptozotocin:
In adult rats, 60 mg/kg is the most common dose of STZ to induce insulin dependent diabetes, but higher doses are also used. STZ is also efficacious after intraperitoneal administration of a similar or higher dose, but single doses below 40 mg/kg may be ineffective. In general, rats are considered diabetic if tail blood glucose concentrations in fed animals are greater than 200–300 mg/dl, 2 days after STZ injection. [47]. STZ is a nitrosurea compound derived from *Streptomyces achromogenes*, which has also been used as an antibiotic and a cancer treatment. It enters pancreatic β cells through glucose transporter 2 (GLUT2) channels in the plasma membrane and causes cellular toxicity and local immune responses that lead to hypoinsulinemia and hyperglycemia in animals. In some models, especially rats, a single dose of STZ is effective at inducing T1 diabetes. In mice, however, multiple low doses (40 mg/kg) are the most effective

at maintaining mouse viability and inducing pancreatic dysfunction in part through immune destruction [50].

Disadvantages of diabetes induction by streptozotocin:
Spontaneous recovery from high blood glucose levels by the development of functioning insulinoma and high incidence of kidney and liver tumors [51].

Alloxan:
The most frequently used intravenous dose of alloxan in rats is 65 mg/kg, but when it is administered intraperitoneally (i.p.) or subcutaneously its effective dose must be higher. For instance, an intraperitoneal dose below 150 mg/kg may be insufficient for inducing diabetes in this animal species. In mice, doses vary among 100–200 mg/kg by intravenous route (i.v.) [47]. Alloxan and its reduced product dialuric acid, establish a redox cycle with the formation of superoxide radicals. These radicals undergo dismutation to hydrogen peroxide with a simultaneous massive increase in cytosolic calcium concentration, which causes rapid destruction of pancreaticβ-cells. The range of the diabetogenic dose of alloxan is quite narrow and even light overdosing may be generally toxic and may cause the loss of many animals [47].

Anti psychotics:
Atypical antipsychotic drugs especially olanzapine and clozapine have been found to induce weight gain, hypercholesterolemia, hypertriglyceridemia and Diabetes Mellitus [52].

Mechanism of action of anti psychotic drugs in diabetes induction:
The development of diabetes has been reported to occur anywhere from 10 days to 18 months after starting therapy. One theory is that diabetes might result from the weight gain caused by these agents. Other studies suggest that these agents affect glucose transport metabolism peripherally, possibly increasing the potential for hyperinsulinemia and peripheral insulin resistance. Further hypotheses point to the activity of atypical antipsychotic drugs at the serotonin receptors of the beta cells in the pancreas, more specifically 5HT1A and 5HT2 receptors. This activity might lead to derangement of beta cell function, with resulting increases in glucose levels in patients [53].

Induction of diabetes in CAT:
Unlike rodents, cats are resistant to the diabetogenic effects of streptozotocin and alloxan, yet they remain susceptible to their toxic side effects. Partial pancreatectomy alone (>75% removed) was effective in inducing diabetes in 70% of cats while partial pancreatectomy in combination with local injection of alloxan was effective in inducing diabetes 100% of cats. These models have been used to evaluate the complications of diabetes rather than the pathogenesis of this disease itself. Partial pancreatectomy (50% removed) combined with growth hormone and dexamethasone treatment was given to cats to induce insulin resistance. All cats which were given with growth hormone and dexamethasone after the partial pancreatectomy were evaluated and all remained hyperglycemic even after growth hormone and dexamethasone therapy were discontinued [54].

The benzothiodiazines are the drugs with diuretic and antihypertensive properties. Unfortunately, hyperglycemia resulted in some individuals treated with these drugs. The benzothiodiazines

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seem to act through 2 specific mechanisms. Primarily these drugs inhibit insulin release, most likely by interrupting the microtubule system in the beta cells so that secretory granules cannot be released. However, it is also apparent that these chemicals have extra islet effects because hyperglycemia in severely diabetic animals, those which are essentially insulin-deficient, is increased after drug treatment. This effect is most likely due to the stimulation of release of epinephrine and other catecholamines which promote glycogenolysis and; thus, elevate circulating glucose levels. The only obvious morphological change in the beta cells from animals treated with benzothiodiazines is an increased accumulation of secretory granules. This change is not permanent. When the drugs are removed, their diabetogenic effects are reversed and the beta cells appear normal.

Cyproheptadine is an antiserotonin, antihistaminic compound which has been used clinically to stimulate weight gain in adults and children. When given to adult rats in repeated high doses, this chemical produces an inhibition of proinsulin synthesis, a reduction in pancreatic insulin, and glucose intolerance. Morphologically, extensive vacuolization can be seen in the beta cells by light microscopy, and a progressive degranulation of beta cells, dilation of endoplasmic reticulum, and loss of ribosomes from the surface of the Rough Endoplasmic Reticulum can be observed using electron microscopy. All these effects are reversed when treatment with the drug is stopped. However, permanent changes in beta cells can be induced in the offspring of pregnant rats given repeated low doses of cyproheptadine during the last 8 days of gestation. This treatment initially causes a 50% reduction in fetal pancreatic and serum insulin concentrations. In contrast, the maternal animals exhibit no changes in insulin levels. By 50 days after birth, the progeny of the drug-treated dams are glucose intolerant, have 2-fold increased levels of pancreatic insulin, and have an accentuated response to the insulin-lowering action of cyproheptadine on the beta cells. These results demonstrate that a permanent postnatal defect in beta cell function can be produced by prenatal exposure to this chemical [49].

Vacor :
Vacor is a rat poison which, when ingested, results in the destruction of the beta cells in the pancreas, in a fashion analogous to that of streptozotocin, and thereby induces diabetes. In addition, Vacor also has extra pancreatic effects that contribute to the hyperglycemia [55].

Nicotinamide and streptozotocin:
Type 2 diabetes was induced by intraperitoneal administration of nicotinamide (110 mg/kg) dissolved in saline. 15 minutes after the administration of nicotinamide, streptozotocin (65mg/kg) was administered by intraperitoneal route dissolved in citrate buffer (PH 4.5). After 7 days following streptozotocin and nicotinamide administration, blood was collected from retro-orbital puncture and serum samples were analyzed for blood glucose [56].

Ferric nitrilotriacetate induction of diabetes mellitus:
This is a rarely used procedure. Rats and rabbits were parenterally treated with a large daily dose of ferric nitrilotriacetate. It manifested diabetic symptoms such as hyperglycaemia, glycosuria, ketonemia and ketonuria after approximately 60 days of treatment. The blood insulin response to oral glucose loading was poor [51].

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Induction of diabetes mellitus by surgical methods
Metabolic surgery:
Incretin is referred to as the higher production of insulin in response to oral glucose load when compared to the response to an intravenous bolus of glucose, which directly results in production of two main hormones. They are: glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), which are produced by highly specialized cells in different parts of the small intestine after stimulation of direct contact of ingested nutrients with the intestinal lumen. GIP is primarily produced in the proximal intestine by K cells, while GLP-1 is produced in the distal ileum by L cells. Based on these fundamental differences, two distinct theories behind the incretin effect have been put forward: the foregut and hindgut theories. The foregut theory suggests that the exclusion of the proximal small intestine from contact with ingested nutrients is the most important reason for the improvement of glucose metabolism after bariatric interventions. The hindgut theory states that the expedited transit of food to the more distal small intestine is the reason for the increase in insulin production and normalization of postprandial glucose levels. Ileal transposition causes a significant elevation of GLP-1. Ileal transposition might enhance glucose uptake by the peripheral tissues independently of insulin action. An elevation of GLP-1 also results in delayed gastric emptying which by itself causes a more efficient postprandial glycemic control [48].

Pancreatectomy:
In this method complete removal of the pancreas is seen in animal species such as rats, pigs, dogs, primates.

Limitations of pancreatectomy: High level of technical expertise and adequate surgical room environment, major surgery and high risk of animal infection, adequate post-operative analgesia and antibiotic administration, supplementation with pancreatic enzymes to prevent mal-absorption and loss of pancreatic counter regulatory response to hypoglycemia [57].
More recently, partial pancreatectomy has been employed, but large resection (more than 80% in rats) is required to obtain mild to moderate hyperglycemia [57].

A method to induce diabetes in adult rats is to mimic the unfavorable intrauterine environment, which in humans leads to low-birth weight and is supposed to confer high risk for the development of diabetes in adult age. This model is known as intrauterine growth retardation by uteroplacental insufficiency. In the rat is based on the premise that uterine malnutrition may also increase the risk of diabetes amongst offspring in later life. This has been achieved by several means, including bilateral uterine artery ligation at 19 days of gestation, i.e. 3 days before term. The diabetogenic effects of manipulating the intrauterine environment are probably mediated by a permanent programming of the developing offspring, e.g. by the mechanism of imprinting. It should also be pointed out that the increased risk of diabetes continues into subsequent generations, which in turn, suggests that changes also affect the germ cell line [47].

In conscious dogs intravenously infused somatostatin (3.3 µg per min for 1 h) caused prompt and sustained declines in mean plasma insulin and glucagons even during alanine infusion and intraduodenal casein hydrolysate feeding; plasma glucose declined, but not significantly. 6.7 µg per min of somatostatin significantly lowered pancreatoduodenal vein glucagon and insulin within 2.5 min and profoundly suppressed their secretion throughout the infusion. Consistent bi

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hormonal suppression occurred at rates as low as 24 ng per kg per min, but was variable at 12 and 2.4 ng per kg per min. When somatostatin-induced (3.3 lig per min) hypoglucagonemia was corrected by exogenous glucagon, hyperglycemia occurred. [58].

It is concluded that in normal dogs pharmacologic doses of somatostatin virtually abolish insulin and glucagons secretion in the basal state and during hyperaminoacidemia. Hyperglycemia occurs during somatostatin- induced insulin lack only if hypoglucagonemia is corrected. Somatostatin suppresses glucagon in diabetic dogs and lowers their plasma glucose approximately 1mg per dl per min, even when the gluconeogenic substrate alanine is abundant. Glucagon suppression can be maintained for several hours in such dogs and hyperglycemia is thereby reduced [58].

Mongrel dogs, weighing between 19 and 27 kg were used. Two or more days before an experiment, a catheter was inserted under Nembutal anesthesia through the jugular vein into the inferior vena cava and anchored in place. In some dogs, a small glass T-cannula connected with Teflon tubing was implanted into the superior pancreaticoduodenal vein at a distance of about 3 cm from its junction with the portal vein so as to permit direct sampling of pancreatic venous effluent. In another group of dogs, a polyethylene catheter was anchored with one end in the duodenal lumen so as to permit intraduodenal administration of casein hydrolysate [58].

Somatostatin was administered in normal saline adjusted to pH 7.4 through catheters implanted in a crural vein. In experiments in which alanine was employed, the amino acid was infused in a dose of 1 mmol per kg of body wt over a 15-min period in a volume of 60 ml of normal saline [58].

Diabetes was induced by an i.p. injection of 110 mg/kg nicotinamide 15 min before an i.v. (penile vein) injection of 65 mg/kg streptozotocin (Sigma Chemicals) in 0.9% sodium chloride solution [59].

Diabetes induced by retrovirus
New born mice (3-6 days old) of both sexes were inoculated intraperitoneally or intracerebrally with 5×10^5 or 5×10^4 plaque forming units (pfu) of virus respectively.

Retrovirus type I (Lang strain) and retrovirus type 3 (Dearing strain) were passaged in SJL mouse pancreatic beta cell cultures. Recombinant virus was passaged into L-929 cells. All titrations were performed on L-929 mono layers using an agar overlay. The D clone of encephalomyocarditis virus was grown and titrated in secondary mouse embryo cells.

Mice infectd with retrovirus 1 developed autoantibodies to cytoplasmic antigens in pancreatic beta islets, anterior pituitary and gastric mucosa. Because these tissues contain hormone producing cells and certain of these hormones are available in pure form the retrovirus infected animals are shown to contain auto antibodies to growth hormone and insulin. Many of the mice infected with retrovirus type 1 developed poly endocrine disease. It affects the alpha, beta and delta cells.

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Mice infected with retrovirus type 1 developed transient diabetes characterized by hyperglycaemia, abnormal glucose tolerance tests and hypoinsulinaemia. Inflammatory cells and viral antigens were seen in alpha, beta and delta cells. Electron microscopy revealed virus particles in growth hormones producing cells and radioimmuno assay showed that the concentration of growth hormone in blood was decreased [60].

CONCLUSION

Diabetes mellitus can be treated by a wide variety of drugs. These drugs shows severe side effects, therefore an optimized therapy is not available. Diabetes mellitus can be induced in different animal models for the discovery of new drugs. A standard animal model should be established for the development of new drugs mainly the drugs which arrest the progressive loss of pancreatic β- cells should be developed.

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