



Review Article

Experimental Models on Diabetes: A Comprehensive Review

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Abstract

Present world scenario globally, upto 2010, around 285 million people suffering from Type 2 diabetes making up about 90% of the cases. According to statistics, by 2030, this number is estimated to almost double. Diabetes mellitus occurs throughout the world, but is more common (especially Type 2) in the more developed countries. The greatest increase in prevalence is, however, expected to occur in Asia and Africa, where most patients will probably be found by 2030. The aim of this review is to summarize several studies done for the discovery of new drug using different animal models for in vivo studies (chemical, surgical, and genetic models) & in vitro models (glucose uptake, pancreatic islet cell lines and insulin secretion).

Keywords: Diabetes mellitus; Malabsorption; Insulin.

Introduction

Diabetes mellitus is a group of metabolic diseases in which a person has high blood sugar, either because the body 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) [1]

There are three main types of diabetes mellitus (DM). Type 1 DM results from the body's failure to produce insulin, and presently 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". The third main form, gestational diabetes occurs when pregnant women without a previous diagnosis of diabetes develop a high blood glucose level. It may precede development of Type 2 DM [2].

Genetic defects of β -cell function

Maturity onset diabetes of the young

Mitochondrial DNA mutations

Genetic defects in insulin processing or insulin action

Defects in proinsulin conversion

Insulin gene mutations

Insulin receptor mutations

Exocrine pancreatic defects

1. Chronic pancreatitis
2. Pancreatectomy
3. Pancreaticneoplasia
4. Cystic fibrosis
5. Hemochromatosis
6. Fibrocalculous pancreatopathy

Pathophysiology

Insulin is the principal hormone that regulates uptake of glucose from the blood into most cells (primarily muscle and fat cells, but not central nervous system cells). Therefore, deficiency of insulin or the insensitivity of its receptors plays a central role in all forms of diabetes mellitus. Insulin is also the principal control signal for conversion of glucose to glycogen for internal storage in liver and muscle cells. Lowered glucose levels result both in the reduced release of insulin from the β -cells and in the reverse conversion of glycogen to glucose when glucose levels fall. This is mainly controlled by the hormone glucagon, which acts in the opposite manner to insulin. Glucose thus forcibly produced from internal liver cell stores (as glycogen) re-enters the bloodstream; muscle cells lack the necessary export mechanism. Normally, liver cells do this when the level of insulin is low (which normally



correlates with low levels of blood glucose). Higher insulin levels increase some anabolic ("building up") processes, such as cell growth and duplication, protein synthesis, and fat storage. Insulin (or its lack) is the principal signal in converting many of the bidirectional processes of metabolism from a catabolic to an anabolic direction, and *vice versa*. In particular, a low insulin level is the trigger for entering or leaving ketosis (the fat-burning metabolic phase) [1].

Present word scenario

Globally, as of 2010, an estimated 285 million people had diabetes, with Type 2 making up about 90% of the cases.[3] Its incidence is increasing rapidly, and by 2030, this number is estimated to almost double. Diabetes mellitus occurs throughout the world, but is more common (especially Type 2) in the more developed countries. The greatest increase in prevalence is, however, expected to occur in Asia and Africa, where most patients will probably be found by 2030. The increase in incidence in developing countries follows the trend of urbanization and lifestyle changes, perhaps most importantly a "Western-style" diet. This has suggested an environmental (i.e., dietary) effect, but there is little understanding of the mechanism(s) at present, though there is much speculation, some of it most compellingly presented. [4]

According to the World Health Organization (WHO), there are approximately 160,000 diabetics worldwide, the number of diabetics has double in the last few years and is expected to double once again in the year 2025 [5]. Due to its high prevalence and potential deleterious effect on a patient physical and psychological state, diabetes is a major medical concern [6]. In India has more diabetics than any other country in the world, according to the International Diabetes Foundation, [7] In India, around 50.8 million people are affected by diabetes. It is only in China that you will find a greater diabetic population, with 92.4 million Chinese adults affected by diabetes. although more recent data suggest that China has even more.[8] The disease affects more than 50 million Indians - 7.1% of the nation's adults - and kills about 1 million Indians a year.[7] The current estimate for 2010 of 285 million adults with diabetes is 67% higher than the 2004 published estimate for the year 2000 [9], and our 2030 estimate of 439 million is 20% higher than the same study's estimate for 2030 [9]. Comparisons with other older estimates show even greater differences.

The economic impacts of diabetes with its complications and associated diseases are large [10]. According to recent estimates the prevalence of diabetes mellitus is 4% worldwide and that indicates 143 million persons are affected which will increase to 300 million by the year 2025[11]. In urban India, mortality rates are twofold higher in people with diabetes compared to non-diabetic subjects [12]. It is estimated that the number of diabetes patients worldwide will reach 380 million by 2025; Asia, in particular, is experiencing a rapidly emerging diabetes epidemic [13]. It has been estimated that the global burden of type 2 diabetes mellitus (T2DM) for 2010 would be 285 million people (2010) which is

projected to increase to 438 million in 2030; a 65 % increase . Similarly, for India this increase is estimated to be 58%, from 51 million people in 2010 to 87 million in 2030[14].

In-vivo studies

Chemical induction of diabetes mellitus

The majority of studies published in the field of ethno pharmacology between 1996 and 2006 employed this model. Streptozotocin (STZ, 69% and alloxan (31%) are by far the most frequently used drugs and this model has been useful for the study of multiple aspects of the disease. Both drugs exert their diabetogenic action when they are administered parenterally (intravenously, intraperitoneally or subcutaneously). The dose of these agents required for inducing diabetes depends on the animal species, route of administration and nutritional status [15].

Streptozotocin model of diabetes mellitus

Streptozotocin is an antibiotic derived from *Streptomyces achromogenes* and structurally is glucosamine derivative of nitrosourea. Rakietyen and his associates⁷⁹ first demonstrated the diabetogenic property of STZ in dogs and rats in 1963. Streptozotocin prevents DNA synthesis in mammalian and bacterial cells. In the bacterial cells, it renders special reaction with cytosine groups, resulting in degeneration and destruction of DNA. The biochemical Streptozotocin enters the pancreatic cell via a glucose transporter-GLUT2 and causes alkylation acid (DNA). Furthermore STZ induces activation of poly adenosine diphosphate ribosylation and nitric oxide release. As a result of STZ action, pancreatic -cells are destroyed by necrosis [16]. In adult rats, 60 mg/kg is the most common dose of STZ to induce insulin dependent diabetes [17], 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 in adult mice, STZ given in multiple lowdoses (40 mg/kg, i.v. for 5 days) [18] induces an insulin dependent diabetes that is quite similar to the autoimmune forms (islet inflammation and cell death) of Type 1 diabetes. On the other hand, a single dose between 60 and 100 mg/kg of STZ [19], administered systemically can also cause insulin may be ineffective dependent diabetes, but it lacks the autoimmune profile [20]. Recently, a new animal model of Type 2 diabetes has been produced by combination of STZ and NAD administration in adult rats 94. The rats administered NAD (230 mg/kg, ip) 15 min before STZ (65 mg/kg, iv) has been shown to develop moderate and stable non-fasting hyperglycaemia without any significant change in plasma insulin level. As NAD is an antioxidant which exerts protective effect on the cytotoxic action of STZ by scavenging free radicals and causes only minor damage to pancreatic beta cell mass producing Type 2 diabetes. Therefore, this model is found to be an advantageous tool for investigation of insulinotropic agents in the treatment of Type 2 diabetes [21].



Alloxan model of diabetes mellitus

Alloxan is the also used chemical for induction of diabetes mellitus. It is a well- known diabetogenic agent widely used to induce Type 1 diabetes in animals [22]. Alloxan is a urea derivative which causes selective necrosis of the pancreatic islet β -cells. It is used to produce experimental diabetes in animals such as rabbits, rats, mice and dogs. With this agent, it is possible to produce different grades of severity of the disease by varying the dose of alloxan used. Thus alloxan induced diabetes mellitus served as a pathological biomodel for testing a substance with supposed antioxidant activities the most frequently used intravenous dose of alloxan in rats is 65mg/kg, but when it is administered Intraperitoneally or subcutaneously its effective dose must be higher. A solution of 2% alloxan (40mg/kg) diluted in 0.9% normal saline was administered to the animals through the iliac vein. The animals were allowed to resume feeding and drinking 30 minutes after the drug administration. In order to assess the effect of alloxan and to chemically establish the diabetic condition, an incision was done in any of the four veins in the tail of the rat with a 15 scapel blade 10 days after induction a blood glucose level was determinsd by using a portable glucose analyzer. The most frequently used intravenous dose of alloxan in rats is 65mg/kg, but when it is administered intraperitoneally or subcutaneously its effective dose must be higher [23]. Alloxan administration in experimental animals has been reported to produce pancreatic lesion which is proportional to the dose of the drug administered. And the size of the lesion also correlates with the pancreatic insulin content [24].

Surgical models of diabetes mellitus

This technique used to induce diabetes is the complete removal of the pancreas. Few researchers have employed this model in the last years to explore effects of natural products with animal species such as rats, pigs, dogs and primates [18,19,25]. Limitations to this technique include (1) high level of technical expertise and adequate surgical room environment, (2) major surgery and high risk of animal infection, (3) adequate post-operative analgesia and antibiotic administration, (4) supplementation with pancreatic enzymes to prevent malabsorption and (5) loss of pancreatic counter regulatory response to hypoglycemia. More recently, partial pancreatectomy has been employed, but large resection (more than 80% in rats) is required to obtain mild to moderate hyperglycemia. In this case, small additional resection can result in significant hypoinsulinemia [26].

Genetic models of diabetes

Spontaneously develop diabetic rat - These models permit the evaluation of the effect of a natural product in an animal without the interference of side effects induced by chemical drugs like alloxan and STZ reported above. Example is the spontaneously diabetic Goto-Kakizaki rat which is a genetic lean model of type 1 diabetes originating from selective breeding over many generations of glucose-intolerant nondiabetic wistar rats [27]. Regarding type 1 diabetes models, the mouse typically presents hyperglycemia between 12 and 30 weeks of age, whereas in BB rats it occurs around 12 weeks of age. One great advantage of these models is that they can be employed as model of atherosclerosis which represents the long term complication of diabetes mellitus and tested against several natural products [28].

Genetically engineered diabetic mice

Although significant advances in this field have arisen in recent years, especially with the advent of transgenic mice, In this case, rodents may be produced to over (transgenic) or under (knockout)-express proteins thought to play a key part in glucose metabolism [25,29]. There have been no studies carried out involving natural products and these models. Certainly, the high costs restrict their study in sophisticated protocols which explore mechanisms of potential therapeutic agents that either stimulate pancreatic cell growth or inhibit pancreatic cell death [31]. Von Herrath and Oldstone (1997) [31] infected mice with lymphocytic choriomeningitis virus (LCMV) to induce IDDM. Similarly, Oldstone *et al.*(1991) developed a transgenic mouse model that expressed IDDM by inserting a viral gene in the animal egg stage[32].

In-vitro studies on insulin secretion

Conventional antidiabetic agents can affect several pathways of glucose metabolism such as insulin secretion, glucose uptake by target organs as well as nutrient absorption. Recently, incretins [33], and transcription factors such as peroxisome proliferator-activated receptors—PPAR are targets of modern therapy. Insulin receptor, glucose transport ers, however, has not been yet the focus of antidiabetic therapy. Although few studies using natural products have been published [34, 35, 37],

Studies using insulin-secreting cell lines

Bioengineered technologies have provided new opportunities to improve and establish more appropriate cultured cell lines help to facilitate studies of mechanisms of both insulin secretion and cell dysfunction, being also the target to the study of natural products. The most widely used insulin-secreting cell lines are RIN, HIT, beta-TC, MIN6 and INS-1 cells. These cell lines release mainly



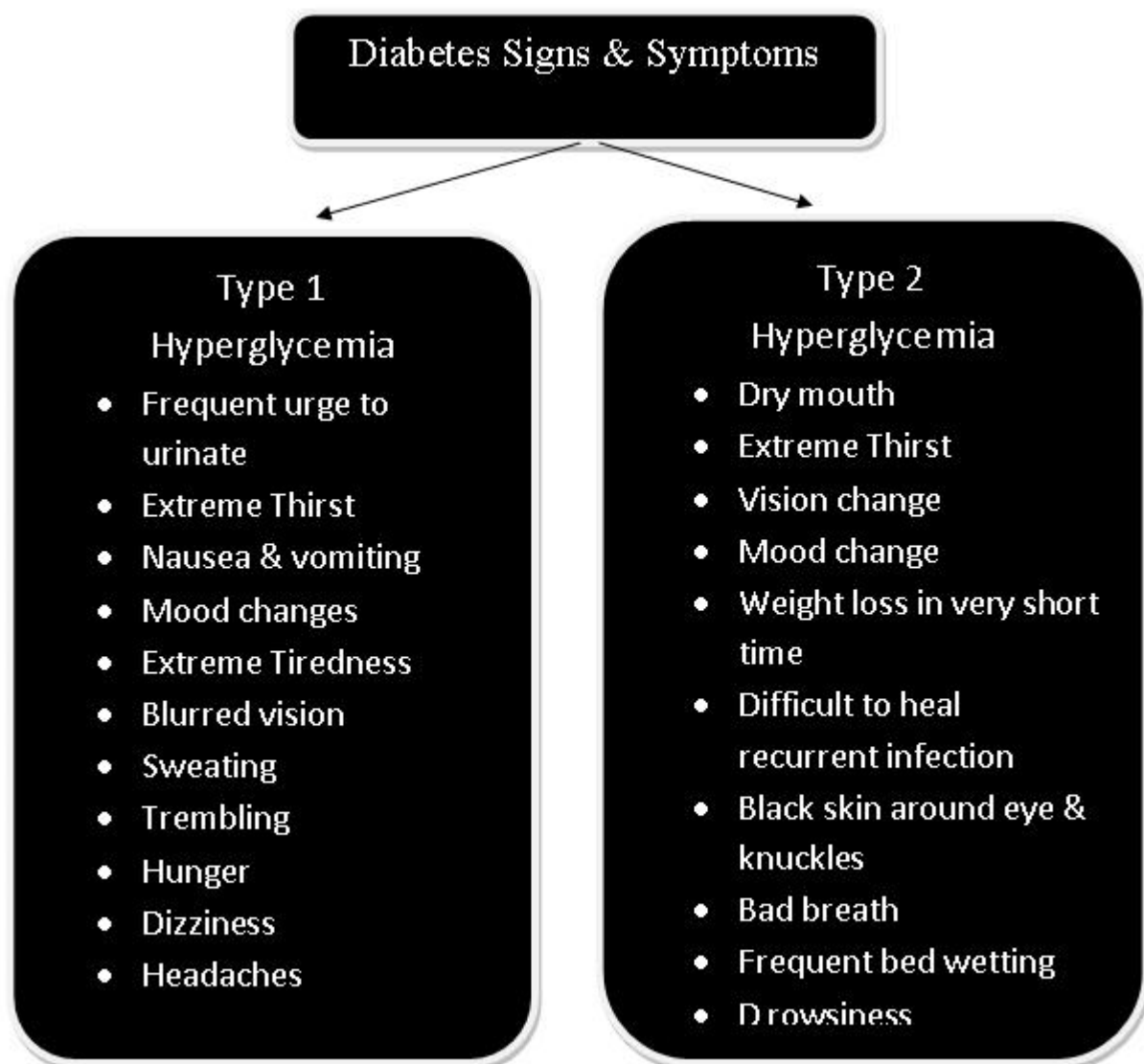


Figure 1 Signs & Symptoms of Diabetes

Table 1 Comparison of Type 1 and 2 Diabetes

Feature	Type 1 Diabetes	Type 2 Diabetes
Age at onset	In children	In adults
Onset	Sudden	Gradual
Body habitus	Thin or normal	Often obese
Endogenous insulin	Low or absent	Increased , decreased or normal
Prevalence	~10%	~90%



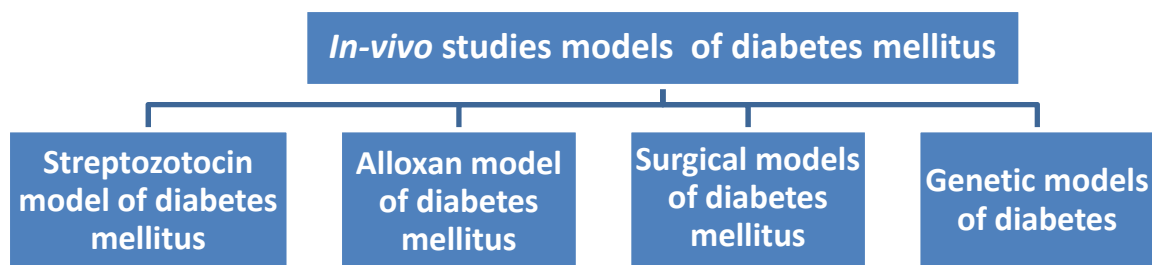


Figure 2: In-vivo study models of diabetes

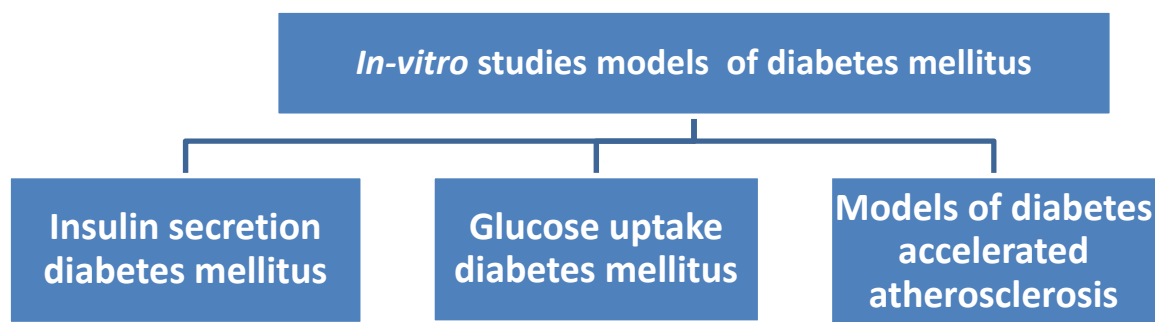


Figure 3: In-vitro study models of diabetes

insulin and small amounts of glucagon and somatostatin. Although the behaviour of none of these cell lines perfectly mimics primary cell physiology, they are extremely valuable tools for the study of molecular events underlying cell function [37]

Studies using isolated pancreatic islet cell lines

Diabetic rat or mouse that can be obtained by collagenase digestion technique followed by adequate separation. These pathway can be studied with isolated pancreatic β -cells from either control or & transference to appropriated culture medium [38]. It is known that insulin secretion occurs when pancreatic cells utilize glucose to generate adenosine triphosphate (ATP) from adenosine diphosphate (ADP) [39]. The resulting increase in cytoplasmic ATP/ADP ratio closes ATP-sensitive potassium channels, causing depolarization of the plasma membrane, which activates voltage-dependent Ca^{2+} channels. This results in elevation of the intracellular Ca^{2+} concentration which triggers insulin secretion [40]. In type 2 diabetes, pancreatic cells exhibit atypical ion channel

activity and an abnormal pattern of insulin secretion [41]. These pathways can be studied with isolated pancreatic cells from either control or diabetic rat or mouse that can be obtained by collagenase digestion technique, followed by adequate separation and transference to appropriated culture medium [35,36]. Afterwards, the experimental protocol is assayed.

In-vitro studies on glucose uptake

Adipose tissue is considered a key link between obesity and Type 2 diabetes by promoting the development of lipotoxicity, i.e. cell damage as a consequence of elevated intracellular lipid concentrations and insulin resistance [41]. Insulin resistance either at the adipocyte or skeletal muscle levels contribute to hyperglycemia. However, adipocytes from different sites of the body may have different biological or pathological effects. Pathways related to insulin resistance may be studied in cell lines of adipocytes such as marine 3T3-L1 cells [42] and rat L6 muscle engineered to over-express GLUT4[43] and may be employed as



tools to evaluate the effects of natural products upon glucose uptake.

Models of diabetes accelerated atherosclerosis

Accelerated cardiovascular disease is a leading cause of both morbidity and mortality in diabetic patients [44]. Aggressive therapy of dyslipidemia is necessary, since the risk of myocardial infarction is the same as in nondiabetic patients with previous myocardial infarction [45]. Currently, rats and mice are the most widely used models to study diabetes and atherosclerosis. Albeit, diabetic mice do not exhibit a high degree of atherosclerosis unless hyperglycemia is associated with severe hyperlipidemia, a fat diet is also present in these protocols. Models of diabetic nephropathy, a microvascular complication have also been developed [44,46].

Oral therapeutics for Diabetes Mellitus

The available therapeutic options for diabetes mellitus target several major sites of action. The pancreas, because it regulates insulin secretion, is a critical organ in the development of diabetes mellitus. The liver is responsible for glucose production, making it a second important target organ in the treatment of diabetes. The intestine mediates glucose absorption into the body, whereas adipose tissue and muscle are active in peripheral glucose uptake. Dysfunction in any one of these organs has been implicated in the development of diabetes mellitus. In response, a diverse array of oral therapeutics has been developed. Sulfonylureas, repaglinide, and nateglinide are insulin secretagogues that stimulate endogenous insulin secretion from the pancreas [47] and are used as hypoglycemic agents for the treatment of Type 2 diabetes.

These agents also improve insulin sensitivity as a result of improved glucose control. The adverse effects of sulfonylureas include severe hypoglycemia (in the event of an overdose), weight gain, erythema, and hepatic dysfunction [48]. Repaglinide and nateglinide, which are meglitinide analogs, decrease ATP-sensitive K⁺ conductance in a glucose-dependent manner. They are taken with meals to prevent postprandial hyperglycemia and to reduce the risk of long-lasting hypoglycemia. Both sulfonylureas and meglitinides can induce weight gain [49].

Conclusion

In spite of the worldwide use of herbs and medicinal plants, the effective treatment of diabetes with phytochemicals has not been validated with scientific criteria which may support their substitution for the current therapy. None of the known single species is exactly equivalent to human diabetes, but each model act as essential tool for investigating genetic, endocrine, metabolic, morphologic changes and underlying aetiopathogenic mechanisms that could also operate during the evolution of Type 2 diabetes in humans. The selection of particular animal model is particularly depending on the investigator's choice whether to use inbred or out bred, availability of particular strain, aim of scientific strategy, type of drug being sought, institutional financial and facility resources in the Type 2 diabetes research and pharmaceutical drug discovery and development programme.

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