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Diabetes mellitus and advances in diabetes research

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Abstract

Diabetes mellitus (DM) is a major global health problem and it affects more than 200 million people worldwide. It is the most common serious metabolic disorder in humans. Diabetes is characterised by hyperglycaemia as a result of a relative or absolute lack of insulin or the actions of insulin on its target tissues or both. The disease is classified into two major groups, Type 1 or Insulin-Dependent Diabetes Mellitus (IDDM) and Type 2 or Non-Insulin Dependent Diabetes Mellitus (NIDDM). Type 1 diabetes represents approximately 5–10 % of all cases of diabetes and it is characterised by an auto-immune mediated destruction of the beta cells of pancreas, leading to a severe reduced capacity of these cells to produce insulin with resultant insulin deficiency. Type 2 diabetes represents more than 90 % of all cases and it is characterised by insulin resistance and relative insulin deficiency. It is mainly due to a genetic disposition, environmental and human behavioural factors, including sedentary lifestyle, overtly rich nutrition, affluence and obesity. Both forms of diabetes are associated with long-term complications that affect most of the major organs of the body, such as the eyes, brain, heart, blood vessels, kidneys and nervous system. Complications can be suppressed or prevented by good metabolic control. During the past few years, tremendous advances have been made in early diagnosis and in controlling and preventing the disease, understanding the complications and developing new forms of treatment. Research has focused on several areas of DM including the insulin pump, ion channelopathies, pancreatic islet transplant, control of obesity, salivary and pancreatic insufficiencies, contractile dysfunction of the heart and understanding the causes of retinopathy, neuropathy and nephropathy. This review focuses on the disease itself and new research into a number of areas of its long-term complications.

Key words: *Diabetes mellitus, pancreas, insulin, momordica charantia, hypoglycaemia, cardiomyopathy, glucose*

Introduction

Diabetes mellitus (DM) is a generalized chronic disorder characterized by certain abnormalities in carbohydrate, fat, electrolyte and protein metabolism. DM occurs either because of a lack of insulin or because of the presence of factors that oppose the action of insulin (Harris & Zimmet, 1997). This in turn results in an increase in blood glucose concentration (hyperglycaemia) that ultimately leads to several acute and chronic complications including neuropathy, nephropathy, retinopathy, cardiomyopathy, microangiopathy, atherosclerosis and foot ulcers (Kumar & Clark, 2002; Williams & Pickup, 1998).

Diabetes is one of the oldest known human diseases. In 600 BC, Indian Physicians described the condition as a “shower of honey”. Its full name – Diabetes Mellitus – comes from the Greek words for siphon and sugar, describing the symptomatic hallmark of uncontrolled diabetes i.e. passing large amounts of sweet urine (glycosuria, polyuria). There are descriptions of the symptoms by the ancient Persians, Indians, and Egyptians, but a proper understanding of the condition has only developed over the last hundred years. In the 18th century Dobson (Dobson,

1776) linked the disease to a sugar abnormality and Crawley (Crawley, 1788) suggested that the pancreas was intimately involved in the sickness. In 1889 von Mering and Oscar Minkowski succeeded in artificially producing diabetes in dogs by removing the pancreas (Bilous, 2002). They established a relationship between diabetes and the pancreas. With the famous discovery of insulin by Banting and Best in 1921, diabetes mellitus was thought to be "cured". But to-date, despite tremendous efforts in research on pharmacology, pathogenesis and prophylaxis; treatment is still not curable, but treatable (Biluos, 2000). The average life expectancy of a diabetic patient has increased dramatically in the post-insulin era (Rynearson & Hilderbrand, 1941), but diabetes still represents a significant health problem in the general population.

In 1900 diabetes was ranked 27th on the list of cases of death. This has risen alarmingly to the point today where, behind cardiovascular diseases and cancer, diabetes has been reported to be the third leading cause of death in the developed world (Notkins, 1979). Globally, the number of people with diabetes have risen from 30 million in 1985 to 143 million in 2000. It is estimated that in the year 2010 it will be 200 million and 300 million in 2025 (King et al., 1988; Amos *et al*., 1997; Zimmet *et al*., 2000). Most cases will be of Type 2 diabetes, which is strongly associated with a sedentary lifestyle, over rich nutrition and obesity (Zimmet, 1998; Zimmet *et. al*, 2001).

The occurrence of congestive heart failure in the diabetic population and failure of the cardiovascular system are considered to be the leading cause of death in diabetics (Julien, 1997; Kannel & McGlee, 1979; Satiel & Khan, 2001; Bracken et al, 2003). The presence of diabetes has reportedly incurred the chance of cardiovascular problems by 2 fold in the male population and 3-5 fold in the female population (Shepherd & Khan, 1999). Three major factors to largely account for the increased incidence of cardiovascular dysfunction during diabetes include major vessel disease in the form of atherosclerosis, microvascular alterations and primary myopathic disorders in cardiac muscle (Grossman & Messerb, 1996; Amos *et al*., 1997; Bracken et al, 2003; Fallow & Singh, 2004).

Insulin

Insulin is a key player in the control of intermediary metabolism. It has profound effects on both carbohydrate and lipid metabolism and significant influences on protein and mineral metabolism. Consequently, derangements in insulin signalling have widespread and devastating effects on many organs and tissues of the body (Satiel & Khan, 2001; Dunne et al, 2004).

Plasma glucose usually remains in a narrow range between 4 and 7 mmol/L in normal individuals. This tight control is governed by the balance between glucose absorption through the intestine, its production by the liver and its uptake and metabolism by peripheral tissues. Insulin increases glucose uptake in muscle and fat and inhibits haepatic glucose production, thus serving as the primary regulator of blood glucose concentration. Insulin also stimulates cell growth and differentiation and promotes the storage of substances in fat, liver and muscle by stimulating lipogenesis, glycogen and protein synthesis and inhibiting lipolysis, glycogenolysis and protein breakdown. Insulin resistance or deficiency results in profound deregulation of these processes, and produces elevations in fasting and postprandial glucose and lipid levels (Satiel & Khan, 2001).

Like the receptors for other protein hormones, the receptor for insulin is embedded in the plasma membrane (Patti & Khan, 1998; Setsi et al, 2001). The insulin receptor is composed of two alpha subunits and two beta subunits linked by disulfide bonds. The alpha chains are entirely extracellular and contains insulin binding domains, while the linked beta chains penetrate through the plasma membrane. The insulin receptor is tyrosine kinase and it functions as an enzyme that transfers the phosphate groups from adenosine triphosphate (ATP) to tyrosine residues on intracellular target proteins. Binding of insulin to the alpha subunits causes the beta subunits to

phosphorylate themselves (autophosphorylation), thus activating the catalytic activity of the receptor. The activated receptor then phosphorylates a number of intracellular proteins, which in turn alters their activity and induces a biological response (Bell *et. al*, 2001; Satiel & Khan, 2001; Setsi et al, 2001).

Several intracellular proteins have been identified as phosphorylation substrates for the insulin receptor, the best studied of which is insulin receptor substrate 1 (IRS-1). When IRS-1 is activated by phosphorylation, a number of events takes place. Among other processes, IRS-1 serves as a type of docking centre for recruitment and activation of other enzymes that ultimately mediate the effects of insulin (White, 1998; Satiel & Khan, 2001; Setsi et al, 2001).

Carbohydrate metabolism

Glucose is liberated from dietary carbohydrates such as starch or sucrose by enzymatic hydrolysis within the small intestines and is then absorbed into the blood. Elevated concentrations of blood glucose stimulate the release of insulin, which in turn acts on cells throughout the body to enhance uptake, utilization and storage of glucose. The effects of insulin on glucose metabolism vary and these depend on the target tissue (Satiel & Khan, 2001; Brownlee, 2001).

In muscle, adipose and most other tissues insulin facilitates the entry of glucose into the cells. The only mechanism by which cells can take up glucose is by facilitated diffusion through a family of hexose transporters. In many tissues (muscle being a prime example) the major transporter (GLUT4) used for uptake of glucose is made available in the plasma membrane through the action of insulin (Klip & Paquet, 1990). In the absence of insulin, GLUT4 glucose transporters are present in cytoplasmic vesicles, where they are useless for transporting glucose. Binding of insulin to receptors on such cells rapidly leads to fusion of the vesicles within the plasma membrane and insertion of the glucose transporters, allowing the cell to efficiently take up glucose. When blood levels of insulin decrease and insulin receptors are not occupied, the glucose transporters are recycled back into the cytoplasm. There are some tissues that do not require insulin for efficient uptake of glucose: important examples are brain and the liver. This is because these cells do not use GLUT4 for importing glucose, but rather, another transporter that is not insulin-dependent.

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 haepatocytes, which convert it into the storage polymer glycogen (Silverman, 1991; Wright, 1991; Thorens, 1993; Shepherd & Khan, 1999). Insulin has several effects in the liver, including the stimulation of 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 use later. As blood glucose concentrations fall, insulin secretion ceases. In the absence of insulin, the bulk of the cells in the body are unable to take up glucose, and they switch to using alternative fuels, like fatty acids for energy. Neurons, however, require a constant supply of glucose, which in the short term, is provided from glycogen reserves (Kumar & Clark, 2002). In the absence of insulin, glycogen synthesis in the liver ceases and enzymes responsible for breakdown of glycogen become active. Glycogen breakdown is stimulated not only by the absence of insulin, but, by the presence of another metabolic endocrine hormone, glucagon, which is secreted when blood glucose levels fall below the normal range (Satiel & Khan, 2001).

Lipid metabolism

The metabolic pathways for utilization of fats and carbohydrates are intricately intertwined (Brownlee, 2001). Considering the profound effects of insulin on carbohydrate metabolism, insulin also has important effects on lipid metabolism (Bergman & Ader, 2000). Insulin, for instance, promotes synthesis of fatty acids in the liver particularly when its glycogen content is high (roughly 5% of liver mass). This in turn results in a suppression of glycogen synthesis (Brownlee, 2001).

When the liver is saturated with glycogen, any additional glucose taken up by hepatocytes is shunted into pathways leading to synthesis of fatty acids, which are exported from the liver as lipoproteins. The lipoproteins are degraded in the circulation, providing free fatty acids for use in other tissues, including adipocytes, which then use them to synthesize triglyceride. This synthesis requires glycerol which is synthesized in adipose cells from glucose.

Insulin also inhibits breakdown of fat in adipose tissue by inhibiting the intracellular lipase that hydrolyzes triglycerides to release fatty acids. Insulin, thus, has a fat-sparing effect. Not only does it drive most cells to preferentially oxidize carbohydrates, instead of fatty acids, for energy, but it stimulates the accumulation of fat in adipose tissue (Satiel & Khan, 2001; Pilkis & Granner, 1992).

Protein metabolism

Insulin also stimulates the uptake of amino acids into most cells, further expecting an overall anabolic effect (Pilkis & Granner, 1992). Conversely, when insulin levels are low (as in the fasting state) intracellular protein degradation occurs.

Ions

Insulin 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 thus kill patients because of its ability to acutely suppress plasma potassium concentrations (Geering, 1994; Lingrel & Kuntzwiler, 1994; Ewarrt & Klip, 1995).

Aetiology and classification

Two principal forms of DM are recognized (Harris and Zimmet, 1997). These include:-

(1) Type I or Insulin-Dependent Diabetes Mellitus (IDDM) which results from a frank deficiency of insulin. The onset of this disease typically is in childhood. Its pathogenesis involves environmental triggers that may activate autoimmune mechanisms in genetically susceptible individuals, leading to progressive loss of pancreatic islet cells (Harrison *et al*., 1999). Predisposition is mediated by a number of genes that interact in a complex manner with each other and the environment (Atkinson and Mclaren, 1994; Harrison et al., 1999). Many of the acute effects of this disease can be controlled by insulin replacement therapy, but inevitably, there are long-term adverse effects on blood vessels, nerves and other organ systems.

The aetiology of IDDM usually results from either hormonal or cellular immunity. Islet cell antibodies are present in most patients and are diagnostic of IDDM. They gradually decline and disappear over time. Antibodies to specific proteins have more recently been identified: these include antibodies to glutamic acid decarboxylase and to tyrosine phosphatase. The presence of these antibodies in a non-diabetic individual indicates an 88% chance of developing diabetes within 10 years (Zimmet *et al*., 2001). The presence of insulinitis at the onset of IDDM is a further reflection of autoimmune function and the action of inflammatory cells in B cell destruction. Macrophages also produce cytokines leading to the activation of lymphocytes known to be present at the onset of IDDM.

Type 2 or Non-Insulin-Dependent Diabetes Mellitus (NIDDM) begins as a syndrome of insulin resistance, in which target tissues fail to respond appropriately to insulin. Typically, the onset of this disease is in adulthood. The nature of the defect has, however, been difficult to ascertain. In some patients the insulin receptor is abnormal, while in others, one or more aspects of insulin signalling is defective, and in others, no defect has been identified. For most patients, insulin release is not usually impaired at least initially and insulin injections are therefore not useful for therapy. Rather, the disease is controlled through dietary therapy and hypoglycaemic agents (Harris & Zimmet, 1997; Miller, 2001; Zimmet *et al*., 2001; Kumar & Clark, 2002).

The aetiology of type 2 diabetes is still uncertain especially as it is a term applied to several categories of diabetic patients, many of which have an undefined cause. Apart from a small number of patients with mutations in proinsulin, glucokinase, the insulin receptor or specific mitochondrial enzymes, type 2 diabetes is generally recognized from a culmination of genetic and environmental risk factors such as obesity and a sedentary lifestyle. Additional genetic risk factors include insulin resistance and the limited ability of the pancreatic beta cells to compensate for the insulin resistant state. The beta cells progressively lose insulin secretion capability and both blood glucose levels and haepatic glucose production increase, leading to further aggravation of the hyperglycaemic state. Hence, Type 2 diabetes is the result of the combined effects of insulin resistance, post-receptor defects and beta cell failure (Zimmet & Lefebure, 1996; Zimmet *et al*., 2001). Of growing interest is a group of non-diabetic patients with a collection of similar symptoms. Similar to these in type 2 diabetes, these non-diabetic individuals have peripheral insulin resistance and impaired glucose tolerance in a disease recognised as Syndrome X. The pancreatic beta cells of these patients respond to insulin resistance by secreting increased quantities of insulin, which results in hyperinsulinaemia (Dunne et al, 2004). It is hypothesized that the chronically elevated circulating insulin levels may result in dyslipidaemia and an increased risk for atherosclerotic heart disease(Bracken et al, 2003).

Signs and symptoms of diabetes mellitus

The symptoms of diabetes mellitus are similar in both types of diabetes (NIDDM and IDDM) but they develop most rapidly and more severely in patients with IDDM (usually develop over weeks but sometimes over a few months). These symptoms include polyuria, polydipsia, polyphagia, weight loss, fatigue, cramps, constipation, blurred vision and candidiasis (Alterman, 1997; Bilous, 2002; Kumar & Clark, 2002).

IDDM usually presents acutely with hyperglycaemic symptoms (polyuria, polydipsia, polyphagia), tiredness and weight loss. Nausea, vomiting and drowsiness usually denote impending ketoacidosis. Minor symptoms include cramps, blurred vision and superficial infections. The acute presentation of IDDM is probably the culmination of chronic autoimmune destruction of the pancreatic B-cells. Subtle abnormalities of insulin secretion and glucose tolerance can be detected during this prediabetic phase.

Long standing IDDM patients are susceptible to microvascular complications including nephropathy, neuropathy, retinopathy that are specific to diabetes as well as to non-specific macrovascular disease including coronary artery, heart and peripheral vascular disease (Kumar & Clark, 2002; Bracken et al, 2003; Fallow& Singh, 2004).

Symptoms in patients with NIDDM are similar but insidious in their onset. Many cases are diagnosed incidentally or because of the presence of diabetic complications.

NIDDM carries a high risk of large vessel complications. Hyperlipidemia and obesity may contribute to atherosclerosis and induce hypertension. Myocardial infarction is also common and accounts for about 60% of the deaths. Generally, cardiovascular complications are the most common cause of morbidity and mortality in diabetic patients (Julien, 1997). Moreover, human studies have shown that DM can be associated with altered cardiac function (Grossman &

Messerli, 1996) and this can occur independent of cardiovascular complications (Kannel & Mcgee, 1999; Howarth & Singh, 1999; Bracken et al, 2003; Fallow & Singh, 2004).

Complications of diabetes mellitus

The complications of diabetes mellitus include:-

Hyperglycaemia: Insulin is vital to patients with Type 1 DM. Without insulin, patients with Type 1 DM can develop severely elevated blood sugar levels. This leads to increased urine glucose, which in turn leads to excessive loss of fluid and electrolytes in the urine. Lack of insulin also causes the breakdown of fat cells, with the release of ketones into the blood. Ketones turn the blood acidic, a condition called diabetic ketoacidosis. Symptoms of diabetic ketoacidosis include nausea, vomiting and abdominal pain. Without prompt medical treatment, patients with diabetic ketoacidosis can rapidly go into shock, coma, and even death. Diabetic ketoacidosis can be precipitated by infections, stress or trauma. Urgent treatment of diabetic ketoacidosis involves the intravenous administration of fluid, electrolytes and insulin, usually in a hospital intensive care unit. Antibiotics are given for infections. With treatment, abnormal blood sugar levels, acidosis and dehydration can be reversed rapidly, and patients can recover remarkably well (Alterman, 1997; Bilous, 2002; Kumar & Clark, 2002).

In patients with Type 2 DM, stress, infection and medications (such as corticosteroids) can also lead to severely elevated blood sugar levels. Accompanied by dehydration, severe blood sugar elevation in patients with Type 2 DM can lead to an increase in blood osmolality (hyperosmolar state). This condition can lead to coma (hyperosmolar coma). A hyperosmolar coma usually occurs in elderly patients with Type 2 DM. Like diabetic ketoacidosis, a hyperosmolar coma is a medical emergency. Immediate treatment with intravenous fluid and insulin is important in reversing the hyperosmolar state. Unlike patients with Type 1 diabetes mellitus, patients with type 2 DM do not generally develop ketoacidosis (Kumar & Clark, 2002).

Hypoglycaemia: In patients with diabetes, blood sugar level may become abnormally low. The most common cause of hypoglycaemia is excessive use of exogenous insulin or other glucose-lowering medications, to lower the blood sugar level in diabetic patients (Kumar & Clark, 2002). When low blood sugar levels occur because of too much insulin, it is called an insulin reaction. Sometimes, low blood sugar can be the result of an insufficient caloric intake or sudden excessive physical exertion. Blood glucose is essential for the proper functioning of nerve cells in the brain. Therefore, low blood sugar can lead to nervous system symptoms such as dizziness, confusion, weakness, and tremors. Untreated, severely low blood sugar levels can lead to coma, seizures and even in irreversible brain death. The treatment of low blood sugar consists of administering glucose drinks, such as orange juice, soft drinks (not sugar-free), or glucose tablets. If the individual becomes unconscious, glucagon can be given by intramuscular injection. Glucagon causes the release of glucose from the liver, and should be part of the emergency kit of a diabetic, especially if the patient uses insulin. Families and friends of those with diabetes need to be taught how to administer glucagon, since the patients will not be able to do it themselves in an emergency situation.

Chronic complications: These complications are related to blood vessel diseases and are generally classified into small vessel disease, such as those involving the eyes, kidneys and nerves (microvascular disease) and large vessel disease involving the heart and blood vessels (macrovascular disease). Diabetes accelerates hardening of the wall of the larger blood vessels (atherosclerosis), leading to coronary heart disease (angina or heart attack), strokes, and pain in the lower extremities because of lack of blood supply (claudication) (Kannel & McGee, 1979; Grossman & Messerli, 1996; Julien, 1997; Howarth & Singh,1999; Bracken et al, 2003).

Eye complications of diabetes (particularly diabetic retinopathy) occur in patients who have had diabetes for at least 5 years. Diseased small blood vessels in the back of the eye cause the

leakage of protein and blood in the retina. Disease in these blood vessels also causes the formation of small aneurysms (microaneurysms) and new but brittle blood vessels (neovascularization). Spontaneous bleeding from the new and brittle blood vessels can lead to retinal haematoma, scarring and retinal detachment, thus impairing vision. The treatment of diabetic retinopathy consists of using a laser to destroy and prevent the recurrence of the development of these small aneurysms and brittle blood vessels. Approximately 50% of patients with diabetes will develop some degree of diabetic retinopathy after 10 years of diabetes, and 80% of diabetics have retinopathy after 15 years of the disease. Poor control of blood sugar and blood pressure further aggravates eye disease in diabetes. Cataracts and glaucoma are also more common among diabetics. It is also important to note that since the lens of the eye is permeable to water significant variations in blood sugar concentrations may cause the lens of the eye to shrink or swell according to fluid changes. Blurred vision is therefore very common in poorly controlled diabetes. Most patients, therefore, are discouraged from getting a new eyeglass prescription until their blood sugars are controlled (Kumar & Clark, 2002; Williams & Pickup, 1998).

The onset of kidney disease (diabetic neuropathy) and its progression is extremely variable. Initially, diseased small blood vessels in the kidneys cause the leakage of protein in the urine. Later on, the kidneys lose their ability to cleanse and filter blood. The accumulation of toxic waste products in the blood may require dialysis and subsequently therapy of renal transplantation. The progression of nephropathy can be significantly slowed by controlling blood pressure and by aggressively treating high blood sugar levels. Angiotensin converting enzyme inhibitors (ACE inhibitors) or angiotensin receptor blockers (ARBs) used in treating high blood pressure may thus benefit kidney disease in diabetic patients (Kumar & Clark, 2002; Williams & Pickup, 1998).

Nerve damage in diabetes (diabetic neuropathy) is also partially caused by small blood vessel disease. In essence, blood flow to the nerves is impaired and they may die or become deranged due to ischaemia. Symptoms of diabetic nerve damage include numbness, burning and aching of the feet and lower extremities. When the nerve disease causes a complete loss of sensation in the feet, patients may not be aware of injuries to the feet, and fail to properly protect them. Seemingly, minor skin injuries should also be attended to promptly, to avoid serious infections. As a result of poor blood circulation, diabetic foot injuries may not heal. Sometimes, minor foot injuries can lead to serious infection, ulcers, and even gangrene, necessitating surgical amputation of toes, feet and other infected parts. Diabetic nerve damage can affect the nerves that are important for penile erection, causing erectile dysfunction (ED). ED can also be caused by poor blood flow to the penis from diseased diabetic blood vessel. Diabetic neuropathy can also affect nerves to the stomach and intestines, causing nausea, weight loss, and diarrhoea and other symptoms known under the term gastroparesis (Kumar & Clark, 2002; Williams & Pickup, 1998). Recently, it has been reported that DM is associated with cognitive deficit and an increased risk of dementia, especially in the elderly. These deficits are associated with neurophysiological and structural changes in the brain (Gispen & Biessel, 2000). It has also been suggested recently that DM may contribute to the pathogenesis of sporadic Alzheimer's disease (Vahanen, 1998). Nerve damage in DM may also result from reduced glucose uptake and cellular dehydration.

Diagnosis of diabetes mellitus

The current number of diabetic cases world wide is around 170 million, but they are several million more cases who are undiagnosed and the figure is rising rapidly. Early diagnosis of DM is of paramount importance both in controlling the disease and in preventing long–term complications. This is a major problem in the developing countries where people do not have access to modern medical facilities as in developed countries. Thus, most diabetic cases in poor

countries are in the chronic stage when they are diagnosed. Many people realise that they are diabetic only when they have to change their spectacles quite often, when they have increased diuresis, they often become tired and an injury takes a long time to heal and in many cases their extremities are numb.

Diabetes is normally diagnosed in symptomatic patients by an elevated random blood glucose or by two fasting values above 6.7 mmol/litre for whole blood and 7.8 mmol/litre for plasma. When in doubt, the patient is asked to perform an oral glucose tolerance test (OGTT). The patient drinks 75 g of glucose solution and venous plasma is measured for glucose 2 hours later. A value greater than 11.1 mmol/litre is confirmed as diabetes. If the value is between 7.8 –11.1 mmol/litre then the patient is said to have impaired glucose tolerance (IGT) and the patient has to be closely monitored for DM (Williams & Pickup,1998).

Management of diabetes mellitus

The main aims of diabetes management are:-

To achieve a normal glycaemic state.
To reduce the risk of long term damage to organs and tissues resulting from sustained hyperglycaemia.
To enable the patient to maintain as near a normal a lifestyle as possible while ensuring adequate control of his or her diabetes.
To establish compliance with the patient relating to his or her management plan.

In order that these aims are achieved it is important to observe the following -:

Educate the patient about diabetes
Control of diet
Regular exercise
Therapy with oral hypoglycaemic agents or insulin (where necessary)
Frequent monitoring of diabetic control
Correction of other cardiovascular risk factors (e.g. hypertension)
Early detection of signs and symptoms of complications

Findings from the Diabetes Control and Complications Trial (DCCT) and the United Kingdom Prospective Diabetes Study (UKPDS) have clearly shown that aggressive and intensive control of elevated levels of blood sugar in patients with Type 1 and Type 2 DM decreases the complications of nephropathy, neuropathy, retinopathy, and may reduce the occurrence and severity of large blood vessel disease (Williams & Pickup, 1998). Aggressive control with intensive therapy means achieving fasting glucose levels between 4-7 mmol/litre; glucose levels of less than 10 mmol/litre after meals; and a near normal haemoglobin A1C levels (see below). Previous studies in Type 1 patients have shown that in intensively treated patients, diabetic eye disease decreased by 76%, kidney disease decreased by 54%, and nerve disease decreased by 60%. However, the price for aggressive blood sugar control is a 2 to 3 fold increase in the incidence of abnormally low blood sugar levels (caused by the diabetes medications). For this reason, tight control of diabetes to achieve glucose levels between 4-7 mmol/litre is not recommended for children under 13 year of age, patients with severe recurrent hypoglycaemia, patients unaware of their hypoglycaemia and patients with far advanced diabetes complications. To achieve optimal glucose control without an undue risk of abnormally lowering blood sugar levels, patients with Type 1 DM must monitor their blood glucose at least 4 times a day and administer insulin at least 3 times per day. In patients with Type 2 DM, aggressive blood sugar control has similar beneficial effects on the eyes, kidneys, nerves and blood vessels. (Alterman, 1997; Bilous, 2002; Kumar & Clark, 2002; Williams & Pickup, 1998).

Treatment of diabetes mellitus

All patients with diabetes should have diet therapy, which is usually complimented with a programme of regular exercise. Good glycaemic control is unlikely to be achieved with exogenous insulin when diet and exercise are neglected, especially when the patient is also overweight. Exogenous insulin is always recommended in a patient who has been in ketoacidosis, and is usually recommended for patients who present with diabetes under the age of 40 years. Exogenous insulin is also recommended for older patients following primary or secondary failure to oral therapy. (Kumar and Clark, 2002).

In older patients the approach to therapy is empirical. Diet and exercise should be tried in the first instance, and dietary knowledge and compliance should always be reassessed with care before proceeding to the next step. This is of particular importance in the obese patient who fails to lose weight. When diet and exercise fail to achieve satisfactory control, thin patients are usually treated with a sulphonylurea drug, and obese patients with a biguanide. Primary failure of oral treatment, occur when these drugs either alone or in combination never achieve the desired level of control. Other patients may show either a good or reasonable initial response followed by progressive loss of control over the succeeding months or years (Alterman, 1997).

Type 1 diabetes (IDDM): For Type 1 diabetes it is essential that the patient is immediately initiated on insulin therapy. The doses and administration of insulin are discussed below.

Treatment of diabetes with insulin: Exogenous insulin is the mainstay of treatment for patients with type 1 DM. Exogenous insulin is also important in Type 2 diabetes when blood glucose levels cannot be controlled by diet, weight loss, exercise, and oral medications. Ideally, insulin medication should be administered in a manner that mimics the natural pattern of insulin secretion by a healthy pancreas. The complex pattern of insulin secretion by the pancreas is difficult to duplicate. Still, adequate blood glucose control can be achieved with careful attention to diet, regular exercise, home blood glucose monitoring, and multiple exogenous insulin injections throughout the day. In the past, the exogenous insulin used was derived from animal sources, particularly cows and pigs. Not only was there a problem with the supply of insulin in meeting the demand, but beef and pork insulin also had specific problems. Being from animals, these types of exogenous insulin caused immune reactions in people. Patients would become either intolerant or resistant to animal insulin. With the acceleration of scientific research in the latter half of this century, beef and pork insulin was replaced by human insulin. In 1977, the gene for human insulin was cloned, and through modern technology, manufactured human insulin was made available. Human insulin is now widely used. Insulin now comes in a variety of preparations that differ in time of onset and length of action. As a result of these differences, combinations of insulin are often used to allow for a more tailored regimen of blood sugar control. For example, a patient may take an injection of long-acting insulin in the morning and evening to provide a baseline of insulin throughout a 24-hour period. In addition, the same patient may take an injection of short-acting insulin just before meals to cover the increase in carbohydrate load after eating (Alterman, 1997; Bilous, 2002; Kumar & Clark, 2002).

Different methods of delivering insulin: The variety of available insulin preparations is growing as are the methods for administering exogenous insulin.

Pre-filled insulin pens: In the past, insulin was available only in an injectable form. This involved carrying syringes (which a few decades age were made of glass and required sterilization), needles, vials of insulin, and alcohol swabs. Needless to say, patients often found it difficult to take multiple shots a day, and as a result, good blood sugar control was often compromised. Many pharmaceutical companies are now offering discreet and convenient methods of insulin delivery. A small pen-sized device holds an insulin cartridge (usually containing 300 units). Cartridges are available in the most widely used insulin formulations. The amount of insulin to be injected is dialled in by turning the bottom of the pen until the required

number of units is seen in the dose-viewing window. The tip of the pen consists of a needle that is replaced with each injection. A release mechanism allows the needle to penetrate just under the skin and deliver the required amount of insulin. The cartridges and needles are disposed of when finished, and new ones are simply inserted. These insulin delivery devices are discreet and less cumbersome than traditional methods (Alterman, 1997).

Insulin pump: Advance in insulin delivery to diabetic patients is the insulin pump (Allerman, 1997). An insulin pump is composed of a pump reservoir similar to that of an insulin cartridge, a battery-operated pump, and a computer chip that allows the user to control the exact amount of insulin being delivered. Currently, pumps on the market are about the size of a bleeper. The pump is attached to a thin plastic tube (an infusion set) that has a soft cannula (or needle) at the end through which insulin passes. This cannula is inserted under the skin, usually on the abdomen. The cannula is changed every 2 days. The tubing can be disconnected from the pump while showering or swimming. The pump is used for continuous insulin delivery, 24 hours a day. The amount of insulin is programmed and is administered at a constant rate (basal rate). Often, the amount of insulin needed over the course of 24 hours varies depending on factors such as exercise, activity level and sleep. The insulin pump allows for the user to programme many different basal rates to allow for this variation in lifestyle. In addition, the user can programme the pump to deliver a "bolus" during meals to cover the excess demands of carbohydrate ingestion. Over 50,000 people worldwide are using the insulin pump. This number is growing dramatically as these devices become smaller and user-friendlier. Insulin pumps allow for tight blood sugar control and lifestyle flexibility while minimizing the effects of low blood sugar (hypoglycaemia). At present, the pump is the closest device on the market to an artificial pancreas. Naturally, the next step would be a pump that can also sense blood sugar levels and adjust the insulin delivery accordingly. Much effort is being concentrated on this area of research and possibly, even within the next few years, a prototype device will be available for trial (Alterman, 1997).

Inhalation: Another promising route of insulin administration is through inhalation. Inhaled insulin is currently being tested but has not been approved by the United States Food and Drug Administration (FDA). Many devices are available that allow for other medications to be used in this manner, the best example of which is asthma therapy. Insulin is not absorbed through the bronchial tubes (airways), and must reach the air sacks at the end of the bronchial tubes (alveoli) to be absorbed. Once in the alveoli, insulin can be absorbed and enter the bloodstream. Currently, powdered inhalers and nebulisers are being studied to determine which delivery system is the most reliable. The safety of inhaled insulin still needs to be established before a product for consumer use can be made available.

Intranasal, transdermal and pill: Other routes for the delivery of insulin have also been tried and intranasal insulin delivery was thought to be promising. However, this method was associated with poor absorption and nasal irritation. Transdermal insulin (skin patch delivery) has also yielded disappointing results to date. Insulin in pill form is also not yet effective since the digestive enzymes in the gut break it down.

Treatment of type 2 diabetes: Patients should never forget the importance of diet and exercise in the management of diabetes and that control of diabetes starts with a healthy lifestyle regardless of what medications are prescribed. Medications including these that increase the insulin output by the pancreas:–

Sulfonylureas and meglitinides: Sulfonylureas: Historically, increasing the endogenous insulin output by the pancreas has been the major area targeted by medications used to treat Type 2 diabetes. Some of these medications belong to a class of drugs called sulfonylureas. Sulfonylureas primarily lower blood glucose levels by increasing the release of insulin from the pancreas. Older generations of these drugs include chlorpropamide and tolbutamide, while newer drugs include – glimepiride (Amaryl), glipize (Glucotrol) and glyburide (Dia Beta). These drugs

are effective in rapidly lowering blood sugars, but run the risk of causing hypoglycaemia. In addition, they are sulfa compounds, and should be avoided in patients with sulfa allergies (Alterman, 2002; Bilous, 2002; Kumar & Clark, 2002; Williams & Pickup, 1998).

Meglitinides also target the pancreas to promote insulin secretion. Unlike sulfonylureas that bind to receptors on insulin producing cells, meglitinides work through a separate potassium-based channel on the cell surface. (Starlix) Nategnilide and (Prandin) Repeglinide are short acting agents that are taken 30 minutes before meals. Unlike the sulfonylureas, which last longer in the body, Prandin and Starlix are very short acting, with peak effects within one hour. For this reason, they are administered up to 3 times a day just before meals. Since these drugs also increase circulating insulin levels, they may also cause hypoglycaemia, but the literature suggests this is less frequent than the hypoglycaemia seen with sulfonylureas (Alterman, 1997; Kumar & Clark,2002).

In a 3-month study, it was shown that prandin can lower fasting blood glucose values by 61 mg dl^{-1} and post meal blood glucose values by 100 mg dl^{-1}. Prandin is short acting and given before meals and it is particularly beneficial in lowering blood glucose after meals and does not tend to lower fasting glucose levels to the same degree. Prandin has been used in combination with other medications, such as Glucophage, with impressive results. In 83 patients with Type 2 diabetes, blood sugar control improved significantly with the addition of Prandin to Glucophage. (Kumar & Clark, 2002).

Prandin does interact with other medications. The physician must be aware of all other medications the patient is taking before prescribing Prandin. The usual starting dose is 0.5 mg before each meal, and can be increased to 4 mg. The maximum daily dose is 16 mg. It is used with caution in people with kidney or liver abnormalities. Since Prandin increases insulin levels, it has the risk of causing abnormally low blood sugars, which results in sweating, tremors, and confusion and may lead to coma and seizure if severe and prolonged. In addition, the use of Prandin has been associated with headaches and muscle and joint aches along with sinus infections in some individuals. This drug should not be used in pregnancy or by nursing mothers. The dose may need to be adjusted in older people, since they may metabolize (eliminate) medications at a slower rate.

Medications that decrease the amount of glucose produced by the liver: Biguanides have also been used for many years in Europe and Canada. Metformin (Glucophage) for instance reduces glucose production by the liver. Briefly, because metformin does not increase insulin level, when used alone, it does not usually cause hypoglycaemia. In addition, metformin tends to suppress appetite, which may also be beneficial in the treatment of type 2 diabetes. Metformin may be used by itself or in conjunction with other oral agents or insulin. It should not be used in patients with kidney impairment and moreover, it should be used with caution in those with liver impairment. The older parent compounds of metformin were associated with a serious condition called lactic acidosis with a dangerous acid build up in the blood resulting from accumulation of the drug and its breakdown products. While metformin is safer in this regard, it is recommended that the drug be discontinued for 24 hours before any dye-related procedure (such as IVP kidney study) or surgery is performed. The dyes may impair kidney function and cause a build up of the drug in the blood. Metformin can be restarted after these procedures once the patient has voided normally (Alterman, 1997; Bilous, 2002; Kumar & Clark,2002).

Medications that increase the sensitivity of cells to insulin: A class of drugs known as thiazolidinediones lowers blood glucose by improving target cell response to insulin (increasing the sensitivity of the cells to insulin). Troglitazone (Rezulin) was the first of this type of compound to be introduced. However, because of severe toxic liver effects, troglitazone has been taken off the market. Sister compounds are now available with a better safety profile. These drugs include (Actos) Pioglitazone and (Avandia) Rosiglitazone.

Pioglitazone (Actos) and Rosiglitazone (Avandia) are new thiazolidinediones that have been approved for use in the United States. While they are sister compounds to Rezulin, extensive studies have failed to show any liver problems associated with this particular drug. Patients should be aware, however, that these drugs are still relatively new, and its long-term safety profile is not yet known. Both Avandia and Actos act by increasing the sensitivity (responsiveness) of cells to insulin. It improves sensitivity to insulin in muscle and fat tissues. These drugs have been effective in lowering blood sugars in patients with Type 2 diabetes, Actos and Avandia act within 1 hour of administration and are dosed daily. It is important to note that it takes up to 6 weeks to see a drop in blood glucose levels on these agents and up to 12 weeks to see a maximum benefit. Actos and Avandia have been approved as first line therapy in diabetes, and for use in combination (Alterman, 1997; Bilous, 2002; Kumar & Clark, 2002).

Traditional plant-based medicines in the treatment of diabetes mellitus: Medicinal plants have been used throughout time to treat diabetes (see Platel & Srinivassan, 1997 and Garau et al, 2003 for review). For the most part, anti-diabetic medicinal plants have been used routinely to treat DM prior to the discovery of insulin in the early 1920s. In more traditional cultures, plant-based remedies continue to be the treatment of choice for diabetes. Amongst this list of plants are:- *Astocarpus altillis* (Breadfruit), *Azadirachta indica* (Neem), *Bidens alba* (Spanish-needle), *Carica papaya* (Papaw), *Cassia occidentalis* (Wild coffee), *Catharanthus roseus* (Periwinkle), *Stachytarpheta jamaicencia* (Burr-vine), *Syzygium cumini* (Jamoon) and *Momordica charantia* (Caryla).

Bearing fruit from a flowering vine: *Momordica charantia* (caryla) is a flowering vine plant belonging to the plant family Cucurbitaceae (Ahmed et al,1998 : Garau et al, 2003; Day, 1990). It is a slender-stemmed tendril climber, the older stem often flattened and fluted, to 6 m or more long. Leaves alternate, cut into 5 – 7 narrow based lobes, the lobes mostly obtuse with marginal points, to about 12 cm long and as broad, very thin-textured and characteristic pungent-aromatic; tendrils laterally inserted at the petiole-base. Flowers are yellow, the female on short peduncles, the male on longer ones, short-lived. It bears oblong-shaped, bitter fruit that ranges in colour from pale to dark green, narrowed to both ends, 8 – 15 cm long, with prominent tubercles on the ribs, opening when ripe, becoming softly fleshy and revealing pendulous seeds covered with red pulp. Although the country of origin is uncertain, the plant is widely cultivated for its fruit on fences and in thickets in tropical regions of India, China, East Africa and Central, the West Indies and South America. In India, the unripe fruit is known as caryla and is a common ingredient in many curries. Caryla is found in many Asian dishes as well. The English know it as bitter gourd or bitter melon. Another local name is balsam pear. In South America and Guyana the plant is known as caryla or wild caryla (Sharma *et al*, 1950).

In folk medicine of South Asia, Africa, South America and the West Indies, the fruit and leaves of the plant have reportedly been used as hypoglycaemic agents, purgatives, emetics and abortifacients (Sharma et al., 1950 :Day et al, 1990; Sharma et al, 1996; Attar-uri- Rahman and Zaman, 1989; Day, 1990; Karunanayake et al, 1984) and the seeds therapeutically utilized against DM (Pons & Stevenson, 1943). The insulin-like hypoglycaemic activity in the seeds was attributed to charantin, the bitter ingredient of Momordica charantia (Olaniyi, 1975).

Laboratory studies have shown that extracts of caryla may block the absorption of sugar molecules in the intestine or improve the body's ability to utilise sugar, which would help to reduce blood sugar levels (Meir & Yaniv, 1985; Shibb *et. al*, 1993; Platel & Srinvassan,1997; Garau et al, 2003; Ahmed et al, 2004). Other studies have shown that caryla extracts may enhance the secretion of insulin from the pancreas (Ahmed *et al*, 1998). More recently, researchers at United Arab Emirates University have found that the juice of the bitter melon fruit may actually help to renew or recover partially destroyed insulin-secreting cells in the pancreas. The fruit juice or its extract can also reduce arterial blood pressure, regulate glucose uptake into jejunal vesicles,

and stimulate glucose and amino acid uptakes into L6 muscle cells (Ahmed, 1998; Ahmed *et al*., 1998, 2004; Cummings, 2000; Cummings *et al*., 2002;2004; Sharma et al 1996). The uptake of glucose into L6 muscle cells can be blocked by wortmannin, an inhibitor of phosphatidyl inositol 3-kinase (Klip & Paquet, 1990; Cummings, 2002; Cummings *et al*., 2002; 2004).These results suggest that the active ingredient in Momordica charantia may act via the same cell signalling pathway as insulin in stimulating glucose uptake into muscle cells.

Research on laboratory animals: Extracts of caryla have been found to improve blood sugar control in several animal models of diabetes (Sitasawad *et al*., 2000; Sarkar *et al*., 1996; Platel & Srinvassan, 1997; Garau et al, 2003; Ahmed, 1999;Ahmed et al, 2004; Sharma et al 1996). An exciting recent finding by Japanese researchers suggests that caryla powder not only reduces blood sugar levels in laboratory rats, but also increases HDL-cholesterol (the good cholesterol) while decreasing total cholesterol and triglycerides in the liver (Jayasooriya et al. 2000). This action suggests that caryla may be helpful in preventing some of the negative cardiovascular effects of diabetes.

Clinical studies: Despite support from laboratory experiments, well-controlled human studies investigating the benefits of caryla are limited. One small study involving 19 subjects with type 2 diabetes found that they responded better to an oral sugar challenge after consuming about 3 to 4 ounces of caryla juice prepared from fresh unripe fruits (Welihinda *et al*., 1996). Similar results were reported over 15 years earlier in the Maharashta Medical Journal: one to one and one-half ounces of fresh juice before each meal "satisfactorily stabilized" blood sugar in over 60 percent of the 161 subjects, all of whom had diabetes (Vad, 1960). Surprisingly, the powdered extract of caryla, which is readily available in the United States as a dietary supplement, has yet to be studied thoroughly in humans.

Momordica charantia (or caryla) has been used as an anti-diabetic remedy in many parts of the world for centuries. Despite a growing body of experimental support, it has yet to be systematically examined in well-controlled human studies. Complicating matters is the lack of information regarding the most effective amount and type of caryla extracts to use (see Platel & Srinivasan, 1997 and Garau et al, 2003 for review). Firstly, it is vitally important to isolate the active ingredient and then characterise it biochemical properties. It has been suggested that Momordical charantia fruit juice contains about 68 different agents (Platel & Srinvasan, 1997). Since diabetes is a costly and deadly disease that affects currently an estimated 170-180 million people worldwide (Zimmet *et al*., 2001) then botanical treatments may one day have a place in conventional diabetic medicine especially in developing countries.

Advances in diabetes mellitus research

There are a number of research projects currently in progress worldwide searching for the causes of diabetes and ways to diagnose the disease as early as possible and to prevent, treat and cure this complicated metabolic disorder. Additionally, it has been observed that changes in human behaviour, environmental factors, obesity, affluence, food indulgence and sedentary lifestyles over the last 25 years have resulted in a dramatic increase in the incidence of DM worldwide. The epidemic is mainly of Type 2 diabetes since this group comprises of about 90-95% of all cases of diabetes. The early diagnosis and prevention of diabetes and the control of major complications require an international approach, thus governments worldwide are obligated to spend more money on education and research into diabetes and its complications (Amos *et al*, 1997; Zimmet *et al*, 2001). Research is currently focusing on a number of measures including – aetiology, prevention, early diagnosis, “tight” blood glucose regulation, cardiovascular complications, identification and control of genes that cause diabetes and obesity, pancreas and kidney transplantation, islet cell transplantation, ion channelopathies, angiopathy-induced neuropathy, cattrach and retinopathy, use of plant-based medicine, new synthetic drugs,

pancreatic and salivary insufficiencies, the benefits of regular exercise, careful diet and weight control, the insulin pump and several others. In most of the above mentioned research projects it is necessary to employ experimental animal models first before exposing human beings.

Animal models in diabetic research

Experimental animal models facilitate the study of the pathogenesis and complications of diabetes, because they permit the evaluation of treatment protocols that are not immediately feasible or ethical in human patients. Furthermore, they provide populations of genetically uniform subjects that can be maintained under environmentally controlled conditions. Hence, new pharmaceutical agents and dietary regimens can firstly be tested in animals in order to evaluate potential toxicity and therapeutic benefit in humans. For the most part, these experimental protocols include the use of agents designed to improve diabetic control and prevent the onset/progression of complications. Although any single animal model may offer unique advantages for the study of human diabetes, it is not likely to be identical to the human disease. Furthermore, if one assumes that all animal models have inherent limitations, then the use of multiple experimental models represents a useful strategy for evaluating new therapeutic modalities for the study, of diabetes and its complications (Mordes *et al*., 1981; Amos et al., 1997; Kumar & Clark, 2002).

Animal models of diabetes can be divided into two categories, one in which animals are rendered diabetic by using specific procedures and the other in which animals develop diabetes spontaneously owing to a genetic predisposition. In both cases the animals can be classified either as NIDDM model or IDDM model and they display obesity, insulin deficiency (hypoinsulinaemia.), insulin resistance and hyperinsulinaemia and variable degrees of hyperglycaemia (Mordes *et al*., 1981; Shafrir, 1997; Gispen & Bressets, 2000).

Model of type 1 diabetes: The BB/Wor rat develops spontaneous and viral-induced syndromes of autoimmune DM, and is the best rat model of human Type 1 diabetes. Salient features include genetic predisposition, abrupt onset of insulin dependent, ketosis-prone diabetes and autoimmune destruction of pancreatic ß-cells. Since the first description of the syndrome in 1977, more than one thousand papers have been published by laboratories throughout the world (Shafrir, 1997). Support for the immune pathogenesis of diabetes in the BB/Wor rat is derived from the following: lymphocytic insulitis prior to and during the acute onset of hyperglycaemia; selective destruction of the pancreatic ß-cells with sparing of the other islet cells; prevention and/or amelioration of ß-cell destruction by immune suppressive agents directed against T cells and macrophages, and measures which correct the effects of genetically induced T cell lymphopaenia; adoptive transfer insulitis and diabetes to naive recipients with Conconavalin-A (CON-A) activated acute diabetic spleen cells. Other features of the BB/Wor rat, which identify it as the best rat model of human IDDM include their inbred status (>75 generations of sib matings) and high (80-95%) incidence of diabetes among both genders, and an average of onset of diabetes at 70 days. All diabetes prone (DP) BB/Wor strains are severely lymphopenic as manifested by a lifelong reduction of T-lymphocytes in peripheral blood, spleens and lymph nodes. Lymphopenia is genetically transmitted, the result of the homozygous lyp gene which was recently mapped on chromosome 4. The BBDP/Wor rats develop more severe and more “human like” complications of diabetes than streptozotocin (STZ) or alloxan models. Two conditions account for this observation. 1) Pancreatic beta cells in the chronically diabetic BBDP/Wor rats are completely destroyed and thus the animals are devoid of C-peptide, 2) The rats possess normal aldose reductase activity and therefore accumulate sorbitol, a compound implicated in diabetic complications (Mordes *et al*., 1981; Shafrir, 1997; Gispen & Bressets, 2000).

In addition to the genetically induced type 1 model, a variety of procedures have been used to induce this type of diabetes. These include pancreatectomy, exposure to viruses, and

administration of cell cytotoxic agents (alloxan, cosamine-nitrosurea, and STZ). These animals display typical Type 1 diabetes mellitus conditions such as severe insulinopaenia, hyperglycaemia, glycosuria, polydipsia and muscle wasting (Mordes *et al*., 1981; Sharma et al, 1996, Ahmed 1999; Howarth & Singh, 1999; Shetriv, 2000; Bracken et al 2003).

Models of type 2 diabetes: A variety of animal models of Type 2 diabetes are available including models which carry a genetic predisposition to develop diabetes e.g. Obese Zucker fatty rats (Bray, 1977), the Goto Kakizaki rats (Goto et al., 1975), the BHE rats (Ber-Danier, 1971), the Sand rat (Kalman et al., 1993; 1996), and other models whose diets are modified from normal to high energy (Shimoni et al., 1998). An alternative is chemical induction of diabetes using STZ. Administration of STZ to young adult rats produces a Type 1 like diabetic condition characterised by severe insulinopaenia, hyperglycaemia, glycosuria, polydipsia and muscle wasting. However, when STZ is administered by intraperitoneal injection (90 mg/kg) to neonatal rats, a type 2-like diabetic condition is developed in the rats (Schaffer, 1991). In both models of diabetes, STZ causes cell destruction, which is accompanied within a few days by hyperglycaemia and severe decreases in plasma insulin level (Portha *et al*., 1989). However, unlike the condition produced in young adult rats exposed to STZ, in the neonatal rats there is normalisation of plasma glucose concentration within a week, which may be due to partial regeneration of the cells in the neonatal rat (Schaffer *et al*., 1991). After a few weeks, the Type 2 diabetic rat becomes severely glucose intolerant and insulin resistant, exhibiting severe hyperglycaemia and hyperinsulinaemia following the administration of glucose (Schaffer *et al*., 1991). Similar to human beings with Type 2 diabetes, the STZ rat models develop a deficiency in the first phase of insulin release in response to a glucose challenge. The abnormal response to a glucose challenge has been attributed to specific defect in the sensitivity of the cell to glucose. By 8 – 12 months of age type 2 rats undergo a transition from hyperinsulinaemia to hypoinsulinaemia (Schaffer & Wilson, 1993). In this review, we describe advances in diabetes research which is currently going on in our laboratories.

Systemic effects of diabetes mellitus

Exocrine pancreatic secretion: Diabetic patients (both Type 1 and Type 2) suffer from a number of symptoms and long-term complications (see above). One complication is indigestion of food which is due to the inability of the pancreas to secrete an adequate amount of digestive juice which contains amylase, a major digestive enzyme. Medically, this dysfunction is described as “exocrine pancreatic insufficiency”. Generally, the pancreas consist of two portions – a small endocrine part (2 – 3% by volume) and a large exocrine part (97 – 98% by volume). The endocrine pancreas is involved in the secretion of a number of regulatory metabolic hormones such as insulin, glucagon, somatostatin and pancreatic polypeptide. In contrast, the exocrine pancreas secretes major digestive enzymes, bicarbonate and fluid. The digestive enzymes help in the breakdown of complex food such as proteins, carbohydrates and fats so that they can be absorbed readily into the blood. The islet hormones help in the storage and release of energy from the products of digestion (e.g. amino acids, lipids and glucose). In DM the pancreas is unable to produce sufficient enzymes for digestion of foods and moreover, the secretion of the hormone insulin is either markedly decreased (Type 1 diabetes) or it is insensitive in exerting its effect (Type 2 diabetes).

Several studies including those in our laboratory have investigated the effects of STZ-induced DM (Type 1) on exocrine pancreatic function. In most of the experiments investigators have unanimously agreed that diabetes is associated with decreased exocrine pancreatic function (Sofrankova & Dockray, 1983; Owyang, 1993; William & Goldfine, 1993; Otsuki *et al*., 1995; Yago et al, 1999; Juma et al, 1997; Singh et al, 1999; 2001). Exocrine pancreatic insufficiency is due to either insensitivity of the secretory acini to the gut hormone (cholecystokinin) CCK

(Otsuki *et al*., 1995), a decrease in amylase secretion (but not trypsinogen) in response to caerulein, CCK-8 and carbamylcholine (Sofrankova & Dockray, 1983; Okabayashi *et al*., 1988), an inhibition of amylase mRNA, amylase synthesis and activity of amylase (but not colipase or lipase) (Duan & Evlanson-Albertsson, 1990,1992a,b) or an inability of pancreatic acinar cells to take up glucose (Yang & Zhu,1995) Similarly, we have also shown (Singh et al., 1998; 1999; 2001; Juma et al, 1997; Yago *et al*, 1999) that 4 – 5 days after the induction of Type 1 diabetes using STZ, the CCK-8 and acetylcholine (ACh)-evoked amylase secretion and cellular calcium ion mobilisation were markedly reduced. Moreover, after 2 months of STZ-induced diabetes mellitus the amount of amylase released in pancreatic juice is virtually zero (Patel et al, 2004a,b). In addition, our studies have shown that the islet hormones failed to potentiate the secretory effects of either ACh or CCK-8 in diabetic rats compared to the response seen in healthy aged-matched control animals. The precise signal transduction mechanisms associated with reduced digestive enzyme secretion (exocrine pancreatic insufficiency) during the interaction of the endocrine and exocrine pancreas in diabetes mellitus is unknown. Current research work is focussed on receptor binding studies and the measurement of such intracellular mediators as cyclic AMP, cyclic GMP, tyrosine kinase, calcium and magnesium. In relation to reduced amylase secretion from pancreatic acinar cells, we are currently investigating the amylase mRNA and CCK receptor gene expressions (Patel et al,2004c) and tritiated glucose uptake by the acinar cells.

Salivary secretion: More recently, we have employed human subjects (Mata et al,2004) and the STZ-induced type 1 diabetic animal model to study salivary insufficiency (Mahay *et al*., 2002; Mahay, 2004). Both type 1 and type diabetic subjects secrete significantly less amylase in saliva compared to healthy controls. Light microscopic studies of STZ-induced diabetic rat parotid glands showed striking differences in morphology compared to age-matched control. Diabetic parotid glands weigh less and they contained a large number of lipid vacuoles, whereas glands from control animals displayed normal structure and with few or no lipid vacuoles. In relation to the physiological function of the salivary gland, our results have demonstrated basal amylase secretion is significantly decreased in diabetes mellitus compared to aged-matched control. Similarly, both ACh and NA evoked marked decreases in total amylase output for diabetic rat parotid segments compared to aged-matched controls (Mahay, et al 2002; 2004). Since intracellular calcium ion concentration is a major trigger and mediator for secretion, we have measured the levels of intracellular free calcium ion concentration in parotid acinar cells from diabetic and aged-matched control rats. Our results have shown that diabetes produces no significant change in basal intracellular calcium concentration. In contrast, during ACh stimulation there was a significant decrease in the plateau phase of the calcium transient but not the initial rise (Mahay, 2004). These results suggest that DM is not affecting the calcium released from intracellular stores but the calcium entering the cell "capacitative calcium influx" from extracellular medium. Like the pancreas, current studies are focussed in characterising calcium and magnesium transport and the measurements of intracellular mediators, glucose uptake by acinar cells and the gene expressions for amylase mRNA and muscarinic cholinergic and adrenergic receptors.

Cardiomyopathy: There is now clear evidence that DM is a major risk factor which worsens the prevalence, severity and prognosis of cardiovascular disease (Julien, 1997; Amos *et al*., 1997; Bracken et al, 2003; Fallow & Singh, 2004). Epidemiological studies have confirmed the relationship between DM and coronary artery disease, hypertension and congestive heart failure. Moreover, cardiovascular complications are the most common causes of morbidity and mortality in diabetic patients. Clinical studies have demonstrated impaired diastolic and systolic function in both Type 1 and Type 2 diabetic hearts (Howarth & Singh, 1999; Bracken *et al*., 2003; Fallow & Singh,2004). During the process of excitation-contraction coupling depolarisation of heart muscle

cell membrane leads to small entry of calcium ions via the L-type calcium channels and possibly the Na/Ca exchange operating in reverse mode. This small entry of calcium ions triggers a much larger release of calcium ions from the main intracellular store, the sarcoplasmic reticulum. This rise in intracellular calcium transient activates the myofilaments to produce normal cardiac contraction. In the diabetic heart, current evidence suggests an altered process that underpins the mechanism of excitation-contraction coupling. This in turn is responsible for the contractile dysfunction observed n the diabetic heart. Research work in several laboratories including ours have demonstrated that both the contraction and relaxation processes are deranged during DM and this contractile dysfunction is associated with a derangement in cellular calcium homeostasis, possibly through the mechanism of calcium entry into the cell, the uptake of calcium within the sarcoplasmic reticulum or calcium extrusion from the cell via the Na/Ca exchanger (Howarth & Singh, 1999; Bracken, 2002; Bracken *et al*., 2003; Bracken et al, 2004; Woodall et al 2004.). It has also been suggested that the cardiac muscle myofilaments may be insensitive to calcium during DM (Howarth & Singh, 1999; Bracken et al, 2004; Woodall et al, 2004). We are currently characterising the calcium and magnesium transport mechanisms in the hearts of both type 1 and type diabetic rats compared to age-matched control. Moreover, we are measuring the gene expressions for the adrenergic and ryonodine receptors and for SERCA and the Na/Ca exchanger in diabetic and control hearts.

Diabetes mellitus and pancreas transplantation

Administration of exogenous insulin ameliorated the symptoms of insulin-dependent diabetic patients, but it can neither cure nor prevent the severe degenerative complications associated with diabetes mellitus. Despite insulin therapy, the severity and frequency of the chronic degenerative late complications are nevertheless high in diabetic patients (Anderson *et al*., 1980). The recognition of the increased frequency and complications associated with diabetes has encouraged researchers to develop transplant of either the pancreas or islets which in turn can produce endogenous insulin.

Due to improvements in organ procurement, preservation and surgical techniques either whole organ (Kallen *et al*., 1990; Nozawa M & Otsu, 1990; Sanchez De Badajoz & Vara Thorbeck, 1990; Grenier *et al*., 1993; Gruessner 1990, 1993; Jannson *et al*., 1993; Nakhleh *et al*., 1993;) or segmental (Dafoe *et al*. 1992; Kaji *et al*. 1992) transplantations are extensively used to cure experimental diabetes in animals or to formulate a clinical model for transplantation.

In whole organ transplantation, pancreaticoduodenal grafts (Nakhleh *et al*. 1993) are grafted unto the splenic, renal or the iliac vessels of the host (Guessner *et al*., 1990; Sanchez De Badajoz & Vera Thorbeck, 1992), and the pancreatic ducts were either drained into the intestine or into the urinary bladder (Gruessner *et al*., 1990; Gruessner *et al*. 1993; Nozawa & Otsu, 1990; Sanchez De Badajoz & Vera Thorbeck, 1990). The urinary bladder is the most common site of drainage of pancreatic secretion. In the transplantation of pancreatic fragment, pancreatic tissue is taken and implanted into different sites including the anterior eye chamber (Adeghate & Donath, 1990; Adeghate, 1998; Adeghate *et al*. 2001), kidney subcapsular space (McEnvoy & Hegre, 1979), subcutaneous region (Adeghate *et al*. 2001; Adeghate, 1999; Kramp & Renold, 1981) and liver (Mossimann *et al*. 1976).

Human pancreatic transplantation: The increase in number of diabetic patients, the proliferation of severe complications such as macro- and micro-angiopathy, neuropathy, nephropathy and retinopathy (West, 1978) and the availability of modern technology have enabled scientists and clinicians alike to find new methods in achieving the transplantation of an insulin-secreting graft. Simultaneous kidney and pancreas transplantations are the accepted methods of treatment in a selected group of type I diabetes mellitus with end-stage renal disease

(Stratta *et al*. 1993). Human pancreatic transplantation can be divided into two groups, segmental and whole organ.

Segmental pancreatic transplantation: Segmental pancreatic transplantation is employed in certain diseases of the pancreas that involves the destruction of pancreatic parenchyma, for example, carcinoma and pancreatitis. This procedure has been used to cure a number of pancreas diseases including alcoholic and idiopathic pancreatitis associated with severe pain (Tamura *et al*. 1992; Tamura *et al*. 1993). The transplants, usually autografts are transplanted into the iliac fossa (Tamura *et al*. 1993; Dafoe *et al*. 1990) with the vessels tied either to the splenic or the iliac vessels. This is usually accompanied by pancreaticojejunostomy (Dafoe, 1990) or pancreaticocystostomy for draining pancreatic secretion. This method is not the most common procedure used in the surgical treatment of type I diabetes mellitus.

Whole organ transplantation: From December 1966 to December 1997, more than 7000 pancreas transplants were reported to the International Pancreas Registry located at the University of Minnesota, Minnesota, U.S.A. These include primary re-transplants. Approximately, half of the cases were reported from Europe and half from North America. Only a small fraction (2%) of the cases were from other continents (Sutherland *et al*. 1989).

These pancreas transplants were performed either alone or in combination with kidney transplants (Remuzzi, 1994). Significant improvement in pancreas transplantation has been achieved because of advances in procurement, preservation, transportation and transplantation techniques (Nakai, 1993). The success of pancreas transplantation cannot be separated from the success achieved in the area of immunosuppressive therapy. Transplant recipients received immunosuppressive drugs including cyclosporin, prednisolone and azathioprine.

Clinical assessment of the viability of pancreas transplants: A number of methods have been employed to test the function and survival of pancreas transplants. These methods include the followings:

Metabolic function: The most common parameters investigated include the plasma/serum and urinary insulin (Kallen, 1990) and C-peptide levels. In addition to this blood and urine glucose values› glucose tolerance test and glycosylated haemoglobin values are usually assessed to determine the functional status of pancreas transplants (Tamura *et al*. 1992).

Enzymes: Serum anodal trypsinogen correlates well with rejection occurring in pancreatic allografts (Perkal et al., 1993) Serum anodal trypsinogen is said to increase during pancreatic graft rejection. An increase in the serum levels of human pancreatic elastase I, and an increase in urine amylase level have also been shown to correlate with damage of the exocrine component of pancreatic graft (Linder *et al*. 1991).

Immunological investigation: Some of the immunological parameters investigated for the viability of pancreas transplants include phagocytosis, antiinsular antiboby, and insulin antibody. Moreover, the level of the serum marker "pancreas specific protein" may predict whether a graft will be rejected or not (Fernstad *et al*. 1989). This protein was said to be elevated in graft rejection. The monitoring of plasma soluble interleukin-2-receptor (SIL-2R) concentrations has been proposed in organ transplantation to detect early signs of rejection. Increase in SIL-2R values may predict impending graft rejection or imminent cytomegalovirus disease (Kinop, 1990; Perkins *et al*. 1990 & Touraine *et al*. 1991).

Clinical: Changes in body weight, insulin requirement, polydipsia, polyuria and bulimia are taken into account in assessing pancreatic graft function.

Complications and problems associated with pancreatic transplantation: The problems associated with pancreas transplantation are numerous and include venous thrombosis (Grewal *et al*. 1993; Gruessner *et al*. 1993), infections of the respiratory system with cytomegalovirus and pneumonia-causing microbes (Elinder *et al*. 1992; Kingsmore *et al*. 1993). Infections of the urinary bladder are more common because pancreas secretion is frequently drained into the

bladder and trypsin, a product of this secretion has been shown to promote infection (Smith, 1992). Surgical complications such as fistula (Bentley & Garrison, 1992) pancreas secretion leakage (Olausson *et al*. 1991), and pseudoaneursym of the graft were frequently observed in patients hosting pancreatic grafts. Dehydration and acidosis (Beden et al., 1993), haematuria (Reisman and Viets, 1992), post-transplantation pancreatitis (Linder et al., 1990), recurrence of autoimmune diseases (Purcell et al., 1993) and cardiovascular diseases are common after pancreatic transplantation.

The complete therapeutic cure and abolition and/or reversal of the complications of diabetes mellitus has not been completely abolished, but nevertheless, there is hope of complete recovery if transplantation is performed quickly before the onset of these severe complications.

Conclusion

Diabetes mellitus is a major global health problem affecting over 2% of the world population. Moreover, it is an increasingly common chronic disorder which is associated with substantial costs in terms of quality of life and demands on health budgets. The diabetic epidemic needs an international approach and particular attention has to be paid to the developing countries where type 2 diabetes is more rife. In the first instance, there is an urgent need to educate people about diabetes, its early signs and symptoms, early diagnosis, the long-term complications, traditional and non-traditional treatments, the care and the prevention strategies including proper diet, regular exercise and the human cost in over indulgence in modern life style. It is also vitally important to continue with the research in understanding the molecular and cell signalling mechanisms associated with the development of the long term complications of diabetes.

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