1. INTRODUCTION
Introduction

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1.1 Diabetes

Diabetes mellitus (DM) is one of the most common and complex diseases worldwide with steadily increasing prevalence over the last two decades (Zimmet et al., 2001). It has been estimated that worldwide prevalence of diabetes is 366 million people in 2011 and the number is set to increase 552 million people by 2030. The estimated prevalence of diabetes in India is 61.3 million people in 2011, which is projected to increase to 101.2 million people in 2030 (David et al., 2011).

The impact of diabetes is substantial as it is a lifelong disease, increase morbidity, mortality and healthcare expenditure (De Groot et al., 2001; Jacobson, 2004). Globally diabetes caused about 4.6 million deaths in the age group of 20-79 years in 2011 accounting for 8.2% of global all-cause mortality in this age group in 2011 (IDF, 2011). In India DM was responsible for 109 thousand deaths, 1157 thousand years of life lost due to this disease in 2004 (Venkataraman et al., 2009) and 2263 thousand disability adjusted life years (DALYs) during 2004 (ICMR, 2006). Healthcare expenditures due to diabetes account for 11% of the total healthcare expenditures in the world in 2011. Most of the countries are believed to spend between 5% and 18% of their total healthcare expenditures on diabetes (IDF, 2011). The healthcare budget of the government in India is very low when we consider global standard. (Indian Budget 2010: http://indiabudget.nic.in/).

1.1.1 Definition

DM is a common, serious, chronic and currently incurable metabolic disorder arising from a relative or absolute deficiency of insulin, characterized by abnormal glucose homeostasis. DM is characterized by interaction between environmental and genetic factors and results in defect in insulin secretion and development of insulin resistance. In a normal individual glucose metabolism is controlled by feedback mechanism that monitors blood glucose level
and allow beta cells of pancreas to produce and release the hormone insulin to metabolize excess glucose. In patients with DM either due to lack of insulin secretion or decreased sensitivity of tissue to insulin, the metabolism of carbohydrate, fat and protein gets impaired, which leads to an increase in blood glucose level and create a condition called persistent hyperglycaemia. Oxidative stress, genetic factors, disturbances of lipid metabolism and various growth factors are also among the major causes of chronic complications.

1.1.2 Diagnosis

Diabetes is characterized by recurrent or persistent hyperglycemia, and is diagnosed by demonstrating any one of the following methods; (1) The World Health Organization (WHO) and the American Diabetes Association (ADA) method that uses a fasting plasma glucose (FPG) of 7 mmol/L(126 mg/dL) or higher to define diabetes, (2) HbA1C $\geq$ 6.5% (48 mmol/mol), (3) a casual (random) plasma glucose level $\geq$ 11.1 mmol/L (200 mg/dL) in someone with typical symptoms of diabetes and (4) a plasma glucose level $\geq$ 11.1 mmol/L (200 mg/dL) 2 hours after a 75 g load of glucose given by mouth (the oral glucose tolerance test - OGTT).

1.1.3 Classification

American Diabetes Association (ADA) and the World Health Organization (WHO) have revised the criteria of diabetes classification and diagnosis in 1997. The terms type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM) were replaced by insulin-dependent and non-insulin dependent diabetes, respectively.

1.1.3.1 Type 1 diabetes mellitus (T1DM)

T1DM can be further classified into two types, namely immune mediated and idiopathic. Immune mediated T1DM develops as a consequence of autoimmune destruction of the insulin producing beta cells of the pancreas (absolute deficiency). As a result the individual with T1DM must administer daily injections of insulin to survive. T1DM is commonly
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diagnosed in children and young adults and accounts for up to 10% of all cases of diabetes mellitus and is likely initiated by the exposure of a genetically susceptible individual to an environmental agent. Candidate genes and environmental factors are reportedly prevalent in the general population, but development of $\beta$-cell autoimmunity occurs in less than 10% of the population and progresses to diabetes mellitus in less than 1% of the population.

1.1.3.2 Type 2 diabetes mellitus (T2DM)

T2DM is the most common form of diabetes. It is a result of both impaired insulin secretion and resistance to its action (relative deficiency). About 90 to 95 percent of people with diabetes have T2DM. This form of diabetes is most often associated with old age, obesity, family history of diabetes, previous history of gestational diabetes, physical inactivity and certain ethnicities. About 80 percent of people with T2DM are obese. An individual with T2DM is capable of producing insulin at least initially, but is deficient in his/her cellular response. It has been estimated 60% or more of T2DM could be prevented.

1.1.3.3 Gestational diabetes mellitus

Gestational diabetes resembles T2DM in several respects, involving a combination of relatively inadequate insulin secretion and responsiveness. It occurs in about 2–5% of all pregnancies and may improve or disappear after delivery. Gestational diabetes is fully treatable but requires careful medical supervision throughout the pregnancy. About 20–50% of affected women develop T2DM later in life.

1.1.3.4 Other specific types of diabetes

Other types of DM is a large group of conditions, which include genetic defects in insulin secretion, genetic defects in insulin action, pancreatitis and other exocrine disorders, hormone-secreting tumours such as acromegaly and Cushing’s syndrome. Some cases are caused by the administration of drugs such as glucocorticoids. Some genetic syndromes are
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sometimes associated with diabetes e.g. Down’s syndrome, Klinefelter’s syndrome and many others.

1.1.4 Pathophysiology of T2DM

The human body wants blood glucose maintained in a very narrow range. Insulin and glucagon are the hormones which make this happen. Both insulin and glucagon are secreted from the pancreas, and thus are referred to as pancreatic endocrine hormones. The pancreas serves as the central player in this scheme. It is the production of insulin and glucagon by the pancreas which ultimately determines if a patient has diabetes, hypoglycemia, or some other sugar problem.

In the fasting state 75% of total body glucose disposal takes place in the brain, liver and gastrointestinal tissues. These are non-insulin dependent tissues (DeFronzo, 1997). The remaining 25% of glucose metabolism takes place in muscle, which is dependent on insulin (Gerich et al., 2001). In the fasting state approximately 85% of glucose production is derived from the liver, and the remaining amount is produced by the kidney (DeFronzo, 1997; Gerich et al., 2001; Ekberg et al., 1999). In the fed state, carbohydrate ingestion increases the plasma glucose concentration and stimulates insulin release from the pancreatic β cells. The resultant hyperinsulinemia suppresses hepatic glucose production and stimulates glucose uptake by peripheral tissues (Mandarino et al., 2001).

The majority (about 80% to 85%) of glucose that is taken up by peripheral tissues is disposed of in muscle (Ekberg et al., 1999; Mandarino et al., 2001), with only a small amount (about 4% to 5%) is being metabolized by adipocytes. Although fat tissue is responsible for only a small amount of total body glucose disposal, it plays a very important role in the maintenance of total body glucose homeostasis.

Small increments in the plasma insulin concentration exert a potent antilipolytic effect, leading to a marked reduction in the plasma free fatty acid (FFA) level. The decline in plasma
FFA concentration results in increased glucose uptake in muscle (Santomauro et al., 1999) and reduces hepatic glucose production (Bergman, 2000). Thus a decrease in the plasma FFA concentration lowers plasma glucose by both decreasing its production and enhancing the uptake in muscle (Boden, 1997; McGarry, 2002).

T2DM is known as a heterogeneous disease and its pathophysiology is characterized by defects in insulin secretion and insulin resistance involving muscle, liver, and the adipocyte. The pathogenesis of T2 DM is shown in Figure 1. The individual with diabetes for a long time shows both impaired insulin secretion and action (Raskin et al., 1994).

**Figure 1: Pathogenesis of T2DM**

1.1.4.1 Defects in insulin secretion

Insulin response to glucose challenge is biphasic in a non diabetic person. An early phase that occurs within the first 10 minutes after glucose ingestion representing the release of insulin
stored within the beta cells and a late phase of insulin secretion representing newly synthesized insulin and exists as long as the hyperglycemia persists. The pancreas in people with a normal-functioning β cell is able to adjust its secretion of insulin to maintain normal glucose tolerance. Thus in a nondiabetic individual, insulin is increased in proportion to the severity of the insulin resistance and glucose tolerance remains normal.

In an individual with diminished glucose tolerance and fasting plasma glucose levels of less than 115 mg/dl, the plasma insulin response after oral or intravenous glucose administration can be either normal or, more often, elevated. However, if the fasting plasma glucose concentration surpasses 115 mg/dl in a person with impaired glucose tolerance, the early phase of insulin secretion is lost or become significantly impaired, and the late phase remains normal or more often is increased (Rifkin et al., 1984). Essentially, the plasma insulin response to glucose is often inversely correlated to the degree of fasting hyperglycemia, so that patients with T2DM with moderate to severe hyperglycemia (>180-200 mg/dl) tend to have all phases of insulin secretion impaired and those with intermediate fasting plasma glucose level (120-180 mg/dl) may have increased, normal, or decreased plasma insulin response (Cambell et al., 1988). Impaired insulin secretion is a uniform finding in T2DM patients and the evolution of β-cell dysfunction has been well characterized in diverse ethnic populations.

1.1.4.2 Insulin resistance

Insulin resistance is an early defect. It is common in individuals with impaired glucose tolerance, and fundamentally resides in all T2DM patients, who have fasting plasma glucose levels greater than or equal to 140 mg/gl. Insulin resistance has been found to positively correlate with elevations in fasting plasma glucose concentrations. Those with greater glucose tolerance, therefore, become more insulin resistant than those who are less glucose tolerant (Moller and Flier, 1991).
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The process of insulin resistance can be best explained by first examining the basic action of insulin in nondiabetic persons at a cellular level. This occurs in two phases. Initially, insulin binds to a specific receptor located on the surface of a cell. Then this interaction begins a series of intracellular sequences which results in enhanced glucose transport and stimulation of a number of intracellular enzymatic pathways (Moller and Flier, 1991).

Binding abnormalities (defect in first stage of insulin action) refer to a reduction in insulin binding. This is followed by a reduction in insulin action. Such an occurrence can be found in patients with mutations in the insulin gene or insulin receptors gene. However, such abnormalities account for less than 1% of the people with T2DM. This may be because most of those with T2DM are obese and hyperinsulinemics and a decrease in binding may be secondary to these conditions (DeFronzo et al., 1992).

Postbinding abnormalities which occur after the insulin has bound to the cell are fundamentally responsible for insulin resistance in those with T2DM and significant fasting hyperglycemia. These defects involve a decrease in glucose transport and other intracellular processes involved in glucose metabolism notably insulin stimulated glycogen synthesis (DeFronzo et al., 1992).

Insulin resistance, such as reduced insulin sensitivity, is present in T2DM, but also in obese subjects, smokers and patients with increased production of insulin antagonistic hormones such as cortisone and growth hormone (e.g. cortisone and thiazide diuretics). By definition, insulin resistance entails the need of higher than normal plasma insulin levels to maintain normoglycaemia. Therefore, hyperinsulinaemia may be regarded as a marker of insulin resistance (Reaven, 1995). The major sites of insulin resistance in T2DM are the liver, skeletal muscle and adipose tissue (DeFronzo et al., 1992; Reaven, 1995).

Liver In the liver, insulin resistance conveys increased glucose production after a night of fasting (post-absorptively) and reduced suppression of the production of glucose after a meal
(post-prandially). Post-absorptively, a major part of the glucose is consumed by non-insulin-dependent tissues (mainly the brain) and only about 25% of the glucose is taken up in insulin sensitive tissues. Hence, under these conditions, insulin resistance in the liver plays a more important role for glucose metabolism than insulin resistance in extrahepatic tissues. Post-prandially, on the other hand, glucose is taken up in similar amounts by the liver and the skeletal muscles. Thus, the insulin sensitivity in liver and muscle play an equally important role after a meal.

The increased hepatic glucose production can be related to enhanced gluconeogenesis (Shulman 1999), and it is likely that increased plasma levels of free fatty acids (FFAs) account for this by activation of some key gluconeogenetic enzymes. T2DM patients also exhibit increased activity of hepatic glucose-6-phosphatase, resulting in increased glucose production through the glucose cycle pathway (Efendic et al. 1988).

Peripheral (Muscle) Muscle is the major site of glucose disposal and approximately 80% of total body glucose uptake occurs in skeletal muscle (DeFronzo, 1997). In response to a physiologic increase in plasma insulin concentration, muscle glucose uptake increases linearly, reaching a plateau value of 10 mg/kg per minute. In contrast, in lean T2DM subjects, the onset of insulin action is delayed for 40 minutes and the ability of insulin to stimulate leg glucose uptake is reduced by 50%. Therefore, the primary site of insulin resistance in T2DM subjects resides in muscle tissue.

Peripheral (Adipocyte) In obese non-diabetic and diabetic humans, basal plasma free fatty acid (FFAs) levels are increased and fails to suppress normally after glucose ingestion. FFAs are stored as triglycerides in adipocytes and serve as an important energy source during conditions of fasting. Insulin is a potent inhibitor of lipolysis, and restrains the release of FFAs from the adipocyte by inhibiting the hormone-sensitive lipase enzyme. It is now recognized that chronically elevated plasma FFA concentrations can lead to insulin resistance
in muscle and liver (DeFronzo, 1997; Santomauro et al., 1999; McGarry, 2002; Kelly et al., 2000) and impair insulin secretion (Boden, 1997; Kashyap et al., 2002; Carpentier, et al., 2000). In addition to FFAs that circulate in plasmain, type 2 diabetic and obese nondiabetic individuals have increased stores of triglycerides in muscle (Goodpaster et al., 2000; Greco et al., 2002) and liver (Ryysy et al., 2000; Miyazaki et al., 2002) and the increased fat content correlates closely with the presence of insulin resistance in these tissues.

1.1.5 Signs and symptoms of diabetes

Hyperglycaemia occurs as a result of the underlying metabolic abnormalities of glucose homeostasis and is responsible for the symptoms. The symptoms usually pronounced in T1DM (but may be absent in T2DM) are frequent urination-polyuria and/or nocturia and usually glycosuria, excessive often unquenchable thirst-polydipsia, lethargy, weight loss, frequent and poorly resolving infections and visual changes.

1.1.6 Management of T2DM

There are two principle goals in the management of T2DM, namely, to avoid hyperglycemia by maintaining blood glucose levels as close to the normal range as possible (80-120 mg/dl) and to prevent microvascular and macrovascular complications (Polonsky, 1994; Raskin et al., 1994). Treatment plan should be geared towards reversing the pathogenic metabolic mechanism of diabetes that result in hyperglycemia, namely insulin resistance and impaired beta cell functions (Raskin et al., 1994). Plans should include diabetes education, dietary modification, exercise regimen, self monitoring of blood glucose or urine and medications. It is important to remember that all treatment plans should be present in an idiographic manner and it is most likely to differ from patient to patient. In addition, it may be useful to note that the management of diabetes is typically the patients’ responsibility because they must follow this complex set of self care behaviors on a daily basis for the rest of their lives (Cox et al., 1992).
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*Education* It is critical that a patient become knowledgeable of the self care skills needed to manage his/her diabetes (Raskin et al., 1994). Often, patients are asked to learn a large amount of information and acquire a number of skills. From the time of diagnosis, patients should grasp such critical skills as self testing one’s blood glucose, administering ones insulin (if necessary) and techniques to treat possible bouts of hypoglycaemia. Furthermore, continuing education and skill building is often necessary as a patient’s disease progresses and as new methods of treatment are introduced (Cox et al., 1992).

*Diet* Medical nutrition therapy is believed to be the most important element in the treatment of T2DM. There are four fundamental goals of the nutrition therapy, namely managing near-normal blood glucose levels, normalizing serum lipid levels, attaining and maintaining a reasonable body weight, and promoting overall health (Raskin et al., 1994; Franz et al., 1994).

As it has been mentioned, approximately 80-90% of T2DM patients are obese (>25 body mass index) and thus caloric restriction is usually the primary component utilized to improve glucose tolerance (Raskin et al., 1994). The loss of as little as 5-10% of one’s body weight can improve glucose uptake, reduce insulin secretion, and decrease hepatic glucose production. Furthermore, there are some indications that weight loss may be most effective in the early stage of T2DM when insulin secretion is greatest (Henry, Wallace and Olefsky, 1986).

A specific dietary plan for T2DM patients typically includes the moderation of protein consumption, limiting the intake of simple carbohydrate, increasing the intake of complex carbohydrate, and reducing fat intake. Furthermore, for individuals using insulin therapy as well, the timing of food consumption is as important as the amount and types of food consumed (Cox et al., 1992).
The recommendation of protein intake for T2DM patients is the United States recommended dietary allowance of 0.8 g/kg daily. If possible, it is important to keep protein intake within the bounds of 0.8-1.0 g/kg daily because excessive protein consumption may aggravate renal insufficiency, a common problem among individuals with diabetes. On such a diet, protein will account for about 10-20% of the total calories a patient consumes. Also, Common sources of protein such as meat, fish and poultry are limited to approximately 3-5 oz/day (Franz et al., 1994). The remaining 80-90% of a T2DM patient’s diet should be divided between carbohydrate and fat. Specially saturated fats should make up <10% of one’s total caloric intake because these products contain more than twice as many calories as either carbohydrate or protein, and help develop and maintain obesity. Coconut and palm oil both highly saturated should be completely avoided. In addition, limiting the intake of meat 3-4 oz/day, drink skim milk and substituting margarine or butter, may further aid a patient in limiting their saturated fat intake below the 10% guideline. The form of unsaturated fat should not exceed 20% of one’s total caloric intake (Franz et al., 1994).

The Food Gudie Pyramid provide a good recommended model for carbohydrate intake among T2DM patients. The emphasis in the pyramid is on whole grains, starches, fruit, vegetable, vitamin and minerals. Although a small portion of the carbohydrate make up of a T2DM patients’s diet may allow for simple carbohydrate such as sucrose, the majority of the dietary carbohydrate make up should be dedicated to complex carbohydrate such as starch. Carbohydrate, therefore, makes up approximately 50-60% of the total caloric intake in patients with T2DM (Raskin et al., 1994; Franz et al., 1994).

**Exercise** Exercise can enhance insulin sensitivity and increase skeletal muscle glucose uptake both during and after significant physical activity. Thus exercising in regular interval can help aid in the reduction in glucose intolerance. In addition, exercise has been purported as significant aid in reducing one’s weight, a problem with which most T2DM patients must
contend. This is important because a reduction in weight can increase insulin action and decrease insulin resistance. Furthermore, a consistent regimen of exercise has also been shown to reduce risk factors associated with cardiovascular disease. This may be a function of exercise being correlated with an increase in HDL cholesterol, a decrease in LDL cholesterol and a decrease in triglyceride and insulin; all of which have been shown to provide protection against cardiovascular diseases. Other benefits of exercise include decrease in blood pressure and heart rate, increase in maximum oxygen uptake, and numerous psychological benefits such as decreased anxiety, improved mood and self-esteem (ADA, 1993).

In suggesting an exercise regimen for a patient, a physician must take a number of precautions into consideration. T2DM patients may have insensitive feet or peripheral vascular insufficiency, untreated or newly treated retinopathy and hypertension. Therefore, plan for activity that include the intensity, duration and frequency of the exercise, should be carefully monitored by both the physician and the patient (Raskin et al., 1994).

While walking is generally a safe form of exercise for most patients, some patients with diabetes may be able to undertake more rigorous form of exercise such as biking, swimming, or running. Lifting weight may also be beneficial for some patients. Generally, a session of aerobic exercise should last anywhere from 20-40 minutes and should sustain a patient’s heart rate at approximately 60-80% of his/her maximal heart rate. Furthermore, maximal benefits of exercise are seen when a patient participates in sessions that are less than or equal to 48 hours apart from each other (Raskin et al., 1994).

Self monitoring of blood glucose (BG) levels Another important aspect of T2DM patients’ treatment may include self monitoring one’s blood. The purpose of this is three fold; it prevents unacceptable BG levels, it monitors overall diabetes control and it evaluates the effectiveness of self treatment (Schiffrin and Belmonte 1982; Skyler, 1981). Daily urine tests are often used to detect the presence of ketone, which is a marker for hyperglycemia. In
addition blood tests are performed, via finger prick, and blood drops are placed on a reagent strip to be examined either visually or by meters. The frequency of blood tests vary depending on the number of injections of insulin a patient is administering. They can range anywhere from three or four tests daily to only a few test per week (ADA, 1987).

The basis of managing T2DM is by improving metabolic control through physical activity and dietary changes. However, glucose-lowering medicines and insulin, if necessary, are also prescribed as the disease advances. Commonly used glucose-lowering medicines include, sulphonylureas that enhance insulin secretion from beta cells; meglitinides that augment insulin secretion; biguanides that inhibit hepatic glucose production and increase glucose uptake in muscles and reduce triglycerides and LDL cholesterol levels; thiazolidinediones (TZDs) that improve insulin sensitivity in muscle and to some extent in the liver and reduce triglyceride levels, alpha-glucosidase inhibitors that inhibit the action of glucose oxidase in the gut, thereby delaying glucose absorption into the blood stream. Insulin, which comes in several forms are rapid acting, short acting, intermediate acting, and long acting. Sometimes combinations of glucose-lowering medicines are required. In addition, a range of other medicines are needed to manage diabetes complications like antihypertensive and lipid-lowering agents and medicine to manage co-morbidities such as arthritis, and inter-current illnesses. Thus, the medicine regimen can be complicated and should be reviewed on a regular basis. Glucose-lowering medicines can have significant side effects, for example hypoglycaemia, weight gain, gastrointestinal symptoms and lactic acidosis, myocardial infarction, intercurrent illnesses and leading to poor quality of life.

1.1.7 Complications

1.1.7.1 Major acute complications

There are two major acute diabetic specific complications, namely metabolic problems and infections. Furthermore, within the category of metabolic problems there are two syndromes,
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Hyperosmolar hyperglycaemic nonketotic syndrome and hypoglycaemia (Raskin et al., 1994). Common infections include cold and flu, cutaneous infection, urinary tract infection, pulmonary infection, ear infections, etc.

Hyperosmolar hyperglycemia nonketotic syndrome, a hyperglycaemic condition that typically occurs in older T2DM patients, encompasses four major clinical features. They include severe hyperglycemia (BG > 600 mg/dl), absence of or slight ketosis, plasma or serum hyperosmolality, and profound dehydration. This life threatening condition often exposes itself by way of excessive thirst, altered sensorium (coma or confusion) and physical sign of severe dehydration. In addition there are almost always precipitating factors that precipitate this condition. They may include the use of drugs or may involve acute or chronic diseases that increase glucose levels. Limited access of water may also initiate this syndrome (Raskin et al., 1994).

Hypoglycemia, another metabolic problem that may occur in diabetes populations, involves an imbalance between the amount of food one digests and the dosage of drug therapy one administer (Raskin et al., 1994). Essentially, it occurs when BG levels become too low to provide the body with the necessary metabolic fuel to maintain normal body functions. Symptoms are usually presented when BG levels are approximately 50-70 mg/dl or lower (Cox et al., 1992). Patients who are hypoglycaemic may have altered mental and neurologic functions, such as change in sensorium and behaviour, coma or seizures, as well as adrenergic response. These include tachycardia, palpitation, increased sweating and hunger. Exercise, intake of alcohol, or other drugs and decreased liver or kidney function can initiate or worsen this condition (Raskin et al., 1994).

Infections and inter-current illnesses, other acute complications are leading causes of metabolic abnormalities that can lead to a diabetic coma. Common infections include colds and flu, cutaneous infection, urinary tract infection, pulmonary infection and ear infection. It
is imperative that these problems are diagnosed and treated as quickly as possible to avoid severe hyperglycemia and its complications.

1.1.7.2 Chronic complications

Two landmark studies, the Diabetes Control and Complications Trial (DCCT, 1993) in T1DM and the United Kingdom Prospective Diabetes Study (UKPDS, 1998) in T2DM, demonstrated the importance of controlling blood glucose and blood pressure, respectively, to reduce the complications of diabetes.

The complications of all forms of diabetes include small blood vessel damage (microvascular), which particularly affects the eyes, kidneys and neural tissue. Common manifestations include retinopathy and nephropathy. Large blood vessel damage (macrovascular) and dyslipidaemia are significantly associated with increased mortality from coronary diseases, peripheral vascular disease, and stroke.

Myocardial infarction is often ‘silent’ (asymptomatic) in T2DM, which delays treatment and increases the associated morbidity and mortality. All people with diabetes should be treated as if they have known cardiovascular disease (Tabibiazar and Edelman, 2003). The American Diabetes Association (ADA) recommendations include cardiac stress tests when an individual has two or more cardiovascular risk factors as well as for those with microalbuminuria, and sedentary people about to commence an exercise programme (ADA, 2006).

Table 1 shows the common long-term complications associated with diabetes. The long-term complications are associated with prolonged hyperglycaemia measured by HbA1C (glycosylated haemoglobin) and hypertension and the associated abnormalities (Diabetes Control and Complications Trial, 1993; United Kingdom Prospective Study, 1998). The current HbA1c target is <7.0 per cent. The causes of long-standing hyperglycaemia are multifactorial and include genetic predisposition, free radical oxidative damage, protein
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glycosylation, and endothelial changes. In addition, lower socio-economic status, psychological issues that lead to lack of motivation, emotional distress, poor eating habits and depression, inadequate knowledge on the part of the person with diabetes and the health professionals caring for them, have also been implicated (DeVries et al., 2004).

Table 1: Chronic Complications

<table>
<thead>
<tr>
<th>Condition</th>
<th>Complication</th>
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<td>Nephropathy</td>
<td>Diabetic nephropathy</td>
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<td></td>
<td>Chronic renal failure</td>
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<td>End stage renal disease requiring dialysis</td>
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<td></td>
<td>Affects medicine excretion</td>
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<td>Retinopathy</td>
<td>Diabetic retinopathy</td>
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<td>Glaucoma</td>
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<td></td>
<td>Cataract</td>
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<tr>
<td>Neuropathy (peripheral and autonomic)</td>
<td>Peripheral:</td>
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<td></td>
<td>Neuropathic pain, which affects quality of life</td>
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<td></td>
<td>Foot pathology such as changed gait that predisposes the individual to trauma, ulcers, amputation and fall</td>
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<td></td>
<td>Autonomic:</td>
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<td></td>
<td>Gastroparesis</td>
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<td></td>
<td>Atonic bladder</td>
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<td></td>
<td>Silent myocardial infarction</td>
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<td></td>
<td>Postural hypotension, which increases falls risk</td>
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<td></td>
<td>Erectile dysfunction</td>
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<td>Cardiovascular diseases</td>
<td>Coronary artery disease</td>
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<td>Cerebrovascular disease</td>
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<td>Peripheral vascular disease</td>
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<td>Pregnancy-related complications</td>
<td>Birth defects</td>
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<td>Macrosomia</td>
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<td></td>
<td>Neonatal hypoglycaemia</td>
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<td></td>
<td>Interventions such as forceps and caesarian deliveries.</td>
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</tbody>
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1.2 Risk factors

The known risk factors of T2DM are embedded in genetic as well as environmental factors. These are mainly categorized into two groups, namely modifiable risk factors and non-modifiable risk factors. Modifiable risk factors include hyperglycemia, hypertension, plasma
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lipid and lipoprotein levels, obesity, dietary habits, oxidative stress and physical inactivity. Non-modifiable risk factors include family history, genetic factors and low/high birth weight. T2DM is an independent risk factor for macrovascular disease and is often accompanied by other cardiovascular disease risk factors. These modifiable risk factors play an important role in the initiation and progression of diabetic complications.

1.2.1 Hyperglycemia

Lowering blood glucose concentration is known to reduce risk of diabetes complications. With the discovery of glycosylated haemoglobin the association between long-term hyperglycaemia and complications was confirmed. Retinopathy and microalbuminuria are good markers of microvascular disease and indicative of a generalized vasculopathy. The numerous studies, that have looked at the relationship between glycaemic control and both the onset and progression of microvascular complications, have produced remarkably consistent results.

1.2.2 Hypertension and lipid abnormalities

Hypertension exacerbates the micro and macrovascular complications of diabetes. Hypertension in people with T2DM, however, is much more common, may precede the diagnosis, and is present in between 30 and 50% of patients at diagnosis. It is a major component of the metabolic syndrome that constitutes T2DM and appears to reflect insulin resistance. The effects on the cardiovascular system are more profound than similar blood pressure levels in a person without diabetes. For example, in the multiple risk factor intervention trial (MRFIT), rising systolic blood pressure was associated with increasing 10 year CHD mortality which was 3-5 times greater in those with diabetes.

Patients with T2DM have an increased prevalence of lipid abnormalities that contribute to higher rates of cardiovascular disorders (ADA standard, 2007). Cardiovascular disease is the major cause of mortality for individuals with T2DM (Haffner et al., 1998; Fox et al., 2007).
Adults with T2DM have cardiovascular death rates 2 to 4 times higher than those of adults without it (Buse et al., 2007). Cardiovascular diseases continue to be the principal cause of morbidity and mortality in developed countries and accounts for up to 80% of all deaths among patients with diabetes (Kannel et al., 1976; Fuller et al., 1983). Epidemiological studies have demonstrated the continuous relationship between serum cholesterol and the risk of atherosclerotic vascular disease, particularly CHD. This was confirmed by the Framingham study and, in the MRFIT study, where in over 300,000 men aged 35–57 years were screened, the relationship between cholesterol and death from CHD was found to be independent of smoking and hypertension and continuous across the age range. A strong relationship between CHD and cholesterol level exists in people with diabetes. The lipid abnormalities associated with diabetes are both qualitative and quantitative.

1.2.3 Oxidative stress

Oxidative stress has been defined as a disturbance in the balance between antioxidants and pro-oxidants (free radicals and other reactive species), with increased levels of pro-oxidants leading to potential damage (Sies 1991; Marzella and Trump, 1987). This imbalance can be an effect of depletion of endogenous antioxidants, low dietary intake of antioxidants and /or increased formation of free radicals and other reactive species.

A free radical is an atom or group of atoms possessing one or more unpaired electron. Radicals may have positive, negative or neutral charges. They are formed as necessary intermediates in a variety of normal biochemical reactions, but when generated in excess or not appropriately controlled, radicals can wreak havoc on a broad range of macromolecules. A prominent feature of radicals is that they have extremely high chemical reactivity, which explains not only their normal biological activities, but how they inflict damage to cells (Staler, 1984).
There are various sources of free radicals, namely environment, internal production, stress factor and chain reaction. Environment sources free radicals include, air pollution, cigarette smoking, smog, soot, automobile exhaust, toxic waste, pesticides, ultraviolet light, background radiation, drugs, and even certain foods that can all generate free radicals in the body. Our body also constantly produces free radicals as a by-product of normal metabolism. Within cells these are generated by the absorption of radiant energy (e.g., ultraviolet light, X-rays), endogenous, usually oxidative reactions that occurs during normal metabolic processes, enzymatic metabolism of exogenous chemicals or drugs. Free radical production may also be increased during various disease states, like inflammation and during unfavorable metabolic conditions such as hypoxia and tissue ischemia. Stress factors such as aging, trauma, medications, disease, infection, and “stress” itself accelerate the body’s production of free radicals and through chain reaction when free radicals steal an electron to balance it, it creates a new free radical in the molecule from which it stole the electron. In many cases the new free radical will seek to balance itself by stealing an electron and so on. Even one free radical is capable of destroying an entire cell or a strand of DNA.

1.2.3.1 Free radical

The major types of free radicals found in the biological system are Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNO) (Wulf, 2002).

*Reactive Oxygen Species* The most important free radicals in biological systems are ROS. Sequential reduction of molecular oxygen (equivalent to sequential addition of electrons) leads to formation of a group of ROS, namely superoxide anion, peroxide (hydrogen peroxide) and hydroxyl radical. These can be produced by the activity of a variety of oxidative enzymes, in different sites like the cell cytosol, mitochondria, lysosomes, peroxisomes and plasma membrane. The superoxide anion is formed by the univalent reduction of triplet-state molecular oxygen ($^3\text{O}_2$). Enzymes such as NADPH oxidases and
xanthine oxidase / nonenzymatically mediate this process by redox-reactive compounds such as the semi-ubiquinone compound of the mitochondrial electron transport chain.

\[
O_2 \rightarrow O_2^-
\]

SODs convert superoxide enzymatically into hydrogen peroxide (Deby and Goutier, 1990; Fridovich, 1978). In biological tissues superoxide can also be converted non enzymatically into the nonradical species, namely hydrogen peroxide and singlet oxygen (\( ^1O_2 \)) (Steinbeck et al., 1993).

\[
O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2
\]

In the presence of reduced transition metals (e.g., ferrous or cuprous ions), hydrogen peroxide can be converted into the highly reactive hydroxyl radical (\( \cdot OH \)) (Chance et al., 1979). Alternatively, hydrogen peroxide may be converted into water by the enzymes catalase or glutathione peroxidase. In the glutathione peroxidase reaction glutathione is oxidized to glutathione disulfide, which can be converted back to glutathione by glutathione reductase in an NADPH-consuming process.

**Fenton reaction** (Halliwell and Gutteridge, 1985),

\[
Fe^{++} + H_2O_2 \rightarrow Fe^{+++} + OH^- + OH^-
\]

**Hydrolysis**

\[
H_2O \rightarrow H. + OH.
\]

**Haber-Weiss reaction**

\[
H_2O_2 + O_2^- \rightarrow OH^- + OH^- + O_2
\]

**Reactive Nitrogen Species** The NO radical (NO\(_r\)) is produced in higher organisms by the oxidation of one of the terminal guanido-nitrogen atoms of L-arginine. This process is catalyzed by the enzyme NOS depending on the micro-environment NO can be converted to various other reactive nitrogen species (RNS) such as nitrosonium cation (NO\(^+\)), nitroxyl anion (NO\(^-\)) or peroxynitrite (ONOO\(^-\)) (Stamler et al., 1992). Some of the physiological
effects may be mediated through the intermediate formation of S-nitroso-cysteine or S-nitroso-glutathione (Gow et al., 1998).

Apart from these, nitric oxide (NO), an important chemical mediator, can act as a free radical and can also be converted to highly reactive peroxynitrite anion (ONOO-) as well as NO₂ and NO₃.

\[
\text{NO.} + \text{O₂.} \rightarrow \text{ONOO}^- + \text{H}^+ \\
\downarrow \\
\text{OH.} + \text{NO}_2 \rightarrow \text{ONOOH} \rightarrow \text{NO}_3^- 
\]

1.2.3.2 Oxidative stress induced cell injury

The human antioxidant defense mechanism may not always be adequate in preventing oxidative damage. When the oxidative stress exceeds the antioxidant capacity of our system, interaction with biological macromolecules may result in the oxidative damage of proteins, DNA and lipids. This may lead to the chemical and functional modification of molecules, cell and tissue injury and malfunction or failure of organs.

_Lipid peroxidation_ Oxidative damage to lipids is known as lipid peroxidation, an autocatalytic process by which polyunsaturated fatty acids and phospholipids undergo degradation by a chain reaction forming lipid hydroperoxide in cell membrane, body fluids, etc. (Farber et al., 1990). Initiation of the reaction occurs when a radical species with significant oxidizing character (OH) abstracts a hydrogen atom from a fatty acid (LH). The resulting alkyl radical (L·) is stabilized by rearrangement to a conjugated diene that combines with molecular oxygen to form a lipid peroxyl radical (LOO·). Peroxyl radicals are able to abstract a hydrogen atom from an adjacent fatty acid, resulting in a lipid hydroperoxide (LOOH) and a second lipid radical (L·). By this propagation reaction, a single free radical may result in the formation of hundreds of hydroperoxides. This chain reaction can be terminated by any reaction with another radical or compound that acts as a free radical trap forming a non-radical product. In this way, decomposition of hydroperoxides leads to the
formaton of stale end products such as endoperoxides, ketones, isoprostanes and aldehydes i.e. malondialdehyde (MDA).

**Oxidative damage to proteins** Oxidative damage to proteins results in site-specific amino acid modification, fragmentation, aggregation, alteration in the electrical charge and protein conformation. After these oxidative changes, for which especially enzymes appear to be sensitive, the proteins become highly susceptible to proteolytic degradation. This is enhanced in the presence of Fe$^{2+}$- ions that react with hydrogen peroxide in the Fenton reaction to form hydroxyl radicals that rapidly oxidize amino acid residues at or near the cation-binding site of the protein (Stadman, 1993). This site specific modification of an amino acid can alter or inactivate the (enzymatic) function of the proteins. However, protein and DNA seem to be less susceptible to free radicals than lipid.

**Lesions in deoxyribonucleic acid (DNA)** Oxidative damage to DNA occurs in the nucleus or in the mitochondria. Agents that generate oxygen free radicals, such as ionizing radiation, induce numerous lesions, mutations and deletion in DNA. Characterization of this damage has indicated that both the sugar and the base moieties are susceptible to oxidation, causing base degradation and cross-linking to proteins (Imlay and Linn, 1988). In this regard, it has been repeatedly shown that 8-hydroxy-2’-deoxyguanosine is an index of oxidative damage to DNA (Dandona et al., 1996). Since DNA is a central to the transfer of information between somatic cell generations, considerable attention is currently being directed to oxidative damage to DNA and its possible relation to aging and cancer (Toyokuni, 1996).

**1.2.3.3 Antioxidant defense system**

Although ROS are produced in abundance in the body, humans are armed with a highly sophisticated and complex antioxidant protection network to either prevent their formation or to neutralize them after they are formed. An antioxidant can be defined as any substance that
when present in low concentrations relative to the oxidisable substrate, significantly delays or reduces oxidation of substrates (Halliwell and Gutteridge, 1995).

The body has developed several endogenous antioxidant systems to deal with the production of reactive oxygen species (ROS). These systems can be broadly divided into enzymatic and non-enzymatic groups. The enzymatic antioxidants include superoxide dismutase (SOD), which catalyses the conversion of O$_2^-$ to H$_2$O$_2$ and H$_2$O; catalase, which converts H$_2$O$_2$ to H$_2$O and O$_2$; and glutathione peroxidase, which reduces H$_2$O$_2$ to H$_2$O. The non-enzymatic antioxidant includes vitamin C, vitamin E, β-carotene, reduced glutathione and numerous phytochemicals. Cells must maintain their levels of antioxidants, often defined as their antioxidant potential, through dietary intake and/or de novo synthesis.

The enzymatic and non-enzymatic antioxidant systems are intimately linked to one another and appear to interact with one another. Both vitamin C and GSH have been implicated in the recycling of alpha-tocopherol radicals. In addition, trace elements like selenium, manganese, copper, and zinc also play important roles as nutritional antioxidant cofactors. Selenium is a cofactor for the enzyme glutathione peroxidase. Manganese, copper and zinc are cofactors for SOD. Zinc also acts to stabilize the cellular metallothionein pool, which has direct free radical quenching ability. The complex interactions of these different antioxidant systems may imply that therapeutic strategies will depend on combination therapy of various antioxidants rather than a single agent. Reactive oxygen species induce membrane lipid peroxidation resulting in a chain reaction that can be interrupted by the direct scavenging of lipid peroxyl radicals by vitamin E and beta-carotene. Vitamin E can then be recycled by both vitamin C and glutathione (GSH). The reducing ability of GSH is catalyzed by the enzyme glutathione peroxidase (GSSG). Glutathione is then recycled by NADPH, which is facilitated by glutathione reductase. The major ROS pathways and antioxidant defenses are summarized in Figure 2.
1.2.3.4 Oxidative stress in diabetes mellitus

Many aspects of the relationship between oxidative stress and chronic diseases have been described. The implication of oxidative stress in the initiation and progression of complications in diabetes has also been described (Baynes, 1991). Much of the evidence concerning the role of oxidative stress in diabetes mellitus comes from the experimental animal models in which diabetes is induced by alloxan and streptozotocin (STZ). Both these chemicals appear to selectively destroy the islets of Langerhans by oxidant production (Wolff, 1993). It has been observed that many biochemical pathways associated with hyperglycemia can increase the production of free radicals and oxidative stress. Increased glucose concentration in a cell-free system (Wolf and Dean, 1987) and in red blood cells
suspension (Jain et al., 1989) generate reactive oxygen radicals and cause the peroxidation of lipoproteins and membrane lipids (Hunt et al., 1990) as well as the depletion of water and fat soluble antioxidants (Jain et al., 1991).

Increased levels of lipid peroxidation products, measures such as lipid hydroperoxides, TBAR, conjugated diene, isoprostanes and oxidized lipoproteins are found in the plasma of diabetic patients (Jain et al., 1989). They are more prominent in those individuals with diabetic complications (Collier et al., 1992; Jennings et al., 1987; Griesmacher et al., 1995).

The effect of improved glycaemic control on plasma TBAR and hydroperoxide concentrations in diabetic ketotic patients has been reported (Faure et al., 1993). Also superoxide anion generation in serum of insulin-dependent diabetes has been described (Ceriello et al., 1991). Markers of oxidative damage to DNA like 8-hydroxy-2’-deoxyguanosine are increased in diabetic patients (Dandona et al., 1996). Moreover, experimental findings suggest that overproduction of ROS and RNS lower antioxidants defense and alter enzymatic pathways in humans with poorly controlled diabetes mellitus which can contribute to endothelial, vascular and neurovascular dysfunction. It has also been reported that patients with diabetes have decreased plasma concentration of ascorbate and increased levels of its primary oxidation product, dehydroascorbate (Egan et al., 1976; Ogino et al., 1978).

Many pathogenic mechanisms are described to explain the adverse effects of hyperglycaemia on the generation of free radicals and reactive oxygen species which include auto-oxidation of glucose, non-enzymatic glycation, mitochondrial overproduction and monocyte alteration, etc.

A number of reasons are possible for the diminished antioxidant defence in diabetic patients such as excessive consumption of antioxidants, inactivation of antioxidant enzymes, decrease in reducing equivalent and disturbance in metabolism. The results of experimental
observation with antioxidant system and antioxidant pharmacotherapy suggest that targeting therapy of a specific antioxidant could become a relevant adjuvant therapy which can improve hyperglycemia, blood pressure, and manage dyslipidemia and can be used for the treatment or prevention of progression of micro or macro vascular complications in diabetes.

1.3 Health outcome measures

It is increasingly recognized that traditional biomedical based outcomes such as clinical and laboratory measures need to be complemented by measures that focus on the patient’s concern in order to evaluate interventions and identify more appropriate forms of healthcare (Slevin et al., 1988). Interest in patient-based measures has been fuelled by the increased occurrence of chronic conditions, where the objectives of interventions are to arrest or reverse decline in function (Byrne, 1992), with major implications for quality of life (QoL) (de Haes and van Knippenberg, 1985; Fowlie and Berkeley, 1987; Devinsky, 1995). Patient-based outcome measures provide a feasible and appropriate method for addressing the concerns of patients in the context of controlled clinical trials. At the same time, increased attention is given to patients’ preferences and wishes in relation to their healthcare (Till et al., 1992). Patients increasingly expect with good reason to be involved in decisions about their care and to be given accurate information to facilitate their involvement (Siegrist and Junge, 1989).

Patient based outcome assessment, very common in clinical research in western countries, has received a great deal of attention during the last two decades and mainly includes functional status and health related quality of life (HRQoL). However, this is not very common in Indian scenario.

In 1988 Ellwood discussed outcome analysis as a “technology of patient experience”. A broader definition of outcome analysis state that it is a comprehensive approach to determine the effect of medical care using a variety of data sources and measurement methods. It includes the rigorous determination of what works in medical care and what does not, and
how different providers compare with regard to their results on patient outcome. Outcomes research examines changes in the health status of patients as a result of services and interventions provided in healthcare settings. It has also been described as a measurable product of structure (the physical and organizational properties of settings in which care is provided) and process (the activities related to patient care) (Merkin, 1994). Outcome research describes, interprets and predicts the impact of various influences, especially interventions on ‘‘final’’ end points that matter to decision makers: patients, providers, private payers, government agencies, accrediting organizations, and the society at large. It measures the quantifiable changes in patients’ health status between two or more time points. Traditionally, outcome was defined in terms of such criteria as mortality and morbidity. Now it is defined as a multidimensional concept that includes not only clinical outcomes but also broad outcome measures such as functionality, quality of life, patient satisfaction, and economic outcomes (Voss and Gallagher-Allred, 1996). The various types of outcome measures are given in Figure 2.

Clinical outcome Clinical outcome include primary and secondary measures. Primary clinical outcome include anatomic measures (height, weight, and anthropometrics), biochemical data (albumin, cholesterol, hematocrit, and total white blood cell count) and study or diagnosis specific outcomes. Secondary clinical outcome include length of stay, rates of infection, readmissions, drug use, and number of physician visits or visits by a home healthcare nurse.

Functional outcome Another type of outcome measure is functional status. Functional outcome are measures of physical capability and may include activities of daily living, such as bathing and dressing, and instrumental activities of daily living, such as using the telephone, grocery shopping or driving. Mental or emotional health status is an important outcome and can be affected by nutrition and hydration. An intervention may improve the patient’s ability to get out of bed, have fewer falls or to be more alert. For a managed care
organization (MCO), this outcome alone may be enough to include nutrition therapy as part of routine treatment.

**Figure 3: Types of outcome**

*Patient satisfaction* The third type of outcome is related to patient satisfaction and includes measures of how well the healthcare episode meets expectations of patients and care givers and relates to how the general quality of life is affected. Patient satisfaction is particularly important to managed care organisation (MCOs). The National Committee on Quality Assurance (NCQA) is the agency that accredits health maintenance organisations (HMOs). NCQA has developed a set of criteria for accreditation called the Health Plan Employer Data Information Set (HEDIS). Within HEDIS, there are five categories that include patient satisfaction.
satisfaction, so MCOs are keenly aware of how satisfied their patient population is. Patient satisfaction data can be used by health maintenance organizations (HMOs) as a marketing tool to potential enrollees. NCQA suggests that benefit managers, employers, and enrollees use the experience of previous users to make their decisions. Although patient satisfaction data are soft, studies demonstrate it is associated with a positive clinical outcome.

Quality of life (QoL) The QoL outcome includes a variety of quality of life measures. There are a number of validated tools for the collection of quality of life data. Self-assessed health status, including levels of well-being, pain, and energy, and even measures of a patient’s role as parent, spouse, or employee can be measured.

Quality of life broadly encompasses how an individual measures the ‘goodness’ of the multiple aspects of his/her life (Diener et al., 1999). It is an important measurement tool that represents the effect of disease on a patient’s life as perceived by the patient and provides medical information to the healthcare professional to achieve their goal of treatment (Davies et al., 2008). Quality of life has, however, been defined in several different ways, with the belief that it is multidimensional and has several contributing factors that include healthcare professional related and patient related factors (Rose et al., 2002).

QoL include all aspects of the human experience like life satisfaction, social and role functioning, sense of community, spiritual fulfillment, economic status, self-esteem, enjoyment, pleasure and appreciation. While assessing the individual QOL, there are certain important concepts in the behavior or understanding of patients that need to be established. These concepts are also known as domains or dimensions, like physical function, emotional function, social function, role performance, pain and other symptoms. These domains can be assessed by two different types of instruments, namely generic and health related or disease-specific quality of life instruments. A specific instrument is used in research depending upon the expected research outcome. Generic instruments are used for the general population to
assess a wide range of domains applicable to a variety of health status, conditions and
diseases (Guyatt et al., 1993). Health Related Quality of Life (HRQoL) usually includes
physical, psychological and social components and is influenced by aspects of the primary
care setting such as the relationship with health professionals (Rose et al., 2002). Disease-
specific QoL instruments include aspects of health, considered to be of importance by
patients and clinicians and are mainly designed to focus to make it more responsive to change
in health, together with detailed and accurate assessment of patient concerns. This makes
these instruments important primary end points to measure HRQoL change in clinical trials
(Garratt et al., 2002).

Economic outcome The fourth type of outcome data is economic outcome, including costs
and charges. It is very difficult to collect actual cost data despite the need to do so. Economic
outcome measures demonstrate the return on investment of a medical intervention
(Drummond et al., 1988). Economic outcome in healthcare can be evaluated by several
different means. They are cost utility (CU) analysis, cost benefit analysis (CBA), cost
minimization analysis (CMA) and cost effectiveness analysis (CEA). Disease severity is
considered to be an important determinant of cost, as mild to moderate the disease, the major
cost incurred in the drug therapy. Healthcare cost or economic outcomes can be grouped into
several categories: direct medical, direct nonmedical, indirect nonmedical and intangible
costs (Eisenberg, 1989).

Direct medical costs are the costs incurred for medical product and services used to prevent,
direct and/or treat a disease. Direct medical costs are the fundamental transactions associated
with medical care that contribute to the portion of gross national product spent on healthcare.
Examples of these costs include drugs, medical supplies and equipment, laboratory and
diagnostic tests, hospitalization and physician visit. Fixed costs are essentially “overhead”
costs (e.g. heat, rent, electricity) that are not readily influenced at the treatment level and thus
remain relatively constant. Direct nonmedical costs are any costs for nonmedical services that are results of illness or disease but do not involve purchasing medical services. These costs are consumed to purchase services other than medical care and include resources spent by patients for transportation to and from healthcare facilities, extra trips to the emergency department, child or family care expenses, special diets and various other out-of-pocket expenses. Indirect nonmedical costs are the costs of reduced productivity (e.g. morbidity and mortality). Indirect costs are costs that result from morbidity and mortality, and are important source of resource consumption, especially from the perspective of the patient. Morbidity costs are costs incurred from missing work, whereas mortality costs represent the years lost as result of premature death. Intangible costs are those of other non financial outcomes of disease and medical care. Examples include pain, suffering, inconvenience and grief. These are difficult to measure quantitatively and impossible to measure in terms of economic or financial costs.

1.4 Supplements

Chronic diseases are estimated to account for 35 million deaths worldwide, which is about two third of total deaths (Alwan et al., 2010). T2DM comprise a major proportion of these diseases in both developed and developing countries after cardiovascular disease and cancer. Many of these chronic diseases share common risk factors and underlying pathologic mechanisms that may be modified by nutrients or dietary supplements like reduction of oxidative damage by antioxidants, cell differentiation, proliferation, and growth regulated by retinol, calcium, and vitamin D, DNA methylation regulated by folate and B vitamins etc. (Huang et al., 2007).

Plants and plant-derived products are part of the healthcare system since ancient human civilizations and a remarkable numbers of modern drugs have been isolated from natural sources, many based on their traditional uses (Farnsworth and Soejarto, 1991). The use of
natural products as an alternative to prescription medicines has become increasingly popular in the last decade (Fabricant and Farnsworth, 2001). Natural products have a vast molecular diversity and functionality and they currently account for a large amount in pharmaceutical and agrochemical markets. The plant-based systems of many other cultures has been extensively documented and play an essential role in healthcare, and it has been estimated by the World Health Organization (WHO) that approximately 80% of the world inhabitants rely mainly on traditional medicines for their primary healthcare (Farnsworth et al., 1985). The use of herbal supplements in the US has become increasingly popular in recent years. These medications fall into the category of alternative/complementary medicines.

Defining complementary and alternative medicine (CAM) is difficult because the field is very broad and continually changing. The National Centre for Complementary and Alternative Medicine (NCCAM) defined CAM as a group of diverse medical and healthcare systems, practices, and products that are not generally considered part of conventional medicine. According to Dietary Supplement Health and Education Act (DHSEA) of 1994 of USA, dietary supplement is a product (other than tobacco) that contains one or more dietary ingredients or their constituents and is intended to supplement the diet and provide nutrients, such as botanical supplements like herbs, vitamins, minerals, other nutritive supplements like amino acids or fish oil capsules, and non-nutritive supplements like glucosamine or chondroitin. These can be taken by mouth as a pill, capsule, tablet, or liquid; and is labeled on the front panel as a dietary supplement.

Increasing number of individuals using complementary and alternative medicine (CAM) for the treatment of common medical conditions (Eisenberg et al. 1998), and patients with DM are more likely to use CAM than individuals in the general population (Egede et al., 2002). An increased use of CAM has been found to be more prevalent in those with chronic and debilitating diseases such as DM (Hasan et al., 2009). The increased use of dietary
supplements and other CAM therapies has been attributed to several different factors, namely dissatisfaction with conventional medical practices, the increased costs associated with conventional medicine, desire for self-treatment of one’s health, and the prevention/treatment of health conditions (Ernst, 2000).

People with diabetes use supplementary therapies for their health. Some use dietary supplements in efforts to improve their blood glucose control, manage symptoms, minimise the risk of developing complications and to improve quality of life. Generally multi-vitamin, multi-mineral (MVMM) preparations are the most commonly used as dietary supplements. Other frequently reported vitamin/mineral (VM) supplements include calcium, vitamin E, and vitamin C (Yeh et al. 2003). A systemic literature review of herbs and dietary supplements used to assess the efficacy, safety and effects on glycaemic control among patients with diabetes reveal that many chemicals used in modern conventional medicines are of plant origins. Vitamin and mineral supplements are commonly used for primary or secondary disease prevention, but information about the efficacy and safety of herbs, vitamins or other dietary supplements used for diabetes management is conflicting.

1.4.1 Vitamin C

Vitamins are a class of nutrient that is required by body for its various biochemical and physiological processes. Human body does not synthesise these vitamins, and hence one must take it through the diet. Vitamins are subdivided into fat soluble vitamins and water soluble vitamins. Fat soluble vitamin are soluble in fat solvent like A,D,E and K. Water soluble vitamins are soluble in water like vitamin C and vitamin B complex (Iqbal et al., 2004).

Vitamin C is an unstable easily oxidized acid and can be destroyed by oxygen alkali and high temperature. Human body requires vitamin C for normal physiological functions. Unlike animals humans can not synthesise vitamin C due to the absence of enzyme L-gulonolactone oxidase from the liver (Burns, 1959). The role of vitamin C is widespread. It helps in the
metabolism of tyrosine, folic acid and tryptophan. It helps to lower blood cholesterol and contributes to the synthesis of the amino acids carnitine and caecholamine that regulate the nervous system. It helps in the formation of neurotransmitter and increase the absorbtion of iron from the gut. It also plays an important role in tissue growth and wound healing and protect the body from harmful effects of free radicals and pollutants.

1.4.1.1 Biochemistry

Vitamin C is an electron donor and, therefore, a reducing agent. All known physiological and biochemical actions of vitamin C are due to its action as an electron donor. When vitamin C donates electrons, they are lost sequentially. The species formed after the loss of one electron is a free radical, semidehydroascorbic acid or ascorbyl radical. As compared to other free radicals (a species with an unpaired electron), ascorbyl radical is relatively stable with a half-life of $10^{-5}$ seconds and is fairly un-reactive. Reduction of a reactive free radical with formation of a less reactive compound is sometimes called free radical scavenging or quenching. Ascorbate is, therefore, a good free radical scavenger due to its chemical properties (Buettner and Mosley, 1993; Bielski et al., 1975).

Ascorbyl radical, with its unpaired electron, is not a long lived compound. Upon loss of a second electron, the compound formed is dehydroascorbic acid. Dehydroascorbic acid stability depends on factors such as temperature and pH, but is often only minutes (Washko et al., 1993). Dehydroascorbic acid may exist in one of several different structural forms (Tolbert and Ward, 1982), but the dominant form in vivo has not been elucidated. Formation of both ascorbyl radical and dehydroascorbic acid is mediated by a wide variety of oxidants in biological systems including molecular oxygen, superoxide, hydroxyl radical, hypochlorous acid, reactive nitrogen species and the trace metals iron and copper.

Once formed, ascorbyl radical and dehydroascorbic acid can be reduced back to ascorbic acid by at least three separate enzyme pathways as well as by reducing compounds in biological
stems such as glutathione. In humans, there is only a partial reduction back to ascorbic acid. All the ascorbic acid that is oxidized is, therefore, not recovered. Some of the dehydroascorbic acid is metabolized by hydrolysis and is lost. If dehydroascorbic acid is not reduced back to ascorbic acid, it is hydrolyzed irreversibly to 2, 3 diketogulonic acid. This compound is formed by irreversible rupture of the lactone ring that is a part of ascorbic acid, ascorbyl radical, and dehydroascorbic acid. 2, 3-Diketogulonic acid is further metabolized into xylose, xylonate, lyxonate and oxalate (Lewin, 1976).

1.4.1.2 Absorption
The absorption of vitamin C occurs in the buccal mucosa, stomach and the small intestine. The buccal absorption is mediated by passive diffusion through the membrane of the buccal cavity. While gastrointestinal absorption is through an efficient and active sodium dependent, energy requiring and carrier-mediated transport mechanism (Wilson, 2009). It has also been explained that the absorption of vitamin C occurs through an active transport system located in the gut and its re-absorption in the kidney (Gabby and Singh, 1991). Since the absorption mechanism in the gut and kidney can reach a saturation point, it is better to take multiple and smaller doses of vitamin C throughout the day than one larger dose. At higher intakes, the process is saturated; up to 180 mg, there is an average absorption of 70% in both smoker and non-smoker, but absorption decreases from 50 to 16% at intakes over the range of 1.5-12 g (Kubler and Gehler, 1970). About 80-90% ascorbic acid is absorbed in the gastrointestinal tract. The absorbed acid circulates freely in the plasma, leukocyte and red blood cell and enters all tissues, with maximum concentrations 68-86 µmol/l plasma being achieved with oral intake of 90-150 mg/day (Olsan and Hodges, 1987). The body uses it in two hours and then excreted from the body within three to four hours. Vitamin C is used up more rapidly under stressful conditions, alcohol consumption and with smoking.
1.4.1.3 Excretion

The kidneys are the key regulators of ascorbic acid homeostatic control. Concentrations are regulated by urinary output. The total body pool size of ascorbate is affected by limited intestinal and renal tubular absorption. Body ascorbate reaches a maximum of 20 mg/kg body weight when ascorbate intake is increased from 30 to 180 mg/day. With intakes less than 100 mg per day, there is little urinary output of ascorbic acid or its metabolites. Intakes near 100 mg/day are associated with a 25% excretion rate, primarily as ascorbic acid metabolites, oxalate and urate (Levine et al., 1996). When intakes exceed 500 mg per day, most absorbed ascorbic acid reappears in the urine as ascorbic acid and, to a lesser degree, ascorbic acid metabolites. Above this level of intake, the excretion in the urine rises rapidly (Kallner et al., 1979).

1.4.1.4 Recommended intakes

Vitamin C is truly a wonder nutrient and there is no doubt that many of the serious degenerative diseases plaguing the civilized world today can be prevented or even reversed through an adequate intake of this essential nutrient. Currently, U.S. dietary recommended intakes of vitamin C for adults are 75 mg daily for women and 90 mg daily for men. These figures are based upon a review (Gey, 1998). The review suggested that plasma vitamin C concentrations of 50 umol/L provide the optimal physiological benefits pertaining to cancer and cardiovascular health (INMA, Food and Nutrition Board, 2009). The proposed new dietary recommended intake of vitamin C is 200 mg/ d while perhaps adequate for healthy, young males, would seem to be quite inadequate for older people and certainly too low for sick people. As a matter of fact, a scientific advisory panel to the US government sponsored Alliance for Aging Research recently recommended that all healthy adult increase their vitamin C intake to 250-1000 mg/d (Voelker et al., 1994)
Many studies have examined potential beneficial effects of vitamin C supplementation, but these studies use amounts varying from doses of just a couple of hundred milligrams up to 8 grams daily. It is difficult, therefore, to know how much vitamin C is actually needed to optimize health, and if there is a level of intake above which no further benefits are noted. One of the studies reported that optimal supplementation is achieved by consuming between 500 and 1000 mg of vitamin C daily (Johnston and Cox, 2001). One point to consider when comparing the findings of different studies is that it is important to compare status markers of the same kind. That is, a person may be classified in different status categories depending upon whether dietary intake or serum concentrations are studied. Various factors such as smoking and medication use may also affect serum vitamin C in addition to intake (Loria et al., 1998). Toxicity, normally, does not occur since vitamin C is water soluble and regularly excreted through body. Excess of vitamin C excreted in the urine gives a false-positive for sugar. High level of vitamin C interferes with copper absorption (Finley and Cerklewski, 1983). An upper limit intake of 2 g per day has been established for vitamin C, using gastrointestinal distress as the marker of adverse effects. With intakes above two grams per day, individuals are more likely to develop osmotic diarrhea and nausea. Other possible adverse effects at high concentrations include increased kidney stone formation in susceptible individuals due to increased urinary oxalic and uric acid. However, a review found that no significant evidence supporting these claims of adverse effects (Hathcock et al., 2005).

1.4.1.5 Deficiency

There are varying degrees of vitamin C deficiency. Adequate vitamin C status means having vitamin C plasma concentrations at least 23 umol/L. Low concentrations of vitamin plasma vitamin C are defined as 11.4-23 umol/L, and values less than 11.4 umol/L are considered deficient (Jacob and Sotoudeh, 2002). Severe vitamin C deficiency is known as scurvy, and is characterized by fatigue, inflammation of the joints, hair and tooth loss, and easily bruising or
bleeding. Since these symptoms are non-specific to scurvy and resemble those of other more common diseases, scurvy is often initially misdiagnosed. Although death occurs from severe scurvy, if a patient is supplemented with vitamin C scorbutic symptoms are usually reversed (Leger, 2008; Vitale et al., 2009). While both DHA and ascorbic acid have been documented to improve symptoms of scurvy, ascorbic acid exerts about twice the antiascorbutic activity of DHA (Otsuka et al., 1986).

1.4.1.6 Antioxidant role

Vitamin C has wide range of physiological functions related to its roles as a reductant and a cofactor in numerous reactions. Vitamin C acts as an antioxidant, halting free radical and lipid peroxidation perpetuation (Canoy et al., 2005). Importantly, ascorbate is one of the first compounds in the body to be oxidized. The order of oxidized compounds are; ascorbate>a-tocopherol>sulphydryls>cholesterol (Vatassery, 1985). The effect of vitamin C and several other compounds on oxidative damage induced by the oxidant tert-butyl hydroperoxide (BHP) has been investigated. It was found that vitamin C was able to partially inhibit the action of BHP (Krukoski et al., 2009).

Vitamin C not only works as an antioxidant, but it also interacts with other antioxidants, such as glutathione. Reduced glutathione (GSH) serves as an antioxidant, protecting the cytosol and mitochondria from the effects of hydroperoxides which are formed during metabolism. GSH is important for maintaining vitamin C concentrations in vivo since it recycles vitamin C by reducing DHA to ascorbic acid, the physiologically active form (Hughes, 1964; Martensson and Meister, 1991). NADH appears to recycle DHA back to ascorbic acid as well, but to a much lesser degree (May et al., 1996).

1.4.1.7 Weight control

Obesity is one of modifiable risk factor for the initiation and progression of diabetes. A study reported that plasma ascorbic acid was inversely related to waist to hip ratios for men and
women, independent of body mass index (BMI) (Canoy et al., 2005). Plasma ascorbic acid was reported to be inversely related to BMI, body fat percentage, and waist circumference in men and women. It was also found to be indirectly related to plasma insulin and to be positively correlated with plasma adiponectin in women (Johnston et al., 2007; Watters et al., 2008). One possible explanation for this could be that vitamin C is a cofactor in the synthesis of carnitine, which is essential for fatty acid oxidation. If vitamin C is insufficient, it may hinder synthesis of carnitine, which would lead to decreased fatty acid metabolism. It has been found that individuals with marginal vitamin C status (<34 umol/L) oxidize 25% less fat than individuals with vitamin C concentrations >34 umol/L during a submaximal treadmill test. Fat oxidation was thus inversely correlated with fatigue. Vitamin C deficiency may affect weight regulation in two ways: through increased fatigue leading to a higher likelihood to stop physical activity sooner and also an impaired fat utilization for fuel (Johnston et al., 2006a).

An interesting biochemical finding pertaining to vitamin C and adiposity is that fat accumulation in overweight rats is associated with vitamin C being in a reduced redox state and lower lipid peroxidation than those concentrations observed in lean rats. This appears to contribute to the accumulation of triglycerides. Redox state refers to the percentage of oxidized vitamin C, meaning that lower redox states have lower ratios of the oxidized forms of vitamin C. Since the purpose of this study was to investigate biochemical differences in adipose tissue of obese compared to lean rats and was not an intervention trial, authors did not suggest ways to alter these biochemical variations observed. It is worth noting, however, that adipose tissue biochemical markers do vary between obese and lean rats, and further studies ought to investigate why these markers vary in this pattern, as well as the effect of antioxidant supplementation on these markers (Galinier et al., 2006).
1.4.1.8 Cardiovascular health

The effect of vitamin C supplementation in rats that were subsequently subjected to induced myocardial infarctions has been studied (Buttros et al., 2009). The study found that the group fed a diet high in vitamin C experienced less myocardial damage and improved autonomic balancing of the heart following isoproterenol-induced myocardial infarction than the control group. Vasodilation appears to be very responsive to vitamin C supplementation, but the timing of supplementation varies vessel response. The effects of vitamin C supplementation on vessel response to endothelin-1 (ET-1) injection have been studied (Bohm et al., 2007). ET-1 administration decreased endothelium-dependent and independent vasodilation and increased venous interleukin-6 (IL-6) concentrations. The authors conclude that since vitamin C operates as an antioxidant, the impaired vascular functioning resulting from ET-1 is due to oxidative stress.

The effect of pretreatment with vitamin C in subjects taking oral methionine-the precursor to homocysteine in metabolism has been studied (Chambers et al., 1999a). While vitamin C did not significantly affect the homocysteine concentrations after oral methionine, the typical decrease in flow mediated dilation was prevented by taking vitamin C. The authors conclude on the basis of these observations that the vascular damage induced by high homocysteine is due to oxidative damage, since vitamin C is known to operate as an antioxidant.

A trial on healthy Romany volunteers has shown a linear correlation between folic acid and Vitamin C on lowering toxic effect of homocysteine with certain doubt but the subsequent findings showed significant effect of vitamin C together with folic acid and vitamin B12 on the inhibition of toxic Hcy influence on vascular endothelium (Krajcovicova-Kudlackova et al., 2001; 2002).

Vitamin C’s effect on vasodilation and forearm blood flow following injection of a low-dose of *Escherichia coli* endotoxin (LPS) has been examined (Pleiner et al., 2002). While the LPS
initially decreased forearm blood flow by 30%, the addition of intra-arterial vitamin C completely reversed these effects. No effect was observed on blood flow among control subjects. Given vitamin C’s documented role in regulating blood flow, it is not surprising that low concentrations of plasma vitamin C are related to a significantly increased risk of ischemic heart disease and stroke (Gey et al., 1993). The data from the 12-year Basel prospective study has been analyzed. It was found that low concentrations of vitamin C increased the risk of these diseases independent of concentrations of other vitamins such as vitamins A or E.

Vitamin C appears to be a key modulator in rates of cholesterol and other lipid synthesis. Guinea pigs fed with suboptimal vitamin C (50 mg/kg) demonstrated numerous hyperlipidemic effects (Montano et al., 1998; Marc, 2008; Cafolla et al. 2002). Plasma concentrations of thiobarbituric acid reactive substances (TBARS) are a commonly used marker of lipid peroxidation and overall circulating oxidation. The differential effect of consuming orange juice versus a vitamin C supplement on TBARS formation has been examined (Johnston et al., 2003). Study participants either drank 8 ounces of orange juice or took about 70 mg of supplemental vitamin C, each treatment providing comparable amounts of vitamin C. Results show that the orange juice and the supplement were equally effective at reducing plasma TBARS formation. One surprising finding is that one cup of orange juice was equally effective than two cups at inhibiting lipid peroxidation in plasma, perhaps due to a leveling effect.

1.4.1.9 Diabetes

Supplementing with mega doses of vitamin C has been shown to delay the insulin response to glucose challenge in non-diabetic individuals. It was hypothesized that this is due to competition between glucose and vitamin C for cellular uptake by glucose transporters (Johnston and Yen, 1994). While research would have to be conducted among diabetics
before definitive conclusions can be drawn, these results hold promise as a means to help control blood glucose.

Patients with insulin-dependent diabetes appear to have impaired tissue storage of ascorbic acid, even if their intake is sufficient. This may lead to intracellular scurvy, which may be responsible for some of the degeneration typical of this disease. Blood samples from diabetics and nondiabetics and the compared trends has been analyzed (Cunningham et al., 1991). Although vitamin C consumption exceeded recommendations among diabetics and their plasma ascorbic acid concentrations were normal, storage of ascorbic acid (as seen in mononuclear leukocytes) was significantly reduced by 33%. These data support the theory that glucose is inhibiting cellular uptake of ascorbic acid in insulin dependent diabetes.

Three months of co-supplementation of Mg, Zn, vitamin E with vitamin C (200mg/day) has been shown to significantly increase HDL-c and apo A1 levels (Maryam et al., 2005). It was suggested from this study that supplementation of these micronutrients could be recommended for T2DM patients based on their daily requirements. Two different dosages of vitamin C, 0.5g/d and 1g/d, shows a dose-dependent effect on the cellular contents of antioxidants and on vitamin E content of LDL in elderly patients with T2DM (Daniel et al., 2009), although mega doses of vitamin C may seems to be toxic in diabetes with certain kidney disorders (Goldburg, 1993; Will and Tyers, 1996).

A recent study has reported that antioxidant supplementation moderately lowers insulin sensitivity and endothelial adhesion molecule levels in overweight young adults (Heather, et al., 2009). It was recommended long-term studies should be carried out to determine whether antioxidants are effective in suppressing diabetes or vascular activation over time. It was also found in animal studies that antioxidant suppresses leukocyte adhesion and thus endothelial dysfunction associated with increase in iris blood flow perfusion in diabetes. It may also be a therapeutic agent for preventing diabetic complications (Amporn et al., 2007). In general
vitamin C shows positive therapeutic signs by implicating antioxidant activity in diabetes, but its relation to management of long term complication and/or on vascular risk factors in diabetes still remains unclear.

1.4.2 Resveratrol

The first known use of grape extracts for the benefit of human health can be dated back over thousand years. Darakchasava, an Ayurvedic medicine wherein the main constituent is *Vitis vinifera L*, is prescribed as cardiotonic in India (Soleas et al., 1997). A major portion of this medicine is resveratrol. This compound is today available in tablet form and is recommended as a dietary supplement. Resveratrol, as a component of red wine, is thought to be responsible for the “French paradox” i.e. low mortality due to coronary heart disease as a result of moderate consumption of red wine (Kopp, 1998). The most recent data has reinforced this theory and indicated that resveratrol play a crucial role in cardiovascular protection provided by grapes and wines (Bertelli and Das, 2009).

Resveratrol is a compound that has received attention recently in diabetes research for its wide ranging beneficial effects on the biological system. The effects have been attributed to its chemical structure (Clement et al., 1998; Fontecave et al., 1998) and possible antiperoxidant effects (Tadolini et al., 2000; Cai et al., 2003). It has been found to act as a powerful antioxidant and antiinflammatory agent, along with beneficial effects against ageing and metabolic diseases (Rivera et al., 2009). Administration of resveratrol has been shown to cause a reduced amount of edema (Lagouge et al., 2006), increased muscle strength and endurance and reduced protein catabolism (Dirks-Naylor, 2009; Das et al., 2008; Wyke and Tisdale, 2006; Russel et al., 2006; Wyke et al., 2004) along with protecting skeletal muscle from the deleterious effects of oxidative stress through alterations in metabolism (Dirks-Naylor, 2009; Lagouge et al., 2006; Wyke and Tisdale, 2006; Russel et al., 2006; Wyke et al.,
Additionally, within skeletal muscle, resveratrol has been found to increase insulin stimulated glucose transport and improve glucose homeostasis (Dirks-Naylor, 2009).

### 1.4.2.1 Chemistry

![Structure of trans - 3, 5, 4'-trihydroxystilbene]

**Figure 4: Structure of trans - 3, 5, 4'-trihydroxystilbene**

Structurally, resveratrol (trans - 3, 5, 4'-trihydroxystilbene), is a stilbene, the parent skeleton structure of a family of compounds including the cis- and trans-isomers (Dirks-Naylor, 2009). Resveratrol is a phytoalexin, meaning synthesis of the compound can be induced by microbial infections, ultraviolet radiation and exposure to ozone (Alarcon de la Lastra and Villegas, 2005). Grapes are the primary source of naturally occurring resveratrol (Fremont, 2000), with good concentrations of the compound found in the leaf epidermis and the skin of grape berries, but not within the flesh (Alarcon de la Lastra and Villegas, 2005; Fremont, 2000; Stervbo et al., 2007). Stilbene synthase, the terminal enzyme in the production pathway is activated in response to exogenous stress factors, ultra-violet radiation and chemical signals from pathogenic fungi. Levels of resveratrol peak approximately 24 h post stress exposure, and decline 48-72 h later due to the activation of catabolic stilbene enzymes (Stervbo et al., 2007). Concentration of resveratrol in grapes is dependent upon maturity and variety of the grape, along with duration of stress exposure. Grape leaves reach concentrations ranging 50-400μg/g fresh weight, while fresh grape skin content ranges 50-100μg/g (Alarcon de la Lastra and Villegas, 2005).

Resveratrol can occur in multiple forms, both of which are counted in these concentrations. Isoforms, *trans-* and *cis-* , have their own properties and functions. The trans conformation
possesses numerous health benefits (Bertelli et al., 1996). Plants can also synthesize
stillbenoid glucosides, forming cis- and trans- resveratrol 3-O β glucoside (Fremont, 2000),
which when cleaved can produce a free glucose molecule. Resveratrol can also be obtained
through organic synthesis; trans-resveratrol is most often formed first, and when exposed to
ultra violet irradiation changes into the cis conformation (Stervbo et al., 2007). Research on
the biological properties of resveratrol such as bioavailability and function were limited until
the compound could be synthesized, as pure extraction from the grape skin and leaves is
difficult to achieve.

1.4.2.2 Bioavailability and metabolism

Bioavailability of a nutrient is defined by the amount available to the target tissue after
administration, including absorption, distribution, and metabolism of the compound.
Resveratrol absorption from the intestine is rapid (Bertelli et al., 1998), allowing for
distribution to various organs, primarily the liver, kidneys, brain, lungs, and muscle (Soleas et
al., 2001; Wenzel et al., 2005). Absorption of resveratrol is not greatly impacted by the
vehicle in which it is administered. Resveratrol is most commonly consumed as red wine, but
can also be found in grape juice and vegetable homogenates. Although resveratrol content
can vary depending on several variables, a study held resveratrol concentration constant
among different matrices to determine absorption rate (Goldberg et al., 2003). The matrix
used to deliver the compound does impact total resveratrol when administered utilizing the
different forms of administration. In order to travel through the body, resveratrol must be
bound to protein or become conjugated, primarily into resveratrol-3-glucoronide or
resveratrol-3-sulfate, to remain at elevated concentrations in the serum due to its low water
solubility (Belguendouz et al., 1997). Albumin is thought to be one of the main plasma
protein transporters of unconjugated resveratrol due to its physical properties allowing it to
bind to hydrophobic compounds (Jannin et al., 2004). The compound is also shown to
interact with lipoproteins in a lipid concentration dependent manner (Belguendouz et al., 1998).

Upon absorption, delivery of protein bound or conjugated resveratrol to the tissues occurs, with the liver having the highest accumulation of the compound (Wenzel et al., 2005; Alarcon de la Lastra and Villegas, 2005). Uptake of resveratrol into the liver occurs via passive diffusion, and carrier mediated transport. Utilization of radiolabeled resveratrol allowed for the examination of time, dose, and temperature dependencies of resveratrol uptake into the organ, finding that carrier facilitated transport is primarily used to allow for efficient and specific uptake of resveratrol (Lancon et al., 2004). The liver seems to be a reservoir of resveratrol and its conjugated metabolites, resulting from metabolism both in the intestine and the liver. The function of this pool is not entirely understood. However, it is believed to be partially responsible for the prolonged effects of resveratrol after a bolus dose. Detectable levels of resveratrol can occur as early as 15 min post resveratrol administration, with peak concentrations occurring 30 min after dosage (Wenzel et al., 2005). Although free resveratrol content begins to decline 1 h post-dosage (Asensi et al., 2002), resveratrol metabolites remain in the circulation for longer periods of time. A second resveratrol peak occur approximately 6 h post administration, due to enteric recirculation of conjugated metabolites. The presence of these metabolites is known to aid in the elevation of detectable levels of resveratrol in the blood over time, which may contribute to the efficacy of resveratrol within the body (Gescher and Steward, 2003). However, the exact function of these structures remains to be determined.

1.4.2.3 Excretion

Removal of resveratrol from the body is accomplished mainly through renal excretion. Decreasing concentration of radiolabelled resveratrol metabolites in the kidneys strongly suggests their primary role in the elimination of these compounds from the body (Bertelli et
Some elimination may occur through biliary binding pathways, although minimal research has been conducted in this area (Alarcon de la Lastra and Villegas, 2005). Resveratrol-3-sulfate and resveratrol-3-glucoronide are found in the urine of rodents and humans (Soleas et al., 2001). However, species variation should be taken into consideration, along with differences due to dose rate and concentration. Free resveratrol has not been found in murine or human urine, suggesting that the majority of free resveratrol is either utilized by the peripheral tissues or conjugated and stored by the liver or excreted (Asensi et al., 2002).

1.4.2.4 Dosage

Despite the extensive research conducted across murine and human models, an effective dose for resveratrol is yet to be established. Administration rates, concentrations, and durations are widely varied, making it difficult to determine appropriate means through which beneficial effects can be seen. Fresh grape skin contains 50-100 mg resveratrol/g (Gusman et al., 2001), making the content of naturally occurring beverages containing the skin vary greatly as well. In red wine, the range of detectable trans-resveratrol spans from non-detectable levels to 14.3 mg/L (62.7 µM), with reported health benefits seen at average levels, 1.9 ± 1.7 mg/L (8.2 + 7.5 µM) (Stervbo et al., 2007). During early resveratrol trials, wine of a known content was used as the main method of dosage. Even then, duration and concentration of supplementation varied greatly; 86 µg resveratrol/kg BW for a single dose, or 43 µg resveratrol/kg BW once daily for 15 days (Bertelli et al., 1996). The trend continues through much of the literature utilizing a more “natural” form of resveratrol, and has transcended through present day.

The development of organic synthesis of trans-resveratrol opened the door to further research on the compound without concern of competition or conflicting results due to competition of other flavinols present in wine. Resveratrol could then be administered in greater doses, over greater time periods, along with the option of injectable administration. Intragastric
administration to mice has been supplemented from values ranging to 0.228 mg/kg BW to 60 mg/kg BW, making it difficult to elucidate the appropriate dose on a weight basis (Benton et al., 2010). In human studies, supplementation levels have been reported at 0.03 mg/kg BW to 0.35 mg/kg BW, with many other values in-between (Goldberg et al., 2003). Duration of supplementation also varies tremendously; some literature touts the benefits of a loading dose of high concentration, followed by a lower maintenance dose, while others remain at a specified concentration over time. Single dose administration seems to be utilized only during trials for which absorption, metabolism, or clearance are the primary areas of evaluation.

1.4.2.5 Biological role

Resveratrol is currently known for its antioxidant, antiinflammatory, anticancer and chemopreventative abilities. Interest in resveratrol began when an inverse correlation was found between red wine consumption and cardiovascular disease occurrence (Fremont, 2000). The compound was first thought to be the biologically active ingredient in red wine in 1992 (Siemann and Creasy, 1992), causing extensive research into the effects of resveratrol within the body. Currently, resveratrol is known to impact numerous mechanisms and pathways within the body, including inhibition of lipid peroxidation, free-radical scavenging, alteration of eicosanoid synthesis, modulation of lipid metabolism, improvement of insulin sensitivity, antiinflammatory activity and estrogenic activity (Fremont, 2000). Resveratrol prevents the peroxidation of membrane lipids by ROS (Fauconneau et al., 1997). Resveratrol acts as a scavenger of free radicals, preventing the oxidation of membrane lipids (Sun et al., 1997). Maintenance of structural integrity of the plasma membrane is crucial to proper cellular function, and can have detrimental effects on exercise performance. Preventative effects are not limited to the muscle cells; it has been shown that resveratrol has prevented damage due to peroxidative stress in dopaminergic neuronal and hepatic cells (Fauconneau et al., 1997; Sun et al., 1997). The compound has also been found to prevent metal induced lipid
peroxidation within microsomes and low-density lipoproteins (LDL) (Kawada et al., 1998). Alterations in the conformation of LDLs can increase the amount of circulating fatty acids in the bloodstream, due to structural changes in apo B, thereby affecting catabolism by the B/E receptor system, aiding in the predisposition of the animal to numerous metabolic diseases (Kahn and Swartz, 2002).

Modulation of lipid metabolism can also occur due to resveratrol supplementation. The impact of resveratrol on insulin secretion has been studied for the first time (Zhang et al., 2004). Several studies have been reported on its beneficial effects in diabetes including glucose-induced insulin secretion (Szkudelski, 2006; Thirunavukkarasu et al., 2007), increased insulin concentration (Chi et al., 2007; Palsamy and Subramaniah, 2008, 2009), insulin sensitivity (Baur et al., 2006; Revera et al., 2009), glucose oxidation and lactate release (Szkudelski, 2008, 2009) and its binding effect on sulfonylurea receptors and ability to block the pancreatic ATP-sensitive K+ channels in β-cell, voltage-gated K+ channels similar to glibenclamide (Hambrock et al., 2007; chen et al., 2007), which act as a channel blocker thereby stimulate insulin secretion. Its antiinflammatory, antioxidant, cardiovascular properties, including inhibition of platelet aggregation and promotion of vasodilation by enhancing the productions of NO have been described (Silan, 2008). Resveratrol has been shown to increase silent information regulator 2 homolog (SIRT) 1 and peroxisome proliferator-activated receptor (PPAR) - γ coactivator-1 (PGC1) in muscle (Lagouge et al., 2006), resulting in an altered metabolism displaying an increased sensitivity to insulin, along with a preferential switch to fat as a fuel source (Benton et al., 2010). Increases in fatty acid transporter content, PPAR-γ pathway activation, and fat oxidation enzymes are responsible for this shift. The glucose sparing effect of resveratrol allows for more glucose to be stored as glycogen, and a decrease in fat available for deposition in the adipocytes. Reduction in circulating fatty acids in the blood stream combined with improved insulin sensitivity makes
resveratrol a potential mediator for T2DM in humans, and a potential area of interest for equine metabolic syndrome. Resveratrol has been shown to improve insulin resistance via enhancement of enzyme activity involved in glucose metabolism, along with increasing the insulin sensitive cell’s ability to respond to the hormone. In streptozotocin-nicotinamide-induced diabetic rats, resveratrol supplemented orally at 5 mg/kg BW for 30 days was able to alter activities of key carbohydrate metabolism enzymes such as hexokinase and pyruvate kinase, which returned back to normal concentrations, allowing for normal flux of the substrate through this catabolic pathway (Palsamy and Subramanian, 2009). Reduction of circulating glucose is achieved by enhancement of GLUT-4 translocation, perhaps due to resveratrol’s actions on PGC-1 (Karlsson et al., 2005). In mice, resveratrol supplementation at 400mg/ kg BW daily increased levels of PGC-1α acetylation, leading not only to an increase in glucose transport, but also to an induction of oxidative phosphorylation genes and mitochondrial biogenesis (Lagouge et al., 2006). Increasing the cells ability for glucose uptake and metabolism can aid to alleviate hyperglycemia along with ensuring substrate availability in the cell for utilization and storage.

Increased circulating levels of ROS, excessive circulating free fatty acid levels, and hyperinsulinemia, can all cause oxidative stress, resulting in chronic inflammation, insulin resistance, and decreased exercise performance (Kronfeld et al., 2005). Resveratrol acts to diminish oxidative stress via induction of mitochondrial MnSOD expression and activity (Robb et al., 2008). A potential mechanism of action for this result begins with resveratrol increasing the SIRT 1 to NAD+ ratio, leading to an increase in FOXO3, resulting in increased MnSOD activity. Other enzymes, such as CuZn SOD, glutathione peroxidase, and catalase observed either remain the same or decrease in activity with resveratrol supplementation (Robb et al., 2008). Resveratrol could be thus a natural alternative therapeutic agent that
could provide beneficial treatment to help type II diabetic or prediabetic individuals improve their glucose tolerance.

Though numerous animal studies have been reported on the wide ranging beneficial effects of resveratrol on diabetes mellitus, only limited clinical data are available concerning its potential effects.