Chapter 1
Introduction
1. INTRODUCTION

And plants at whose name the verse feels loath,

Filled the place with a monstrous undergrowth,

Prickly and pulpous, and blistering, and blue,

Livid and Starr’d with a lurid dew,

And agarics, and fungi, with mildew and mould,

Started like mist from the wet ground cold;

Pale, fleshy as if the decaying dead

With a spirit of growth had been animated.

Their mass rotted, off them flake by flake,

Till the thick stalk stuck like a murderer’s stake,

Where rags of loose flesh yet tremble on high,

Infecting the winds that wander by.

In ‘Mont Blanc’ (Written in 1816), a poem that explains the relationship between humankind and nature, Percy Bysshe Shelly paints a vivid picture of mushrooms growing on the forest floor and he reveals the prejudices of his time and place against mushroom.

Mushroom is the fruit body of the fungus, the reproductive part of the fungus that grows above ground and release spores, the seed like element from which new fungus are made. Mushroom produces prodigious number of spores that guarantee the spread of the fungus in the environment. When the spores, carried by wind and water land in a hospitable place it may germinate and start a new fungus colony. At that point the spore grows hyphae, the fine threadlike strands from which the mycelium is made. The mycelium is the feeding body of the mushroom.
How fast and how large the mycelium grows depends on the environmental factors such as soil, temperature and the accessibility of food. Researchers have reported finding a mycelium beneath the soil of Michigan that is 1500 years old and 35 acres wide and weighs 100 tons. Mapping by molecular methods have revealed that the mycelium germinated from a single spore (George, 2007).

Fungi make up about a quarter of the biomass of the earth. In nature fungi are the great recyclers. To feed itself, but also to assist plants in getting the nutrients they need, a fungus breaks down organic matter into essential elements. According to recent estimates by David Hawksworth, there are over 1,500,000 species of fungi on earth. Mushrooms constitute at least 14,000 and perhaps as many as 22,000 known species, but this may be less than 10% of the total. Assuming that the proportion of useful mushrooms among the undiscovered mushrooms will be only 5%, there may be thousands of as yet undiscovered species that will be of possible benefit to humankind. Even among the known species the proportion of well investigated mushrooms is very low. About 700 species have been used as food and 50 or so species are poisonous.

Mushrooms are potent medicine and contain many nutrients. For many years, mankind has benefited from green plants as a source of drugs and herbal remedies. Fungi on the other hand have not been considered in any significant way. However, this is changing very rapidly. The prominence of fungi can be now seen increasingly as evidenced by their use as a major source of pharmaceuticals and medicinal foods. Among the fungi, mushrooms have been used for untold centuries as food and medicine. Some were considered valuable enough to be used in place of currency. There are even deities around the world that are related in some way to the more precious mushrooms. Edible and medicinal mushrooms can not only convert the huge lignocellulosic biomass waste into human food, but most remarkably can produce notable mycopharmaceuticals and mycocosmeceuticals.

There are a limited number of medicinal mushrooms that both traditional medicine and contemporary scientific investigation hold in high esteem. Among the most respected is *Ganoderma lucidum* (Reishi). Because of this, it is no surprise that Reishi was the first medicinal mushroom that the American Herbal Pharmacopoeia

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(AHP) chose for comprehensive review in its series of therapeutic compendiums (Steven Bailey, 2001).

The Chinese, inter alia, have used preparations as medicines from these fungus for millennia (cited as early as 100 B.C. in the Shen Nong’s Herbal classic which is widely considered as the oldest book on oriental medicine and foundation of traditional Chinese medicine (Shiao et al., 1994)) and have given the organism the name “Lingzhi”. The name *Ganoderma* is derived from the Greek *ganos* which means brightness or sheen, hence shining and *derma* means skin (Liddell et al., 1980), while the specific epithet *lucidum* in Latin for shining.

In Japan the names include Munnertake meaning 10,000 year mushroom, Sachitake and Reishi, whereas in Korea it has the name Youngzhi (Russell, 2006) and Tuwonbiri in Hausa (Mohammed et al., 2007). In Thailand this mushroom is called monkey’s seat (Quimio, 1986).

1.1 About the mushroom

The Genus *Ganoderma* was established in the west by Finnish Botanist, P. Karsten in 1881 (Ryvarden, 1991) and more than 120 species have been reported in the world since then (Mao and Lin, 2001). The book, which is known in Japan as ‘Shinnoh Honsohkyo’ is now accepted as being the original textbook of oriental medical science. Among 365 kinds of medicines classified and explained in it, 120 of them are declared to be superior medicines and called ‘God’s Herbs’. The book states that for superior medicines any amount can be taken as desired on a continuous basis with no unfavourable effects. Of the superior medicines listed in the text, *(Ganoderma)* was rated number one (Linda Mc Glasson, 1992). In Latin, *lucidum* means shiny or brilliant and aptly describes this mushroom’s fruiting body, which has a modeled, sculptured, varnished appearance.

*Ganoderma* is a species of basidiomycetes which belongs to the order of *Aphyllophorales*, family *Polyporaceae* (or *Ganodermaceae*), class *Hymenomycetes* (Kendrick, 1985) which are generally called polyporus. They differ from the ordinary mushrooms which belong to the order *Agaricales*, due to presence of pores on the
underneath surface of their fruiting bodies in contrast with the gills of the mushrooms of the Agaricales. The fruiting bodies are usually laterally stiped and are also tough and leathery. Species of *Ganoderma* are especially unique among the polypores because of their shellacked or lacquered smooth fruiting bodies, usually reddish, which assume shelflike forms on trunks, logs or stumps (Quimio, 1986).

The cultivation and utilization of this fungus is popular in China and Japan due to their medicinal properties and the luck they are reputed to bring to people who possess them. They are believed to help in the maintenance of youth and long life (Quimio, 1986).

It is taken as a powder in hot water or in whisky, or by boiling the fruiting body and drinking the *Ganoderma* “tea” (Quimio, 1986). *Ganoderma* preparations does indeed contain bioactive ingredients such as triterpenes, polysaccharides, nucleosides, steroids, fatty acids, alkaloids, proteins, peptides, aminoacids and inorganic elements (Shiao et al., 1994). Among these ingredients triterpenes and polysaccharides possess diverse and potentially significant pharmacological activities. Since the first isolation of two new bitter triterpenes, ganoderic acids A and B from the dried epidermis of *Ganoderma lucidum* in 1982 by Kubota et al., more than 130 oxygenated triterpenes (mostly lanostane-type triterpene) have subsequently been isolated from the fruiting bodies, spores, mycelia and culture media of lingzhi (Kim and Kim 1999, Chairal and Hayashi 1994). It should be noted that Lingzhi is the only noted source of these bioactive ganoderic acids.

Mau et al., (2001a) have found that the fruit bodies of *Ganoderma* spp. contained low amounts of total soluble sugars. So they would not give a palatable sweet perception. Aspartatic and glutamic acids were monosodium glutamate-like (MSG-like) components, which gave the most typical mushroom taste, the umami taste or palatable taste that was the characteristic flavour of MSG and 5'-nucleotides (Yamaguchi, 1979). Contents of MSG-like components and 5'-nucleotides were relatively lower and insignificant in Ling chih. In addition, bitter triterpenoids, which showed profound medicinal effects, also were present and dominated the taste of medicinal mushrooms. The bitter taste from bitter components and triterpenes

### 1.1.1 The Legend of *Ganoderma*

*Ganoderma* has a colourful past. According to legend, Taoist priests in the first century were the first to experiment with *Ganoderma*. They are supposed to have included the mushroom in magic portions that granted longevity, eternal youth and immortality. The Taoist priests of the period practiced alchemy and were known for casting spells and mixing concoctions. They were looked upon as magicians or wizards; by present day standards, they might be considered charlatans. But it should be remembered that alchemy was the beginning of chemistry and Shamans, who treated the sick by summoning the forces of nature to the aid of their patients, were the first doctors.

A poem by the first century philosopher Wang Chung remarks on the Taoist priests’ use of mushrooms in their quest to attain a higher state of consciousness.

*They does themselves with the germ of gold and jade*

*And eat the finest fruit of the purple polypore fungus*

*By eating what is germinal, their bodies are lightened*

*Any they are capable of spiritual transcendence.*

*Ganoderma*’s reputation is the ‘Mushroom of immortality’ reached Emperor Ti of the Chin Dynasty about 23 centuries ago. The Emperor is supposed to have outfitted a fleet of ships manned by 300 strongmen and 300 beautiful women to sail to the East, where *Ganoderma* was believed to be growing and bring back the mushroom. The ships were lost at sea. Legend has it that the shipwrecked castaways washed ashore on an island and founded a new nation there. The island, the story goes, is called Japan.
1.1.2 Habitat and Identification

Ganodermataceae is comprised of poroid species that have distinctive basidiospores and often a pileus surface that appears varnished. The basidiospores are ovoid, usually truncate at one end, and golden brown in colour. The two walls of the spore are separated by columns. Because the outer wall sags between the supporting columns, the spore surface appears punctuate. Although family assignment can be made on the basis of a single spore, all species also have a trimitic hyphal system and clamp connections in the generative hyphae. Species of white rotters and often are capable of selective delignification. Few reports suggested that *Ganoderma lucidum* may produce two types of Basidiospores. One type produced in the season is said to generate only after insect ingestion and probably is dispersed in this manner. The second type of basidiospore is the common one produced throughout the rest of the growing season. This type is widely air spread and germinate readily on agar without special treatment (Michael Kuo, 2004).

*Ganoderma lucidum* can be considered as one of the most beautiful mushrooms in the world. When very young its varnished surface is Chinese red, bright yellow, and white. Later the white and yellow shades disappear, but the resulting varnished, reddish to reddish brown surface is still quite beautiful and distinctive. While *Ganoderma lucidum* is annual and does not actually grow more each year like some polypores, its fruiting body is quite tough and can last for months (Michael Kuo, 2004).

1.1.3 Description

It is saprobic; growing alone or in groups on decaying hardwood logs and stumps (rarely on conifers); annual; widely distributed but more common in eastern North America (Michael Kuo, 2004). In nature, Lingzhi grows at the base and stumps of deciduous trees, especially maple (National Audubon Society; Field guide to Mushrooms, 1993). Only two or three out of 10,000 such aged trees will have Lingzhi growth, and therefore its wild form is generally rare. Today, Lingzhi is effectively cultivated both indoors under sterile conditions and outdoors on either logs or
Woodchip beds. This annual mushroom grows on a wide variety of dead or dying trees, e.g., deciduous trees especially oak, maple, elm, willow, sweet gum, magnolia, and locust (Quercus, Acer, Alnus, Betula, Castanea, Corylus, Fagus, Fraxinus, Populus, Pyrus, Magnolia, Tilia). *Ganoderma lucidum* is less frequently found on coniferous trees (e.g., Larix, Picea, Pinus) in Europe, Asia, and North and South America (in temperate rather than subtropical regions). In the Orient, it grows primarily on plum trees. It is also found on stumps, generally near the soil surface, and occasionally on soils arising from buried roots. Cap is 2-20 cm; at first irregularly knobby or elongated, but by maturity more or less fan-shaped; with a shiny, varnished surface often roughly arranged into lumpy "zones"; red to reddish brown when mature; when young often with zones of bright yellow and white toward the margin. Pore surface is white, becoming dingy brownish in age; usually bruising brown; 4-7 tiny (nearly invisible to the naked eye) circular pores per mm; tubes to 2 cm deep.

Stem is sometimes absent, but more commonly present; 3-14 cm long; up to 3 cm thick; twisted; equal or irregular; varnished and colored like the cap; often distinctively angled away from one side of the cap. Flesh of this mushroom is brownish; fairly soft when young, but soon tough. The Spore Print is brown. In chemical reactions with KOH it is black or blackish on all surfaces. Spores are 7-13 x 5-9 µm, more or less elliptical, sometimes with a truncated end; appearing smooth at lower magnifications, finely spiny at high magnification. *Polyporus lucidus* is a former name. (Leysser ex Fries) Karsten. (Arora, 1986; Gilbertson & Ryvarden, 1986 and Lincoff, 1992.)

*Ganoderma lucidum* generally occurs in two growth forms, one, found in North America, is sessile and rather large with only a small or no stalk, while the other is smaller and has a long, narrow stalk, and is found mainly in the tropics. However, many growth forms exist that are intermediate to the two types, or even exhibit very unusual morphologies, (David Arora 1986) raising the possibility that they are separate species. Environmental conditions also play a substantial role in the different morphological characteristics lingzhi can exhibit. For example, elevated carbon dioxide levels result in stem elongation in lingzhi. Other forms show "antlers", without a cap and these may be affected by carbon dioxide levels as well.

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The *Ganoderma lucidum* is extremely rare and difficult to find in the wild. Because the husks of the spore are very hard, the spores can’t germinate as readily is the spores of other mushrooms. To germinate, the right combination of oxygen and moisture conditions is needed. Fortunately, we are now able to recreate favorable growth conditions. It can be cultured on logs that are buried in shady, moist areas. *Ganoderma lucidum* can also be inoculated onto hardwood stumps. Under commercial cultivation conditions, *Ganoderma lucidum* is normally grown artificially on sawdust and logs. The mushroom that was once the provenance of the emperors of China can now be purchased in health food stores (George, 2007).

### 1.1.4 Therapeutic action

The mycelia and fruiting bodies are used as Chinese traditional medicine to treat diseases such as tumors (Peng *et al.*, 2005), hypertension, hyperglycemia, hepatitis, chronic bronchitis, bronchial asthma, (T.K, 1999; Kyo *et al.*, 2002), Liver fibrosis( Wu *et al.*, 2004), Lupus erythematosis, nephritis, dysmenorrhoea, anorexia, migraine, arthritis, haemorrhoids, hypercholesterolaemia, constipation (Shiao *et al.*, 1994; Jong and Birmingham, 1992), neurasthenia, insomnia (Lin., 2002) gastric ulcer (Kim and Kim, 1999), cough (Yan *et al.*, 1999) inflammation, cardiovascular disorders and acts as antiviral (eg., antiHIV), antibacterial, antiparasitic, immunomodulator, kidney toxic, nerve toxic, sexual potentiator (Wasser and Weis, 1999), antiaging (Gan *et al.*, 1998), antiangiogenic, anti-metastasis and antiangiogenesis (Kimura *et al.*, 2002; Shiao, 2003) and wound healing agent (Lia *et al.*, 2001). So this mushroom is considered as symbol of success and well being meaning “marvelous herbs” or mushroom of immortality (Statneta, 1993).

### 1.2 DIABETES MELLITUS

Diabetes mellitus (DM) is prevalent in all countries of the world. More than 30 million people are said to be affected throughout the world with this disease. It is presently the most common non-communicable disease worldwide and is the fourth or fifth leading cause of death in most developed countries. There is also substantial evidence that it is an epidemic in many developing and newly industrialized countries. Complications from diabetes such as cerebrovascular
diseases, peripheral vascular diseases, stroke, diabetic neuropathy, amputations, renal failure and blindness are on the increase (Amos, 1997). Therefore, it is certain that diabetes will be one of the most challenging health problems in the new millennium. Prevention and control programs are needed to stem the rising epidemic of diabetes and its complications.

In 1997, an estimated 124 million people worldwide had DM, 97% of these had non-insulin dependent diabetes mellitus. By the year 2010, the total number of people with diabetes is projected to reach 239 millions (McCarty, 1994). Regions with greatest potential are Asia and Africa, where DM rates could rise to 2 to 3 folds than the present rates.

The projected increase in DM incidence in Asia is likely to be 3.6 to 11.4, 28.8 to 57.5, 8.6 to 19.5 and 21.7 to 44 millions in Western Asia, South Central Asia, South East Asia and East Asia respectively. Increase in complications will undoubtedly follow the prevalence of DM.

Diabetes was known even in ancient times. The name of this disease, which is characterised by excessive flow of urine and insatiable thirst, was coined by the Graeco-Roman physician Aretaeus of Cappadocia (approx. 80-130 A.D.) and is derived from the Greek word diabainen ('to flow through'). The adjective 'mellitus', which comes from Latin and means 'honey-sweet', was added by the German physician Johann Peter Frank who was in 1790, by introducing yeast fermentation test for the quantitative determination of urinary glucose, relieved the physicians of his time from the need to taste their patient's urine.

In diabetes insipidus an excessive amount of urine is produced as a result of a disturbance of the hormonal control of reabsorption of water in the kidneys. Untreated diabetes mellitus, in contrast, is characterised by high blood glucose levels either due to diminish or absent of insulin production or reduced effectiveness of insulin in the body.

Diabetes is a chronic disorder of carbohydrate, fat and protein metabolism characterized by fasting elevations of blood sugar (glucose) level and a greatly increased risk of heart disease, stroke, kidney disease, and loss of nerve function.
Diabetes can occur when the pancreas does not secrete enough insulin, or if the cells of the body become resistant to insulin; hence, the blood sugar cannot get into the cells which then leads to serious complications. The classic symptoms of diabetes are frequent urination, excessive thirst and appetite. Because these symptoms are not very serious, many with diabetes do not seek medical care. In fact, of the more than 10 million Indians with diabetes, fewer than half know that they have diabetes or ever consult a physician.

1.2.1 CLASSIFICATION

DM is divided into two major categories: type I and type II. Type I or Insulin-Dependent Diabetes Mellitus (IDDM) occurs most often in children and adolescents. Type II or Non-Insulin Dependent Diabetes Mellitus (NIDDM) usually has an onset after 40 years of age.

1.2.1.1 Insulin-Dependent Diabetes Mellitus

IDDM is associated with complete destruction of the beta-cells of the pancreas which secretes the hormone insulin. IDDM patients require lifelong insulin for the control of blood sugar levels. The type I diabetic patients must learn how to manage his or her blood sugar levels on a day-by-day basis, modifying insulin types and dosage schedules as necessary, according to the results of regular blood sugar testing. About 10% of all diabetics are type I. Although the exact cause of type I diabetes is unknown, current theory suggests it is due to injury to the insulin producing beta-cells coupled with some defect in tissue regeneration capacity.

Type I diabetes appears to have an autoimmune component at its origin as antibodies for beta-cells are present in 75% of all cases of type I diabetes compared to 0.5 to 2.0% of normal. It is probable that the antibodies to the beta cells develop in response to cell destruction due to other mechanisms (chemical, free radical, viral, food allergy, etc.). It appears that normal individuals either do not develop as severe as antibody reaction, or better able to repair the damage once it occurs.
1.2.1.2 Non-Insulin Dependent Diabetes Mellitus

About 90% of all diabetics are type II. Insulin levels are typically elevated indicating a loss of sensitivity to insulin by the cells of the body. Obesity is a major contributing factor to this loss of insulin sensitivity, with approximately 90% of individuals with type II diabetes being obese. Achieving ideal body weight in these patients is associated with restoration of normal blood sugar levels in most cases. In type II diabetes, diet is of primary importance and should be implemented diligently before a drug is used. Most type II diabetics can be controlled by diet alone. Despite a high success rate with dietary intervention, physicians often use drugs or insulin instead.

Table 1.1: Comparison of Type I and Type II Diabetes

<table>
<thead>
<tr>
<th>Features</th>
<th>Type I</th>
<th>Type II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at onset</td>
<td>Usually under 40</td>
<td>Usually over 40</td>
</tr>
<tr>
<td>% of all diabetics</td>
<td>Less than 10%</td>
<td>Greater than 90%</td>
</tr>
<tr>
<td>Seasonal trend</td>
<td>Fall and winter</td>
<td>None</td>
</tr>
<tr>
<td>Family history</td>
<td>Uncommon</td>
<td>Common</td>
</tr>
<tr>
<td>Appearance of symptoms</td>
<td>Rapid</td>
<td>Slow</td>
</tr>
<tr>
<td>Obesity at onset</td>
<td>Uncommon</td>
<td>Common</td>
</tr>
<tr>
<td>Insulin level</td>
<td>Decreased</td>
<td>Variable</td>
</tr>
<tr>
<td>Insulin resistance</td>
<td>Occasional</td>
<td>Often</td>
</tr>
<tr>
<td>Treatment with Insulin</td>
<td>Always</td>
<td>Not required</td>
</tr>
<tr>
<td>Beta-cells</td>
<td>Decreased</td>
<td>Variable</td>
</tr>
<tr>
<td>Ketoacidosis</td>
<td>Frequent</td>
<td>Rare</td>
</tr>
<tr>
<td>Complications</td>
<td>Frequent</td>
<td>Frequent</td>
</tr>
</tbody>
</table>
Table 1.2: Classification of the various forms of diabetes mellitus on the basis of causes and natural history proposed by the American Diabetes Association (Fiedler, 1999; Briese, 1998)

<table>
<thead>
<tr>
<th>Name</th>
<th>Causes, Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 1 A diabetes</td>
<td>Destruction of insulin-producing β-cells leading to absolute deficiency of insulin</td>
</tr>
<tr>
<td>Type 1 A</td>
<td>Destruction of β-cell by an autoimmune process, generally at an early age (Juvenile diabetes); antibodies can be demonstrated. (This category includes patients with disease onset after 30 years of age, formerly known as ‘Latent Autoimmune Diabetes mellitus of Adult’ (LADA).)</td>
</tr>
<tr>
<td>Type 1 B</td>
<td>TID that develops spontaneously with no identifiable cause; probably a genetic ethnic component, relatively rare, fluctuating insulin-dependency</td>
</tr>
<tr>
<td>Type 2 diabetes</td>
<td>Metabolic disorder characterised by insulin resistance of cells and insulin deficiency</td>
</tr>
<tr>
<td>other forms of diabetes due to</td>
<td></td>
</tr>
<tr>
<td>A) genetic mutations that result in defects of the insulin-producing β-cells</td>
<td>Previously known as ‘maturity-Onset Diabetes of the Young’ (MODY); distinction between MODY1 - MODY6, MODY 2 : defects of chromosome 20, affects glucokinase</td>
</tr>
<tr>
<td>B) genetic defects of insulin activity</td>
<td>Cause insulin resistance, e.g. insulin receptor defects</td>
</tr>
<tr>
<td>C) diseases of the pancreas</td>
<td>Inflammation (pancreatitis), cystic fibrosis, pancreatectomy, hemochromatosis</td>
</tr>
<tr>
<td>D) endocrine diseases</td>
<td>Acromegaly, Cushing’s syndrome, hyperthyroidism, Conn’s syndrome</td>
</tr>
<tr>
<td>E) drugs or chemicals</td>
<td>e.g. Nicotinic acid, glucocorticoids, δ-interferon, thyroid hormones</td>
</tr>
<tr>
<td>F) infections</td>
<td>e.g. Transplacental infection of the foetus with rubella, cytomegalovirus</td>
</tr>
<tr>
<td>G) immune reactions</td>
<td>Anti-insulin receptor antibodies</td>
</tr>
</tbody>
</table>
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<table>
<thead>
<tr>
<th>H) hereditary diseases that can be associated with diabetes mellitus</th>
<th>e.g. Down’s syndrome, porphyria</th>
</tr>
</thead>
</table>

Appearance of impaired glucose tolerance as a result of metabolic changes (increased glucose and insulin levels due to mobilization of energy reserves) during pregnancy; various causes, can occur after pregnancy in association with persistently elevated blood glucose levels; subdivided into T1D, T2D, IGT (‘impaired glucose tolerance’) and PAGT (‘previous abnormality of glucose tolerance’), however only about 4% of patients with gestational diabetes remain manifest diabetics after pregnancy (Briese, 1998).

The cause of diabetes mellitus is not fully understood. Recently, increasing evidence suggests that free radicals formations are involved in the pathogenesis of diabetes and the development of diabetic complications (Colman et al., 1989; Giugliano et al., 1995). The oxidative stress is significantly increased in diabetes because prolonged exposure to hyperglycaemia increases the generation of free radicals and reduces capacities of antioxidation defence systems (Colman et al., 1989).

Carbohydrates are major source of energy for all living organisms including humans. Glucose is the only fuel used by few specialized cells i.e. brain and erythrocytes. The major pathways of carbohydrate metabolism either begins or ends with glucose and it is the major form in which carbohydrate from the intestinal tract is presented to rest of the body cells. Glucose is continuously delivered to all tissues by the blood, which normally contains 80-90 mg/dl before meal and 80-120 mg/dl after meal. The dependence of various tissues on glucose varies widely.

Consumption of highly enriched diet with saturated fats or simple sugars (e.g. glucose) can increase insulin concentrations, enhance adipose tissue deposition, reduce insulin sensitivity and impair glucose tolerance. These effects are seen even if total energy intake is not increased, but are more pronounced greater in enriched diet. High fat feeding can also enhance diabetic features in rodents treated...
neonatal with streptozotocin or with ventromedial hypothalamic lesions (Pascoe et al., 1990).

**Table 1.3: Origin of diabetes**

<table>
<thead>
<tr>
<th>Events</th>
<th>Type 1 diabetes</th>
<th>Type 2 diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genetic predisposition</td>
<td>High-risk HLA alleles DR3 and DR4 on chromosome 6</td>
<td>Susceptibility genes on chromosome 2</td>
</tr>
<tr>
<td>Possible environmental factors</td>
<td>Autoimmune disease promoted by β-casein of cow’s milk early childhood vitamin D deficiency in early childhood (?) coxsackie viruses chemicals</td>
<td>Overweight reduced muscular activity poor diet:</td>
</tr>
<tr>
<td>Changes in characteristic markers in peripheral blood</td>
<td>Presence of auto antibodies against β-cell components (Glutamic Acid Decarboxylase (GAD), tyrosine phosphatase (IAA) up to 10 years before clinical manifestation</td>
<td>- too fatty</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- too sweet</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- too much stress</td>
</tr>
<tr>
<td>Cause of hyperglycaemia</td>
<td>Progressive destruction of insulin producing β-cells by mal-functioning cytotoxic CD8+ T-lymphocytes and macrophages, immune cells that are regulated by IL-12-dependent Th 1-lymphocytes</td>
<td>Peripheral insulin resistance, i.e. reduced sensitivity of cells to insulin, increasing disturbance of insulin secretion, exhaustion of β-cells by over-stimulation, increased gluconeogenesis in the liver</td>
</tr>
</tbody>
</table>

In untreated or inadequately treated clinically manifest type 1 and type 2 diabetes, blood glucose level over 120 mg/dl (6.7 mmol/1) fasting and over 180 mg/dl (10 mmol/1) after meals, elevated HbA1c values (Ravindra and Thomas, 1995).
1.2.2 EPIDEMIOLOGY

Chronic hyperglycaemia in diabetes is associated with damage of tissue, dysfunction and ultimate functional failure of various organs, especially in the eyes, kidneys, nerves, heart and blood vessels (American Diabetes Association, 1998). Several pathogenic processes are involved in the development of diabetes. Autoimmune destruction of β-cells of the pancreas leads to insulin deficiency/release. It may also occur due to diminished tissue response to insulin at one or more points in the complex pathway of hormone action. Inadequate insulin release and impairment of insulin action frequently co-exist. Long term complications of diabetes include retinopathy with potential loss of vision, nephropathy leading to renal failure, peripheral neuropathy with risk of foot ulcers, amputation and charcal joints. Autonomic neuropathy may also cause sexual dysfunction along with gastrointestinal, genitourinary and cardiovascular symptoms. Hypertension, abnormalities of lipoprotein metabolism and periodontal disease are often found in diabetes patients.

The clinical course and prognosis for diabetic patients are influenced predominantly by the duration of the disease and degree of metabolic control exercised (WHO, 1980). Major microvascular complications of diabetes include retinopathy, nephropathy and neuropathy. Diabetes is the most common cause of adult blindness in developed countries (Klein and Moss, 1992), either due to retinopathy and cataract or due to glaucoma. Diabetic patients are 17 times more prone to kidney disease and diabetes is now the leading cause of end stage renal disease. Microvascular disease including coronary heart disease, cerebrovascular disease or stroke and peripheral vascular disease are the common causes of morbidity and mortality among people with diabetes.

1.2.3 THE HYPOGLYCAEMIA-DIABETES LINK

Blood sugar problems are strongly associated with the so-called "Western Diet" (Burkitt and Trowell, 1981; Vahouny and Kritchevsky, 1982). This diet is rich in refined sugar, fat, and animal products, and low in dietary fibre. It is widely accepted that refined carbohydrates are among the most important contributing factor.
to diabetes and reactive hypoglycaemia (as well as obesity). Refined sugars are quickly absorbed into the bloodstream causing a rapid rise in blood sugar. The body's response to this is to greatly increase the secretion of insulin by the pancreas. The excessive secretion of insulin drives the blood sugar down and often causes the symptoms of hypoglycaemia to appear.

In response to the rapid fall in blood glucose levels, the adrenal glands secrete epinephrine (adrenaline) which causes a rapid increase in the blood glucose level. In time, the adrenal glands become "exhausted" by the repeated stress and cannot mount an appropriate response. This lack of response leads to reactive hypoglycaemia. If blood sugar control mechanisms are further stressed, the body will eventually become insensitive to insulin or the pancreas will also become "exhausted," and the reactive hypoglycaemia will turn into diabetes.

1.2.4 DIAGNOSIS

1.2.4.1 Fasting blood glucose level

The standard method of diagnosing diabetes involves the measurement of blood glucose levels. The normal fasting blood glucose level is between 70 and 105 mg/dl. A fasting blood glucose measurement greater than 140 mg/dl on two separate occasions is diagnostic of diabetes. Levels below 50 mg/dl indicate fasting hypoglycaemia (Wyngaarden et al., 1992).

1.2.4.2 The glucose tolerance test

A more functional test of blood sugar control is the oral glucose tolerance test (GTT). The GTT is a very sensitive test for diabetes. However, it is also very stressful to the patient and has a relatively low specificity.
### Table 1.4: Glucose Tolerance Test Response Criteria

<table>
<thead>
<tr>
<th>Type</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>No elevation greater than 150mg&lt;br&gt;Below 150 mg at the end of first hour&lt;br&gt;Below 120 mg at the end of second hour</td>
</tr>
<tr>
<td>Flat</td>
<td>No variation more than $\pm$ 20 mg from fasting value</td>
</tr>
<tr>
<td>Pre-diabetic</td>
<td>Over 120 mg at the end of second hour</td>
</tr>
<tr>
<td>Diabetic</td>
<td>Over 180 mg during the first hour&lt;br&gt;200 mg or higher at the end of first hour&lt;br&gt;150 mg or higher at the end of second hour</td>
</tr>
<tr>
<td>Reactive hyperglycaemia</td>
<td>Normal response for the first 2 or 3 hours&lt;br&gt;A decrease of 20 mg or more from the fasting level during the final hours</td>
</tr>
<tr>
<td>Probable reactive hyperglycaemia</td>
<td>An elevation of 20 mg or less&lt;br&gt;Followed by a decrease of 20 mg or more below fasting level</td>
</tr>
<tr>
<td>Pre-diabetic hyperglycaemia</td>
<td>A 2-hour response like the prediabetic&lt;br&gt;Hypoglycaemic response during the final 3 hours</td>
</tr>
<tr>
<td>Hyperinsulinism</td>
<td>A marked hypoglycaemic response, with a value of less than 50 mg during the third, fourth or fifth hour</td>
</tr>
</tbody>
</table>

The National Diabetes Data Group recommends giving a 75 gram glucose dose, dissolved in 300 ml of water, for adults (1.75 g/kg ideal body weight for children) after an overnight fast in subjects who have been consuming at least 150 grams of carbohydrate daily for three days prior to the test. The patient is considered normal if the two-hour plasma glucose is less than 140 mg/dl and no value exceeds 200 mg/dl. A confirmatory diagnosis of diabetes requires that plasma levels be above 200 mg/dl at both two hours and at least once between zero time and two hours. Medications that impair glucose tolerance (diuretics, glucocorticoids, nicotinic acid, and phenytoin) may invalidate the results.
1.2.4.3 The Glucose-Insulin Tolerance Test

Relying on blood sugar levels alone is often not adequate in diagnosing blood sugar disorders. Several studies have shown that the glucose-insulin tolerance test (GITT) is more sensitive in the diagnosis of both hypoglycaemia and diabetes than the standard GTT. The GITT uses a standard 6 hour glucose tolerance test coupled with measurements of insulin levels. The GITT appears to be one of the best diagnostic indicators for faulty sugar metabolism.

As many as two-thirds of subjects are of suspected diabetes or hypoglycaemia that have normal glucose tolerance tests will demonstrate abnormal insulin tolerance tests.

1.2.4.4 Skin Tags

Skin tags (fibroepithelial papillomas) are frequently found in diabetic men. The presence of multiple, large, hyper pigmented, lateral skin tags in a male patient may be suggestive of diabetes and warrants laboratory testing to rule out the disease.

Table 1.5: Glucose Insulin Tolerance Test Response Criteria

<table>
<thead>
<tr>
<th>Pattern</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pattern 1</td>
<td>Normal fasting insulin 0-30 units. Peak insulin at 1/2-1 hour. The combined insulin values for the second and third hours are less than 60 units. This pattern is considered normal.</td>
</tr>
<tr>
<td>Pattern 2</td>
<td>Normal fasting insulin. Peak at 1/2-1 hour with a delayed return to normal. Second and third hour levels between 60-100 units usually is associated with hypoglycaemia and is considered borderline for diabetes, values greater than 100 units considered definite diabetes.</td>
</tr>
<tr>
<td>Pattern 3</td>
<td>Normal fasting insulin. Peak at second or third hour instead of 1/2-1hour. Definite diabetes.</td>
</tr>
<tr>
<td>Pattern 4</td>
<td>High fasting insulin. Definite diabetes.</td>
</tr>
<tr>
<td>Pattern 5</td>
<td>Low insulin response. All tested values for insulin less than 30. When associated with elevated blood sugar levels, it indicates probable insulin dependent diabetes (juvenile pattern).</td>
</tr>
</tbody>
</table>
1.2.5 CAUSES OF DIABETES

1.2.5.1 Etiological Factors in IDDM

Insulin Dependent Diabetes Mellitus is generally recognized to be due to an insulin deficiency (Wyngaarden et al., 1992). Although the exact cause is unknown, current theory suggest a hereditary β-cell predisposition to injury coupled with some defect in tissue regeneration capacity. Causes of injury are most likely hydroxyl and other free radicals, viral infection, and autoimmune reactions. Alloxan, the uric acid derivative used to induce experimental diabetes in animals, is a potent beta-cell toxin, causing destruction via hydroxyl radical formation.

(i) Streptozotocin

The N-nitroso derivative of glucosamine has now replaced alloxan as the preferred agent for destruction of β-cells in the induction of experimental diabetes (Leslie and Elliott, 1994). Circumstantial epidemiologic evidence suggests that dietary intake of the N-nitroso-compounds found in smoked/cured meats is diabetogenic in susceptible individuals, producing beta-cell damage by the same mechanism as streptozocin (Helgason and Johasson, 1981). Many other chemicals, such as rodenticide vector, have also been implicated in β-cell damage (Schroeder et al., 1983).

(ii) Viral Infection

Recent epidemiological and experimental evidence has strengthened the hypothesis of a viral etiology of IDDM in some cases (Leslie and Elliott, 1994 and Schroeder et al., 1983). A viral etiology was initially suspected due to the seasonal variation in the onset of the disease (October to March). During these months, viral diseases, such as mumps, hepatitis, infectious mononucleosis, congenital rubella and Coxsackie’s virus infections are much more prevalent. Viruses are capable of infecting pancreatic β-cells and inducing diabetes.
(iii) Autoimmunity

Autoimmune factors may also be etiological in many cases, especially HLA-B8 individuals. Antibodies to pancreatic cells (all types) are present in 75% of all cases of IDDM compared to 0.5 to 2.0% of normal (Leslie and Elliott, 1994). The antibody levels decline progressively after the first few weeks of the disease, suggesting p-cell destruction and depletion of antigenic stimulus. It is probable that the antibodies to the islet cells develop in response to cell destruction due to other mechanisms (chemical, viral, etc.,) when normally concealed cellular antigens are exposed. It appears that normal individuals either do not develop as severe an antibody reaction, or are more able to repair the damage once it occurs.

(iv) Early Weaning and Cow's Milk Exposure

Recent studies have provided some strong evidence that exposure to a protein in cow's milk (bovine albumin peptide) in neonates may trigger the autoimmune process and subsequent type I diabetes. Although animal and laboratory evidence exists to justify these concerns, studies involving humans and clinical end points have generated conflicting results (Gerstein, 1994). A critical review and analysis of all relevant citations in the medical literature indicated that early cow's milk exposure may, in fact, be an important determinant of subsequent type I diabetes and may increase the risk about 1.5 times (Gerstein, 1994). In case-controlled studies, patients with type I diabetes were more likely to have been breast-fed for less than 3 months and to have been exposed to cow's milk or solid foods before 4 months.

1.2.5.2 Etiological Factors in NIDDM

Central to the pathogenesis of NIDDM is insulin insensitivity as evidenced by typically high levels of circulating insulin and the reversibility of hyperglycaemia by dietary changes and/or weight loss sufficient to restore insulin sensitivity.
(i) Obesity

Obesity plays a major role in the etiology of NIDDM for many patients (Smith, 1984; Hughs et al., 1984; Krotkiewski et al., 1983; Wyngaarden et al., 1992; Campbell and Carlson, 1993). Obesity is associated with insulin insensitivity, and adipose size and distribution also seem to be important.

The heterogeneity of human obesity has resulted in an attempt to identify subgroups. Using cellular criteria, two types have been identified: hypertrophic obesity (enlarged fat cells) and hyperplastic obesity (increased number of fat cells). The hypertrophic form is more closely associated with the metabolic complications associated with obesity, i.e., diabetes, hyperinsulinemia, glucose intolerance, hypertension, hyperlipidemia, etc.

Another system is based on fat distribution and also results in two subgroups: android type (deposition in the upper body, i.e., abdomen, typical in the obese male) and gynecoid type (with distribution in the lower body, i.e., gluteal and femoral, most common in the obese female). Activity of the abdominal adipocyte is affected by metabolic variables, such as plasma insulin and triglyceride levels, while the gluteal and femoral adipocytes are more sensitive to certain steroidal hormones such as corticosteroids and estrogens. The incidence of diabetes is higher in both men and women with the hypertrophic, android-type obesity. Weight loss, in particular a significant decrease in body fat percentage, is a prime objective in treating the majority of NIDDM patients since it improves all aspects of diabetes and may result in cure.

(ii) Dietary Fat

The percentage of calories from fat, especially saturated fat, in the diet has been shown to be associated with NIDDM as well as to predict the conversion from impaired glucose tolerance to NIDDM (Marshall et al., 1991; Marshall, 1994). These results suggest that a high fat, low-carbohydrate diet increases the risk of subsequently developing NIDDM. Ironically, such a diet was used in the past for the dietary management of diabetes.
(iii) Chromium

Considerable experimental and epidemiological evidence now indicates that chromium levels are a major determinant of insulin sensitivity (Riales and Albrink, 1981; Offenbacher and Stunyer, 1980; Mertz, 1969; Levine et al., 1968; Mooradian, 1994; Anderson, 1992). Chromium, an essential micronutrient, functions as a cofactor in all insulin-regulating activities. Its deficiency is widespread throughout the world. Trivalent chromium (Cr³⁺) is the only form of chromium that exhibits biological activity (Mertz, 1969) and is an integral component of the so-called "glucose tolerance factor" (GTF). This low molecular weight compound also contains two molecules of nicotinic acid plus cysteine, glutamine, and glycine. Supplemental chromium has been shown to significantly improve glucose tolerance; decrease fasting glucose, cholesterol and triglyceride levels; and increase the HDL cholesterol level by increasing insulin sensitivity in normal, elderly and NIDDM patients (Mertz, 1969; Levine et al., 1968; Mooradian, 1994; Anderson, 1992). Chromium is not, however, a cure for NIDDM.

(iv) Uremia

Uremia is a potent inhibitor of insulin sensitivity due to a circulating small molecular weight peptide unique to patients with kidney failure. Renal function should be assessed and monitored in all diabetics.

(v) Prenatal Factors

Recent epidemiological evidence supports the concept that prenatal malnutrition, in particular hyperglycaemia, may be a promoter of both types 1 and 2 DM later in life. Studies done in Berlin have shown that adults born during the "hypocaloric war and post-war period (1941-48)" have significantly less DM than those born during the relatively hypercaloric years before and after.

This is not a minor correlation; the data shows a greater than 50% drop in the incidence of DM. Another study shows a significantly lower incidence of childhood diabetes during periods in which maternal hyperglycemia was carefully controlled and the foetus protected from hyperinsulinism (Drner et al., 1984).
Although the data in this study are based on a number of subpositions, they again indicate a greater than 50% drop in the incidence of childhood DM.

1.2.6 MONITOR & CONTROLLING THE DIABETES

There appears to be a causal relationship between hyperglycaemia and the development of the complications of diabetes. Therefore, monitoring and controlling the degree of hyperglycaemia is critical to the prevention of the major diabetic complications. The adequacy of control can be determined by several simple laboratory techniques.

(i) Urinary Glucose

Measurement of urinary glucose is an archaic and insufficient measure of diabetic control since diabetics have a higher renal threshold for glucose than the normal 180 mg/dl.

(ii) Home Glucose Monitoring

The advent of home glucose monitoring, using reagent strips and capillary blood, has brought about major improvement in the care of diabetes. A sample can be obtained by the use of a simple spring-triggered device equipped with a disposable lancet. The best sites are the lateral sides of the distal phalanges (taking care to avoid the nail beds).

Glucose levels are then determined by placing the sample on a reagent strip and measuring the colour change using a commercially available reflectance meter. There are also reagent strips that give satisfactory values by visual inspection using a dual-colour scale.

(iii) Glycosylated Haemoglobin

Proteins that have glucose molecules attached to them (glycosylated peptides) are elevated several fold in diabetics. The measurement of the level of glycosylated haemoglobin (HgbAlc) is used for monitoring blood sugar levels over a long period. Normally about 5 to 7% of haemoglobin is combined with glucose. Mild elevations in blood sugar results in an HgbAlc concentration of 8 to 10%, while

Studies on the Effect of Artificially Cultivated *Ganoderma lucidum* on Streptozotocin Induced Diabetic rats.
severe elevations may result in concentrations up to 20%. Since the average life span of a red blood cell is 120 days, the HgbAlc assay represents time-averaged values for blood glucose over the preceding 2-4 months, thus providing a simple, useful method for assessing treatment effectiveness and patient compliance. HgbAlc determination can also be used in some cases to diagnose diabetes. Although the oral glucose tolerance test is more sensitive than the HgbAlc assay in the diagnosis of diabetes, it is also more stressful to the patient. Because an elevated HgbAlc level almost always indicates diabetes, many physicians will simply measure the HgbAlc level rather than or before subjecting patients, particularly pregnant women, to the stress of a GTT.

In general, measuring glycosylated haemoglobin (and possibly other glycosylated proteins) is an important and useful test for diabetic control and the prevention of Complications.

(iv) Conventional Medical Treatment of IDDM

A type I diabetic requires insulin. Insulin preparations have been used in the treatment of diabetes since 1922. Since insulin is not absorbed orally, it must be injected. Insulin preparations come in concentrations of 100 units per millilitre (U-100) and 500 units per millilitre (U-500), but can differ in their source (beef, pork, or human synthetic insulin), duration of action (rapid, intermediate, and long-acting), and solubility (crystalline versus soluble). The human synthetic insulin is gaining wider acceptance as the preferred source.

Conventional insulin therapy involves administering crystalline insulin, usually a mixture of rapid- and intermediate-acting insulin, once or twice daily. This method is being replaced by intensified insulin therapy where the insulin is given in increasingly sophisticated and complex regimens. Increasing evidence is demonstrating that intensified insulin therapy significantly reduces the development of the chronic complications of diabetes (Amiel, 1993). Intensified insulin therapy is designed to mimic as closely as possible the continuous variations in plasma insulin levels produced by a healthy pancreas.
(v) Conventional Medical Treatment of NIDDM

When type II diabetes cannot be controlled satisfactorily with diet therapy, oral hypoglycaemic agents or, when necessary, insulin is utilized for additional support. Since most type II diabetics are obese and secrete large amounts of insulin each day a trying to overcome the resistance to the action of insulin by the cells of the body, additional insulin is usually of only limited value. While healthy individuals secrete approximately 31 units of insulin daily, the obese type II diabetic secretes an average of 114 units daily. This amount is nearly four times normal.

In contrast to obese type II individuals, lean type II individuals produce about 14 units daily and individuals with type I diabetes secrete only 4 units of insulin daily. If a diabetic is over-producing insulin, it makes more sense to work on increasing the sensitivity to insulin by following the recommendations given below. The other drug treatment of type II diabetes utilizes oral hypoglycaemic agents. These agents are sulphadrugs (sulfonylureas) that appear to stimulate the secretion of additional insulin by the pancreas as well as enhance the sensitivity of body tissues to insulin. Some common examples of this class of drugs are

1. Chlorpropamide (Diabinese)
2. Glipizide (Glucotrol)
3. Glyburide (DiaBeta, Micronase)
4. Glibenclamide
5. Tolazamide (Tolinase)
6. Tolbutamide (Orinase)

As a group, these drugs are not very effective. After three months of continual treatment at an adequate dosage, only about 60% are able to control blood sugar levels using these drugs. Furthermore, these agents generally lose their effectiveness over time. After an initial period of success, these drugs will fail to produce a positive effect in about 30% of cases. The overall rate of achieving adequate control by long-term use of sulfonylureas is 20% to 30% at best. In addition to being of limited value, there is evidence to indicate that these drugs actually produce harmful long-term side effects.

Studies on the Effect of Artificially Cultivated *Ganoderma lucidum* on Streptozotocin induced Diabetic rats.
1.2.7 COMPLICATIONS OF DIABETES

Diabetes gives rise to the development of numerous complications due to hyperglycaemia. The likelihood of developing complications, whether acute or chronic, is ultimately a reflection of the level of blood sugar control. A large body of evidence indicates that good blood sugar control significantly reduces the development of complications. Therefore, as mentioned before, monitoring and controlling the degree of hyperglycaemia is critical to the prevention of the major diabetic complications.

1.2.7.1 Acute Complications

Diabetics are susceptible to three major acute complications:
- Hypoglycaemia,
- Diabetic ketoacidosis (primarily affects type I diabetics), and
- Nonketogenic hyper-osmolar syndrome (primarily affects type II diabetics).

(a) Hypoglycaemia

The problem of hypoglycaemia is much more common in type I than type II because the type I diabetic is injecting insulin. Taking too much insulin, missing a meal, or over-exercising can result in hypoglycaemia. It is imperative that a good relationship exists between the patient and the physician prescribing the insulin so that dosages can be gauged correctly.

Daytime hypoglycaemic episodes are usually recognized by the following symptoms: sweating, nervousness, tremor, and hunger. Night time hypoglycaemia may be without symptoms or manifest as night sweats, unpleasant dreams, or early morning headache. In response to the hypoglycaemia, secretion of several hormones which raise blood glucose levels is increased: epinephrine, nor epinephrine, growth hormone and cortisol. As a result, blood sugar levels will rebound and often lead to hyperglycaemia.

This phenomenon is commonly referred to as the Somogyi phenomenon. It is very important to recognize this response when monitoring insulin needs. The Somogyi phenomenon should be suspected whenever there are wide swings in blood
glucose over short periods of time during the day. For example, blood glucose of 70 mg/dl before breakfast and 400 mg/dl before lunch suggest early morning hypoglycaemia with post-breakfast hyperglycaemia due to increased counter regulatory hormone activity. The advent of the improved insulin therapy, the insulin pump, and home glucose monitors have led to better blood sugar control and a decreased frequency of the Somogyi phenomenon.

(b) Diabetic Ketoacidosis

Another acute complication more likely to occur in the type I diabetic is ketoacidosis, a condition caused by a lack of insulin leading to a build-up of ketoacids. If progressive, ketoacidosis can result in numerous metabolic problems and even coma. Since ketoacidosis is potentially a medical emergency, prompt recognition is imperative. The coma is usually preceded by a day or more of increased urination and thirst as well as marked fatigue, and nausea and vomiting. A simple urine dipstick can be used at home by diabetics to measure the level of ketones in the urine.

(c) Non-ketogenic Hyperosmolar Syndrome

With a mortality rate of over 50%, non-ketogenic hyperosmolar syndrome constitutes a true medical emergency. It is usually the result of profound dehydration secondary to deficient fluid intake or precipitating events such as pneumonia, burns, stroke, a recent operation, or certain drugs such as phenytoin, diazoxide, glucocorticoids and diuretics. The onset of the syndrome may be insidious over a period of days or weeks, with symptoms of weakness, increased urination and thirst, and progressively worse signs of dehydration (weight loss, loss of skin elasticity, dry mucous membranes, rapid heart beat, and low blood pressure).

1.2.7.2 Chronic Complications

On a long-term basis, the diabetic's health condition is complicated by repeated elevations in blood glucose levels. Seven major chronic complications of diabetes are

- Atherosclerosis,
• Diabetic retinopathy,
• Diabetic neuropathy,
• Diabetic nephropathy, and
• Diabetic foot ulcers.

There are two primary mechanisms behind the development of most chronic complications of diabetes.

• **Glycosylated proteins and**
• **Intracellular accumulation of sorbitol.**

**(i) Glycosylated Proteins**

The binding of glucose to proteins, a process referred to as glycosylation, leads to changes in the structure and function of many body proteins. For example, glycosylated LDL molecules (found in high levels in diabetics) do not bind to LDL-receptors or shut off endogenous cholesterol synthesis. In diabetes, excessive glycosylation also occurs with the proteins of the red blood cell, lens and myelin sheath.

Excessive non-enzymatic glycosylation has many adverse effects: inactivation of enzymes, inhibition of regulatory molecule binding, cross linking of glycosylated proteins, trapping of soluble proteins by glycosylated extra cellular matrix, decreased susceptibility to proteolysis, abnormalities of nucleic acid function, altered macromolecular recognition, and increased immunogenicity (Brownlee et al., 1984).

**(ii) Sorbitol**

Sorbitol is a by-product of glucose metabolism formed within the cell through the action of aldose reductase. In non-diabetics, once sorbitol is formed it is metabolized by polyol dehydrogenase to fructose. This conversion to fructose allows the sorbitol to be excreted from the cell.
Unfortunately, in the diabetic with frequent hyperglycaemia, sorbitol accumulates and plays a major role in the development of the chronic complications of diabetes. The mechanism by which sorbitol is involved in the development diabetic complications is best understood by considering its involvement in cataract formation. Although the lens does not have any blood vessels, it is an actively metabolizing tissue that continuously grows throughout life. Hyperglycaemia results in shunting of glucose to the sorbitol pathway. Since the lens membranes are virtually impermeable to sorbitol and lack the enzyme polyol dehydrogenase, sorbitol accumulates to high concentrations which persist even if glucose levels return to normal.

This accumulation creates an osmotic gradient that draws water into the cells to maintain osmotic balance. The cells also release small molecules like amino acids, inositol, glutathione, niacin, vitamin C, magnesium and potassium to maintain osmotic balance. Since these latter compounds function to protect the lens from

Fig. 1.1 The Production of Polyols

\[
\begin{align*}
\text{Galactose} & \xrightarrow{\text{gak}} \text{Galactose-1-phosphate} \\
\text{Galactitol} & \xrightarrow{\text{ar}} \text{Glucose-1-phosphate} \rightarrow \text{Glycogen} \\
\text{Glucose} & \xrightarrow{\text{glk}} \text{Glucose-6-phosphate} \rightarrow \text{Pentose phosphate shunt} \\
\text{Sorbitol} & \xrightarrow{\text{pd}} \text{D-fructose-1-6-phosphate} \rightarrow \text{Glycolysis} \\
\text{Fructose} & \xrightarrow{\text{frk}} \text{Fructose-1-phosphate} \rightarrow \text{(multiple steps)} \\
\end{align*}
\]

ar  Aldose reductase
frk  Fructokinase
glk  Glucokinase
pd  Polyol dehydrogenase
damage, their loss results in increased susceptibility to damage. As a result, the
delicate protein fibres within the lens become opaque and a cataract forms.
Intracellular accumulation of polyols is a major factor in the development of the
majority of the complications of DM.

Elevated sorbitol levels are found in high concentrations in the tissues
commonly involved in the major diabetic complications: the lens epithelium, the
Schwaan cell of the peripheral nerve, the papilla in the kidney, the islets of
Langerhans in the pancreas, and the mural cell of the retinal blood vessels.
Measurement of the erythrocyte sorbitol concentration may become a valuable
indicator of diabetic control, since RBC and nerve cell sorbitol concentrations
correlate well (Gegersen et al., 1983).

(iii) Atherosclerosis

The diabetic has a two to three fold higher risk of dying prematurely of
atherosclerosis than a non-diabetic individual. Therefore, the physician and
diabetic patient must be aggressive in reducing the risk factors linked to heart attacks
and strokes. Foremost is the reduction of LDL-cholesterol levels and triglycerides
while increasing HDL-cholesterol levels. Recommended blood cholesterol and
triglyceride levels are

<table>
<thead>
<tr>
<th>Test</th>
<th>Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>Less than 200 mg/dl</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>Less than 130 mg/dl</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>Greater than 35 mg/dl</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>50 to 150 mg/dl</td>
</tr>
</tbody>
</table>

The most important approach to lowering a high cholesterol level is to
follow a healthy diet and lifestyle. The dietary recommendations are
straightforward: instruct the patient to eat less saturated fat and cholesterol by
reducing or eliminating the amounts of animal products in the diet; increase the
consumption of fibre-rich plant foods (fruits, vegetables, grains, and legumes); and
lose weight, if necessary. The lifestyle changes include getting regular aerobic
exercise, stopping smoking, and reducing or eliminating the consumption of coffee (both caffeinated and decaffeinated).

(iv) Diabetic Neuropathy

Diabetic neuropathies are among the most frequent complications of long-term diabetes. Loss of peripheral nerve function, tingling sensations, and numbness, loss of function, pain, and muscle weakness may occur as a result of diabetic retinopathies.

Occasionally, the neuropathy can affect deeper nerves and result in impaired heart function, alternating bouts of diarrhoea and constipation, inability to empty the bladder, and impotence. The earliest and best measured signs of diabetic neuropathy are decreased sensory and nerve conduction velocities.

There is substantial evidence that diabetic neuropathy is also due to sorbitol accumulation (Wyngaarden et al., 1992 and Cogan et al., 1984). In rats, increasing the sorbitol concentration in the sciatic nerve is directly related to decreasing nerve conduction velocity, possibly as a result of decreased myoinositol concentrations (Yue et al., 1984). Sorbitol accumulation leads to myoinositol loss. Although some studies have shown inositol supplementation to improve nerve conduction velocity, addressing the underlying accumulation of sorbitol is of greater importance (Gegersen et al., 1983).

Diabetic neuropathy is usually limited to the peripheral nerves with the most common being peripheral polyneuropathy (Wyngaarden et al., 1992; Campbell and Carlson, 1993). Typically bilateral, this condition is characterized by paresthesias, severe hyperesthesias and pain. The neurological exam reveals dulled perception of vibration, pain and temperature, particularly in the lower extremities. Bilateral atrophy of the first interosseous muscle of the hand is characteristic.

Nerve conduction is delayed and there may be a delayed response of the Achilles reflex similar to that seen in hypothyroidism. Occasionally, autonomic neuropathy is present in diabetes. This can present in a variety of ways, such as postural hypotension, decreased cardiovascular response to the Valsalva maneuver.
resting tachycardia, alternating bouts of diarrhoea and constipation, inability to empty the bladder, and impotence.

(v) Diabetic Retinopathy

Diabetic retinopathy is a serious eye disease that can result in blindness. In fact, diabetic retinopathy is still the leading cause of blindness in the India. One in 20 type I and one in 15 type II diabetics develop retinopathy (Wyngaarden et al., 1992). The retinopathic lesions are divided into background or "simple" retinopathy (consisting of micro aneurysms, haemorrhages, exudates and retinal oedema) and proliferative or "malignant" retinopathy (with newly formed vessels, scaring, retinitis proliferens, vitreous haemorrhage, and retinal detachment). The development of laser photocoagulation therapy will probably reduce the prevalence of diabetes-induced blindness.

(vi) Diabetic Nephropathy

This is a common complication and a leading cause of death in diabetes (Wyngaarden et al., 1992). Four types of possibly overlapping lesions develop: glomerulosclerosis arteriosclerosis of the efferent and afferent arterioles; Arteriosclerosis of the renal artery and its intra-renal branches; and peritubular deposits of glycogen, fat and muco-polysaccharides. The glomerular lesions contain of oedema, hypertension, proteinuria, and renal failure seen as a complication of diabetes. Periodic monitoring of a diabetic patient's kidney function (BUN, uric acid, creatinine and creatinine clearance) is important.

(vii) Diabetic Foot Ulcers

Ischemia and peripheral neuropathy are the key factors in the development of diabetic foot ulcers. The incidence of gangrene of the feet is 20 times that of matched controls (Campbell and Carlson, 1993). However, foot ulcers are largely preventable through proper foot care, the avoidance of injury and tobacco in any form, and employing methods to improve local circulation. Proper foot care includes keeping the feet clean, dry and warm and wearing only well fitted shoes. Circulation can be improved by avoiding sitting with the legs crossed or in other
positions that compromise circulation, and by massaging the feet lightly upwards and carefully applying hydrotherapy

1.2.8 DIETARY TREATMENT OF DIABETES

Dietary modification and treatment is fundamental to the successful treatment of diabetes, whether it be type I or II. Since diabetics have a higher incidence of death from cardiovascular disease (60-70% versus 20-25% in matched non-diabetic controls) the incidence of diabetes is highly correlated with the fibre-depleted, high-refined carbohydrate diet of "civilized" man (Burkitt and Trowell, 1981). Re-establishing a traditional diet and lifestyle reverses the carbohydrate and lipid metabolism abnormalities associated with the "foods of commerce" and eventually results in a low prevalence of diabetes. The epidemiological evidence indicting the Western diet and lifestyle as the ultimate etiological factor in diabetes is overwhelming (Burkitt and Trowell, 1981 and National Research Council, 1989).

The diet recommends that high in cereal grains, legumes and root vegetables and restricts simple sugar and fat intake. It is called the high-complex carbohydrate, high-fibre diet or HCF diet for short. Clinical trials of dietary treatment with a more primitive diet high in plant cell-wall materials and complex carbohydrates and low in fat and animal products have consistently demonstrated superior therapeutic effects over oral hypoglycaemic agents, insulin (when less than 30 units per day), and other previously recommended dietary regimes (carbohydrate restriction, high protein and the ADA diet) (Anderson, 1988; Anderson and Ward, 1979; Anderson, 1977; Kay et al., 1981; Simpson et al., 1981 and Jenkins et al., 1980).

(i) The Glycemic Index

The "glycemic index" was developed by Jenkins to measure the rise of blood glucose after eating a particular food (Jenkins et al., 1981). The standard value of 100 is based on the rise seen after the ingestion of glucose. The glycemic index ranges from about 20 for fructose and whole barley to about 98 for a baked potato. The insulin response to carbohydrate-containing foods is similar to the rise in blood sugar. The glycemic index is used as a guideline for dietary recommendations for
people with either diabetes or hypoglycaemia. Basically, people with blood sugar problems are advised to avoid foods with high values and choose instead carbohydrate-containing foods with lower values.

However, the glycemic index should not be the only dietary guideline. For example, while high fat foods like ice cream and sausage may have a low glycemic index because a diet high in fat has been shown to impair glucose uptake, these foods are not good food choices for people with hypoglycaemia or diabetes.

(ii) Fruits and Fructose

Many physicians have recommended that individuals with diabetes or hypoglycaemic avoid fruits and fructose. However, recent research challenges this approach. Fructose does not cause a rapid rise in blood sugar levels. Because fructose must be changed to glucose in the liver in order to be utilized by the body, blood glucose levels do not rise as rapidly after fructose consumption compared to other simple sugars. While most diabetics and hypoglycaemic cannot tolerate sucrose, most can tolerate moderate amounts of fruits and fructose without loss of blood sugar control.

In fact, fructose and fruits are not only much better tolerated than white bread and other refined carbohydrates, they produce less sharp elevations in blood sugar levels compared to most sources of complex carbohydrates (starch) (Koivisto and Yki-Jarvinen, 1993). As a bonus, fructose has actually been shown to enhance the sensitivity to insulin by 34% when fed to non-insulin dependent diabetics over four weeks.

(iii) Nutritional Supplements

The treatment of diabetes requires nutritional supplementation, as these patients have a greatly increased need for many nutrients. Supplying the diabetic with additional key nutrients has been shown to improve blood sugar control as well as help prevent or ameliorate many of the major complications of diabetes. Good blood sugar control combined with nutritional supplementation will go a long way in helping prevent many of the major complications of diabetes.
(iv) Chromium

Chromium is vital to proper blood sugar control as it functions as a key constituent of the "glucose tolerance factor". Chromium works closely with insulin in facilitating the uptake of glucose into cells. Without chromium, insulin's action is blocked, and glucose levels are elevated (Mooradian, 1994). In some clinical studies in diabetics, supplementing the diet with chromium has been shown to decrease fasting glucose levels, improve glucose tolerance, lower insulin levels, and decrease total cholesterol and triglyceride levels, while increasing HDL-cholesterol levels (Mooradian, 1994; Anderson, 1992).

Although some studies have not shown chromium to exert much effect in improving glucose tolerance in diabetes, there is no doubt that it is an important mineral in blood sugar metabolism. Reversing a chromium deficiency by supplementing the diet with chromium has also been demonstrated to lower body weight while increasing lean body mass.

All of the effects of chromium appear to be due to increased insulin sensitivity. A chromium deficiency may be an underlying contributing factor to the large number of Americans suffering from diabetes, hypoglycaemia, and obesity. Chromium levels can be depleted by refined sugars, white flour products, and lack of exercise. In addition to the regular consumption of chromium rich foods, the diabetic and hypoglycaemic should supplement the diet with 200-400 meg of chromium. Chromium polynicotinate, chromium picolinate, and chromium-enriched yeast are the suitable forms for supplementation.

(v) Vitamin C

A primary function of vitamin C is the manufacture of collagen, the main protein substance of the human body. Since collagen is such an important protein for connective tissue, vitamin C is vital for wound repair, healthy gums, and the prevention of excessive bruising. In scurvy or severe vitamin C deficiency, the classic symptoms are bleeding gums, poor wound healing, extensive bruising, and increased susceptibility to infection, hysteria, and depression. In addition to its role in collagen metabolism, vitamin C is also critical to immune function, the
manufacture of certain nerve transmitting substances and hormones, and the absorption and utilization of other nutritional factors.

Since the transport of vitamin C into cells is facilitated by insulin, many diabetics do not have enough intracellular vitamin C. Therefore, a relative vitamin C deficiency exists in many diabetics despite adequate dietary consumption (Cunningham, 1991). A chronic, latent vitamin C deficiency will lead to a number of problems for the diabetic including an increased tendency to bleed (increased capillary permeability), poor wound healing, microvascular disease, elevations in cholesterol levels, and a depressed immune system. Vitamin C at high doses (2,000 mg/day) has been shown to reduce the accumulation of sorbitol in the erythrocytes of diabetics and to inhibit the glycosylation of proteins (Davie et al., 1992; Vinson, 1989).

(vi) Niacin and Niacinamide

Niacin (vitamin B₃)-containing enzymes play an important role in energy production; fat, cholesterol, and carbohydrate metabolism; and the manufacture of many body compounds including sex and adrenal hormones. Niacin, like chromium, is an essential component of the glucose tolerance factor making it a key nutrient for hypoglycaemia and diabetes (Urberg et al., 1987).

Supplementing the diet of diabetics with niacin in the form of niacinamide has been shown to exert many favourable effects. Foremost is its possible application in preventing the development of type I diabetes (Pocoit et al., 1993; Cleary, 1990; Pozzilli and Andreani, 1993; Mandrup Paulsen, 1993). Niacinamide, also called nicotinamide, has been shown to prevent the development of diabetes in experimental animals. This observation led to several pilot clinical trials which suggest that niacinamide can prevent type I diabetes from developing, or, if given soon enough at the onset of diabetes, help restore beta-cells or at least slow down their destruction. Some newly-diagnosed type I diabetics have experienced complete resolution of their diabetes with niacinamide supplementation (Schroeder et al., 1983).
(vii) Biotin

Biotin functions in the manufacture and utilization of carbohydrates, fats and amino acids. Since biotin is manufactured in the intestines by gut bacteria, it is not often discussed as a needed nutrient. A vegetarian diet has been shown to alter the intestinal bacterial flora in such a manner as to enhance the synthesis and promote the absorption of biotin. Biotin supplementation has been shown to enhance insulin sensitivity and increase the activity of the enzyme glucokinase, the enzyme responsible for the first step in the utilization of glucose by the liver.

Glucokinase concentrations in diabetics are very low. In one study, 16 mg of biotin per day resulted in significant lowering of fasting blood sugar levels and improvements in blood glucose control in type I diabetics (Reddi et al., 1988). In a study in type II diabetics, similar effects were noted with 9 mg of biotin per day (Maebashi et al., 1993). If high-dose biotin is used in type I diabetics, insulin requirements must be adjusted.

(viii) Vitamin B6 & B12

Vitamin B₆ supplementation appears to offer significant protection against the development of diabetic neuropathy as diabetics with neuropathy have been shown to be deficient in vitamin B₆ and benefit from supplementation (Jones and Gonzalez, 1978). Individuals with long standing diabetes or who are developing signs of peripheral nerve abnormalities should definitely be supplemented with vitamin B₆. The standard dose for this application is 150 mg. It is interesting to note that the neuropathy of a vitamin B₆ deficiency is indistinguishable from diabetic neuropathy.

Vitamin B₆ may also prove to be important in preventing other diabetic complications because it inhibits glycosylation of proteins (Solomon and Cohen, 1989). Vitamin B₆ supplementation should be tried as a safe treatment for gestational diabetes. In one study of 14 women with gestational diabetes, taking 100 mg of vitamin B₆ for two weeks resulted in eliminating the diagnosis in 12 of the 14 women (Coelingh-Bennick and Schreurs, 1975).
A vitamin B₁₂ deficiency is characterized by numbness of the feet, pins and needles sensations, or a burning feeling, i.e., symptoms typical of diabetic neuropathy (Bhatt et al., 1983). Vitamin B₁₂ supplementation has been used with some success in treating diabetic neuropathy. It is not clear if this is due to the correcting of a deficiency state or the normalization of the deranged vitamin B₁₂ metabolism seen in diabetics (Bhatt et al., 1983).

(ix) Vitamin E

Diabetics appear to have an increased requirement for vitamin E. High dose vitamin E (900 IU) not only improves insulin action, but also exerts a number of beneficial effects that may aid in preventing the long-term complications of diabetes (Paolisso, 1993).

(x) Magnesium & Potassium

Magnesium is involved in several areas of glucose metabolism and there is considerable evidence that diabetics need supplemental magnesium. Magnesium deficiency is common in diabetics and magnesium may prevent some of the complications of diabetes like retinopathy and heart disease (White and Campbell, 1993). Potassium supplementation yields improved insulin sensitivity, responsive insulin secretion; insulin administration induces a loss of potassium; and a high potassium intake reduces the risk of heart disease, atherosclerosis, and cancer (Norbiato et al., 1984; Khaw and Barrett-Connor, 1984). Most diabetics can consume a high potassium diet, but their kidney function should be properly evaluated before prescribing a potassium supplement.

(xi) Manganese & Zinc

Manganese is a cofactor in many enzyme systems involved in blood sugar control, energy metabolism, and thyroid hormone function. In guinea pigs, a deficiency of manganese results in diabetes and the frequent birth of offspring who develop pancreatic abnormalities or have no pancreas at all. Diabetics have been shown to have only one-half the manganese of normal individuals. A good daily dose of manganese for a diabetic is 30 mg.
Zinc is involved in virtually all aspects of insulin metabolism, synthesis, secretion and utilization. Zinc also has a protective effect against p-cell destruction and has well documented anti-viral effects. Diabetics typically excrete excessive amounts of zinc in the urine and therefore require supplementation (Mooradian and Motley, 1987), which has been shown to improve insulin levels in both type I and type II diabetes (Hegazi, 1992). In addition, zinc helps improve the poor wound healing observed in diabetics (Engal et al., 1981)

(xii) Flavonoids

Flavonoids appear to normalize the body's reaction to allergens, viruses, and carcinogens as evidenced by their anti-inflammatory, anti-allergic, anti-viral, and anticarcinogenic properties (Chu, 2000; Koleva et al., 2002; Mantle et al., 2000; Oke and Hamburger, 2002). Recent research also suggests that flavonoids may be useful in diabetes. Flavonoids, such as quercetin, promote insulin secretion and are potent inhibitors of sorbitol accumulation. These effects may help explain the favourable effects of many botanical medicines, many of which are high in flavonoids, traditionally used in the treatment of diabetes.

The nutritional effects of flavonoids include: the increase of intracellular vitamin C levels (Lyawe et al., 2006), a decrease in the leakiness and breakage of small blood vessels, the prevention of easy bruising, and immune system support, all of which are of benefit in diabetes. In addition to consuming a diet rich in flavonoids, the diabetic should take an extra 1-2 grams of mixed flavonoids per day.

(xiii) Carnitine

Carnitine supplementation of diabetic patients has resulted in significantly decreased total serum lipid and increased HDL-cholesterol levels (Abdel-Aziz et al., 1984). In addition, it increases d-oxidation of fatty acids, possibly playing a role in preventing diabetic ketoacidosis.

(xiv) Inositol

Inositol supplementation has shown some success in the treatment of experimental animal diabetic neuropathy since it helps re-establish normal
myoinositol levels in the deficient neuron (Leslie and Elliott, 1994). The neuronal myoinositol deficiency is believed to be due to a combination of glucose competition with myoinositol for active transport and accumulation of sorbitol within the cell resulting in the loss of intracellular myoinositol. Oral supplementation in human diabetics has not, however, resulted in significant clinical improvement (Gegersen et al., 1983).

(xv) Botanical Medicines

Before the advent of insulin, diabetes was treated with plant medicines. In 2000, the WHO urged researchers to examine whether traditional medicines produced any beneficial clinical results. In the last 10 to 20 years, scientific investigation has, in fact, confirmed the efficacy of many of these preparations, some of which are remarkably effective. Covered here are those plants which appear most effective, are least toxic, and have substantial documentation of efficacy. Even though the herbs discussed possess blood sugar lowering effects, proper and effective natural treatment of the diabetic patient requires the careful integration of diet, nutritional supplements, lifestyle, and botanical medicine.

The treatment of DM is based on oral anti-hyperglycaemic agents and insulin. However, DM is also treated in Indian traditional medicine using anti-diabetic medicinal plants (Nagarajan et al., 1987). The oral anti-hyperglycaemic agents currently used in clinical practice have characteristic profiles of serious side effects (Pickup and Williams, 1991). This leads to increasing demand for herbal products with anti-diabetic activity and less side effects.

(xvi) Exercise

An appropriate exercise training program is vitally important in a DM treatment plan (Koivisto and Yki-Jarvinen, 1993; Cunningham, 1991; Davie et al., 1992). Exercise improves many parameters and is indicated in both IDDM and NIDDM. Physically trained diabetics experience many benefits: enhanced insulin sensitivity with a consequent diminished need for exogenous insulin, improved glucose tolerance, reduced total serum cholesterol and triglycerides with increased HDL levels that result in a more anti-atherogenic state and, in obese
diabetics, improved weight loss (Koivisto and DeFronzo, 1984; Selby et al., 1987 and Pollack et al., 1984).

However, a physical fitness program does present some risk to the diabetic and must be carefully adapted to the fitness of the patient. Exercise should be avoided during periods of hypoglycaemia. Besides its well-known and documented value, exercise may have a more specific beneficial effect for diabetics: exercise increases tissue levels of chromium (in rats) (Vallerand et al., 1984) and increases the number of insulin receptors in IDDM patients (Pedersen et al., 1980). It is possible, then, that many of the beneficial effects of exercise are directly related to improved chromium metabolism.

1.2.9 RESEARCH

Recent years have witnessed a renewed interest in plants as pharmaceuticals because they synthesize a variety of secondary metabolites with antioxidant potential which can play a major role in protection against molecular damage induced by reactive oxygen species (ROS) (Cao et al., 1997; Vaya et al., 1997). Hundreds of plants are used traditionally for the management of Diabetes mellitus. To date, however, only a few of these medicinal plants have received scientific scrutiny, despite the fact that the World Health Organization has recommended that medical and scientific examinations of such plants should be undertaken (WHO, 1980).