REVIEW OF LITERATURE

Diabetes Mellitus

**Definition:** Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels [23].

**Epidemiology:** Diabetes mellitus is pandemic in both developed and developing countries. Worldwide, 3.2 million diabetes-related deaths are reported annually, a number equivalent to that of HIV/AIDS-related deaths. In India, this increase is estimated to be 58%, from 51 million people in 2010 to 87 million in 2030 [4]. Global Morbidity and Mortality associated with Diabetes is close to four million deaths in the age group of 20-79 years in 2010 (International Diabetes Federation (IDF) Report 2009) [5]. The prevalence of type 2 diabetes is increasing in epidemic proportions worldwide. The long-term complications associated with diabetes are major causes of morbidity and mortality, imposing a high financial burden on health care costs.

Once considered primarily as a risk factor for heart disease, diabetes has now become a high profile public health concern in its own right, due to the escalating epidemic of diabetes in older people, and the emergence of type 2 diabetes in children. With a high genetic predisposition and the high susceptibility to the environmental insults, the Indian population faces a high risk for diabetes and its associated complications. Early diagnosis of high-risk groups and appropriate intervention by lifestyle modification may be the solution to reduce disease burden and associated complications [24].

**History:** The term diabetes was coined by Aretaeus of Cappadocia meant "one that straddles," Diabetes is first recorded in English, in the form diabete, in a medical text written around 1425. Sushruta (6th century BCE) identified diabetes and classified it as Madhumeha [24]. He further identified it with obesity and sedentary lifestyle, advising exercises to help "cure" it [25]. In medieval Persia, Avicenna (980-1037) provided a detailed account on diabetes mellitus in The Canon of Medicine, describing the abnormal appetite and the collapse of sexual functions and also documented the sweet taste of diabetic urine. He also described diabetic gangrene and treated diabetes using a mixture of lupine, Trigonella (fenugreek), and zedoary seed, which produces a considerable reduction in the excretion of sugar, a treatment which is still prescribed in modern times [26].

The first oral hypoglycemic agents suitable for clinical use were the sulphonylureas, developed by Auguste Loubatieres in the early 1940s; carbutamide was introduced in 1955 and tolbutamide in 1957. The biguanide phenformin became available in 1959, and Metformin in 1960 [27]. In 1997, Thiazolidinediones were introduced as the second major class of insulin sensitizers. Among these Troglitazone was withdrawn because of hepatotoxicity [28]. There are several other groups of antidiabetic drugs now such as Glitkins, GLP-1 analogs, SGLT2 inhibitors and Alpha-glucosidase inhibitor.

**Classification of diabetes mellitus [29]:** Revised classification suggested by the expert committee on the diagnosis and the classification of diabetes (constituted by American Diabetes Association) and the World Health Organization (WHO) is given below:

1. **Type 1 diabetes** - (previously known as Insulin Dependent Diabetes Mellitus or juvenile onset diabetes) cell destruction, usually leading to absolute insulin deficiency. This form accounts for 5 – 10% of all the cases.

   **Type 1A:** - Immune-mediated diabetes mellitus (the vast majority)
Here there is a cell-mediated autoimmune destruction of pancreatic islet beta cells, resulting in failure of the pancreas to produce insulin, due to absolute lack of insulin.

**Type 1B:** - Idiopathic

**II. Type 2 diabetes** - (may range from predominantly insulin resistance with relative insulin deficiency to a predominantly insulin secretory defect with insulin resistance)

**III. Other specific types of diabetes**

**A. Genetic defects of cell function characterized by mutations in:**
1. Hepatocyte nuclear transcription factor (HNF) 4α (MODY 1)
2. Glucokinase (MODY 2)
3. Hepatocyte nuclear transcription factor -1α (MODY 3)
4. Insulin promoter factor-1 (IPF-1; MODY 4)
5. Hepatocyte nuclear transcription factor -1β (MODY 5)
6. NeuroD1 (MODY 6)
7. Mitochondrial DNA
8. Subunits of ATP-sensitive potassium channel
9. Proinsulin or insulin conversion

**B. Genetic defects in insulin action:**
1. Type A insulin resistance
2. Leprechaunism
3. Rabson-Mendenhall syndrome
4. Lipodystrophy syndromes

**C. Diseases of the exocrine pancreas**- pancreatitis, pancreatectomy, neoplasia, cystic fibrosis, hemochromatosis, fibrocalculous pancreatopathy, mutations in carboxyl ester lipase.

**D. Endocrinopathies** - acromegaly, Cushing’s syndrome, glucagonoma, pheochromocytoma, hyperthyroidism, somatostatinoma, aldosteronoma

**E. Drug or chemical induced**- Vacor, pentamidine, nicotinic acid, glucocorticoids, thyroid hormone, diazoxide, -adrenergic agonists, thiazides, phenytoin, -interferon, protease inhibitors, clozapine

**F. Infections**— congenital rubella, cytomegalovirus, Coxsackie

**G. Uncommon forms of immune-mediated diabetes**"stiff-person" syndrome, anti-insulin receptor antibodies

**H. Other genetic syndromes sometimes associated with diabetes** - Down's syndrome, Klinefelter's syndrome, Turner's syndrome, Wolfram’s syndrome, Friedreich’s ataxia, Huntington's chorea, Laurence-Moon-Biedl syndrome, myotonic dystrophy, porphyria, Prader-Willi syndrome

**IV. Gestational diabetes mellitus (GDM):** It is glucose intolerance that is first identified during pregnancy. It is diagnosed approximately in 7% of all pregnancies in the U.S. the elevated level of human placental lactogen (HPL) raise insulin resistance [30]. Impaired glucose tolerance (IGT) and impaired fasting glucose (IFG) are risks for diabetes. These conditions are also referred to as prediabetes as 25% of affected individual eventually develop type 2 DM.

**Etiology and pathophysiology of type 1 diabetes mellitus [31]:**
Type 1 diabetes patients are usually young children or adolescents and not obese when they first develop symptoms.
**Primary causes of type 1 diabetes:**
Genetic factors: It has a less genetic predisposition and involves multiple genes. The genes for type 1 DM is located in Human leukocyte antigen (HLA) region on chromosome 6. This region contains 11 genes that code the class 2 major histocompatibility complex (MHC) molecules, which present antigen to T helper cells and are then involved in initiating an immune response. Most individuals have HLA DR3, HLA DR4, and HLA DQ genes.

Environmental factors: Includes viral infection with Coxsackie virus or Echovirus, which may damage pancreatic beta cells and exposes antigen [32].

Autoimmunity: Autoantibodies to islets cells, glutamic acid decarboxylase (GAD), GAD 65 & 67, proinsulin, carboxypeptidase H, 2 ganglioside antigens (GT3 & GM 2-1)ICA 69 and ICA 512. Cells involved in attachment of beta cell include natural killer cells activated cytotoxic T lymphocytes (CD8+) and macrophages. Cell destruction may be due to the release of cytokines, such as IL-1 and TNF-alpha from activated macrophages. Lymphocytic rich inflammatory infiltrate (insulitis) is frequently observed in the early course of the clinical manifestations [33].

**Secondary causes for type 1 diabetes:** Are pancreatitis, pancreatectomy and neoplastic diseases of pancreas, hemochromatosis and cystic fibrosis.
Pathogenesis of type 1 diabetes mellitus [34]:

**Figure 1: Pathogenesis of type I Diabetes**

Etiology and pathophysiology of type 2 diabetes mellitus

Usually presents in adult life. Affected individuals always have a family history, particularly on the maternal side. Type 2 diabetes historically affects people over the age of 40 (but is now seen as young as 3 years old), many of whom are obese and autoimmune destruction of pancreatic islet beta cells does not occur. Less than 1/3 of these patients will ultimately require insulin therapy. Treatment is initially diet, exercise and weight loss that improves insulin resistance and occasionally appears to reverse type 2 diabetes in some obese people.

**Primary causes of type 2 diabetes:-**

Genetic factors: - has a strong genetic predisposition. 60-80 % is seen in identical twins. Individuals with a parent with type2 diabetes have an increased risk of diabetes; if both parents have type 2 diabetes the risk approaches 40%. Various genetic loci contribute to susceptibility and environmental factors (such as nutrition and physical activity) further modulate the phenotypic expression of the disease [35-37].

Environmental factors: obesity and sedentary lifestyle is an extremely important environmental influence in type 2 DM.

Type 2 Diabetes mellitus is characterized by three pathophysiologic abnormalities:

1. Impaired insulin secretion: due to an abnormality in glucose transporters (GLUT2)on beta cells, they do not respond to the high concentration of glucose in the blood.
2. Peripheral insulin resistance: down-regulation of insulin due to obesity and genetic factors.
3. Excessive hepatic glucose production.
Obesity particularly visceral or central as evidenced by the waist-hip ratio is very common in type 2 DM. Adipocytes secrete a number of biological products (leptin, TNF-alpha, free fatty acids, resistin, and adiponectin) that modulate insulin secretion, insulin action and body weight and may contribute to the insulin resistance.

In the early stages of the disorder, glucose tolerance remains normal, despite insulin resistance, because the pancreatic beta cells compensate by increasing insulin output. As insulin resistance and compensatory hyperinsulinemia progress, the pancreatic islets in certain individuals are unable to sustain the hyperinsulinemic state. Impaired glucose tolerance characterized by elevations in postprandial glucose, then develops. A further decline in insulin secretion and an increase in hepatic glucose production lead to overt diabetes with fasting hyperglycemia [38].

**Secondary causes of Type2 DM:**
Secondary causes of Type2 DM are due to excessive production of endogenous hormonal antagonists to insulin-like growth hormone, corticosteroids, thyroid hormone, catecholamines and glucagon.

**Pathogenesis of NIDDM:**

![Pathogenesis of type II Diabetes](image)

The earliest manifestation in Type 2 DM is loss of regular periodicity of insulin secretion. At diagnosis virtually all persons with Type 2 DM have a profound defect in first phase insulin secretion in response to glucose challenge. The responses to other secretagogues (ex: - isoproterenol or arginine) are preserved although there is less potentiation by glucose. These abnormalities of beta cells are secondary to desensitization by chronic hyperglycemia. Patients with FBS of 108-180 mg/dl have fasting and stimulated insulin
values equal to those of euglycemic control subjects i.e hyperglycemic subjects are frankly hypoinsulinemic [39].

**Figure 3: The Ominus Octet Hypothesis, Pathophysiology of hyperglycemia in type II DM[39]**

**Insulin resistance [40]:**
Insulin resistance may be defined as existing when normal insulin concentrations fail to produce a normal biological response. The major insulin responsive tissues are skeletal muscle, adipose tissue and liver. Insulin resistance in muscle and fat is generally marked by decrease in transport of glucose from the circulation. Hepatic insulin resistance generally refers to a blunted ability of insulin to suppress glucose production. Insulin resistance in adipocytes causes increased rates of lipolysis and release of fatty acids into circulation, which can contribute to insulin resistance in liver and muscle, hepatic steatosis, and dyslipidemia.

**Mechanism of Insulin resistance in Type 2 DM [41]:**
1. Reduced level of insulin receptors and tyrosine kinase activity in skeletal muscles, which are secondary to hyperinsulinemia and not a primary defect.
2. Defective PI-3 kinase signaling which reduces translocation of GLUT- 4 to the plasma membrane.
3. Elevated levels of free fatty acids, a common feature of obesity can impair glucose utilization in skeletal muscle.

**Clinical features [42]:**
Clinical features of diabetes mellitus vary from patient to patient, most often symptoms of hyperglycemia, polydypsia, polyuria and polyphagia are the presenting features. Occasionally they may present with complications like diabetic coma or neuropathy, in the absence of symptomatic hyperglycemia.

Type 1 diabetes occurs at an early age of less than 25yrs. Onset of symptoms may be abrupt, with thirst, excessive urination, increased appetite and weight loss developing over some days. In some patients, it first manifests as ketoacidosis following trauma, infection or surgery. Occasionally this initial episode may be followed by a symptom free interval (Honeymoon period), when no treatment is required. Once symptoms develop, insulin therapy is required.
Type 2 diabetes usually begins in the middle life or later. Typically the patient is overweight and diagnosis is usually made during routine lab testing for blood glucose. These patients do not develop diabetic ketoacidosis but they develop hyperosmolar, non ketogenic coma. In this type, if weight loss can be induced, patient may be managed by diet alone. Most patients who fail to respond to diet alone, usually respond to oral hypoglycemic therapy. However, insulin will be required at one or other stage of the disease.

Table 1- Difference between type 1 and type 2 diabetes[41,43]:

<table>
<thead>
<tr>
<th>Sl No</th>
<th>Characteristic features</th>
<th>Type 1 DM</th>
<th>Type 2 DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Prevalence (India)</td>
<td>1.8%</td>
<td>4% (rural), 8% (urban)</td>
</tr>
<tr>
<td>2</td>
<td>Age of onset</td>
<td>&lt;20</td>
<td>&gt;45</td>
</tr>
<tr>
<td>3</td>
<td>Primary defect</td>
<td>Autoimmune</td>
<td>Genetic, environmental factors</td>
</tr>
<tr>
<td>4</td>
<td>Onset of symptoms</td>
<td>Acute onset</td>
<td>Subtle symptoms, goes unnoticed for years</td>
</tr>
<tr>
<td>5</td>
<td>Ketoacidosis</td>
<td>Seen</td>
<td>Not common</td>
</tr>
<tr>
<td>6</td>
<td>Heritability</td>
<td>50%</td>
<td>80%</td>
</tr>
<tr>
<td>7</td>
<td>Family history</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Genetic locus</td>
<td>Ch 6</td>
<td>Unknown</td>
</tr>
<tr>
<td>9</td>
<td>Plasma insulin</td>
<td>Low/ absent</td>
<td>High/normal</td>
</tr>
<tr>
<td>10</td>
<td>Plasma glycogen</td>
<td>High, suppressible</td>
<td>High, resistant</td>
</tr>
<tr>
<td>11</td>
<td>Autoantibodies</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>Other autoimmune diseases</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>Body weight</td>
<td>Normal/ wasted</td>
<td>Obese</td>
</tr>
<tr>
<td>14</td>
<td>Pancreatic diseases</td>
<td>Destroyed</td>
<td>Normal</td>
</tr>
<tr>
<td>15</td>
<td>Treatment</td>
<td>Insulin injections</td>
<td>Diet, exersice, weight loss, oral hypoglycaemic agents, insulin</td>
</tr>
</tbody>
</table>

Screening:
Recommendations for Diabetes Screening of Asymptomatic Persons [42]:
- Testing should be considered for age group 45 or above, particularly in those with Body mass index [BMI] = >25 kg/m², if test is normal it should be repeated at 3 years intervals
- Testing should be considered at a younger age or be carried out more frequently in overweight (BMI = >25 kg/m²).
  Individuals with additional risk factors:
  - First degree relatives with diabetes
  - Habitually physically inactive
  - Members of high-risk ethnic population (ex. African Americans, Hispanic Americans, native Americans, Asian Americans, pacific islanders)
  - Delivery of baby weighing >4.03 kg or history of gestational diabetes
  - Hypertension (BP ≥ 140/90 mm/Hg)
  - HDL cholesterol < 35mg/dl or triglyceride >250 mg/dl
  - Polycystic ovary syndrome
  - Impaired glucose tolerance or impaired fasting glucose on previous testing
  - History of vascular disease
**Diagnosis** [44,45]:
The National Diabetes Data Group and World Health Organization have issued diagnostic criteria for DM based on the following: -
1. The spectrum of fasting plasma glucose (FPG) and response to an oral glucoseload varies among normal individuals and
2. DM is defined as the level of glycaemia at which diabetes specific complications occur rather than on deviations from a population based mean.

<table>
<thead>
<tr>
<th>Category</th>
<th>Fasting plasma glucose (mg/dl)</th>
<th>2 hour plasma glucose (mg/dl)</th>
<th>Casual plasma glucose (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normoglycemia</td>
<td>&lt;100</td>
<td>&lt;140</td>
<td>-</td>
</tr>
<tr>
<td>IFG</td>
<td>100-125 / -</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>IGT</td>
<td>-</td>
<td>140 –199</td>
<td>-</td>
</tr>
<tr>
<td>Diabetes</td>
<td>&gt; / =126</td>
<td>200</td>
<td>&gt;11.5mmol/L with symptoms of the disease</td>
</tr>
</tbody>
</table>

With HbA1c ≥6.5%

**Mechanism of complications** [46]:
Four main theories have been put forth to explain the complications of diabetes:
1. Advanced Glycation End Products (AGEs): Increased intracellular glucose leads to formation of AGEs via non-enzymatic glycosylation of intra and extra cellular proteins through interaction of glucose with amino group of proteins. These AGEs have been shown to cross link proteins, accelerate atherosclerosis, promote glomerular dysfunction, reduce nitric oxide synthesis and induce endothelial dysfunction etc. The serum levels of AGEs correlate with the level of glycemia.
2. Sorbitol pathway: Hyperglycemia increases glucose metabolism via sorbitol pathway, which is an alternate pathway to glycolysis. Increased sorbitol concentrations in cells alter redox potential, increases cellular osmolality, generates reactive oxygen species and causes cellular dysfunction.
3. Through protein kinase C (PKC): Hyperglycemia increases the formation of diacylglycerol leading to activation of PKC. Along with other actions, it alters the
transcription of genes for fibronectin, type IV collagen etc in endothelial cell and neurons.

4. Through hexosamine pathway: Hyperglycemia increases the flux through hexosamine pathway which generates fructose 6-phosphate that in turn alters the function of proteins like nitric oxide synthase or gene transcription for transforming growth factor (TGF)-β. Growth factors appear to play an important role in Diabetic complication. Vascular endothelium growth factor is increased locally in diabetic retinopathy and TGF-β is increased in diabetic nephropathy. But the exact role is unknown. A unifying mechanism is that hyperglycemia leads to increased production of reactive oxygen species (ROS) in mitochondria which in turn activates all the above cited pathways.

![Figure 4: Mechanism of complications](image)

Methods of inducing diabetes[47,48]

1. Surgical:
   - By Pancreatectomy
   - By Ligation of Pancreatic duct
2. Chemical
   - By alloxan
   - Streptozotocin
   - Others- florizine, dithizone, monosodium glutamate
3. Hormone induced diabetes: Growth hormone, Corticosteroid (dexamethasone) induced diabetes.
4. Insulin deficiency due to insulin antibodies: Guinea pig anti insulin serum
5. Virus induced diabetes: Coxsackie B4, Mengo-2T, Reovirus, Rubella virus etc.

Streptozotocin is selected as a method to induce diabetes in the present study for the following reasons:
- It produces a selective toxic effect on β cell and induces diabetes mellitus in most lab animals.
- Streptozotocin is preferred because it has more specific β cell cytotoxicity
Diabetic animals constitute an excellent biomedical tool for the investigation of carbohydrate metabolism and diabetic complications and for understanding the nature of the disease.

1) Surgical induced diabetes [49]:
Induction of diabetes can be achieved through removal of all or part of pancreas. In partial pancreatectomy more than 90% of the organ must be removed to produce diabetes. Total removal of the pancreas results in type 1 diabetes. The portion of pancreas usually left behind following a subtotal pancreatic resection is typically anterior lobe or a portion thereof. This model has now been replaced by chemical and genetic models.

2) Chemical induced Diabetes: - most commonly used diabetic model. They are reclassified into 3 categories and include agents that
- Specifically damage β cell.
- Temporary inhibit insulin production and or secretion and
- Diminish metabolic efficacy of insulin in target tissue.

The first group includes Alloxan, Streptozotocin, glyoxol, dithizon and recently asparginase, pentamidine isothionate and N-3-pyridyl methyl N-P nitrophenylurea (PNU). Second group includes diazoxide, diphenylhydantoin, cyproheptadine and mannohetulose [50,51]. In animals Alloxan and Streptozotocin cause β cell cytolysis within 4-8 hours. This effect is mostly due to interaction with protein synthesis associated with cell membrane and may be mediated by depression of nicotinamide adenine nucleotide levels in β cells. This β cell destructive property of Streptozotocin is used in the treatment of malignant insulinomas and other malignant endocrine disorders.

**Alloxan:** It is 2, 4, 5, 6 –tetroxypyrimidine 5, 6 dioxyuracil. Alloxan or mesoxalyurea is an organic compound based on a pyrimidine heterocyclic skeleton. The name is derived from allantoin, a product of uric acid excreted by the fetus into the allantois and oxaluric acid derived from oxalic acid and urea found in urine.

**Mechanism of action:** It acts on the insulin-producing pancreatic beta cell and selectively kills these cells. Most likely this is due to selective uptake of the compound because of alloxan’s structural similarity to glucose. The beta cell has a highly efficient uptake mechanism (GLUT2) for glucose and alloxan gets into the cell in the same way as glucose.

Alloxan and the product of its reduction, dialuric acid, establish a redox cycle with the formation of superoxide radicals. These radicals undergo disputation to hydrogen peroxide. Thereafter highly reactive hydroxyl radicals are formed by the Fenton reaction. The action of reactive oxygen species, decrease in superoxide dismutase activity with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of β cells [52].

**Advantages:**
1) Selective loss of pancreatic beta cells by Alloxan leaving other cells.
2) Residual insulin secretion makes animal live long without insulin treatment.
3) Comparatively cheaper, easier to develop and maintain

**Disadvantages:**
1) High mortality in rats.
2) Causes ketosis in animals due to free fatty acid generation.
3) Diabetes induced is reversible.

**Streptozotocin (STZ)**
It is a deoxy-S [(methyl-nitrosoaminocarbonyl)-amino]–D glucopyranose is a broad spectrum antibiotic which is produced from Streptomyces achromogens. Rakieten et al first described the diabetogenic property of STZ [53].

**Mechanism of causing beta cell damage:**
- By process of methylation.
- Free radical generation and
- Nitric oxide production.

Streptozotocin is probably the most widely used agent producing Insulin dependent diabetes mellitus (IDDM) in experimental animals. Injection of STZ into the peritoneal cavity of 2-day-old rats resulted in non-insulin dependent diabetes mellitus (NIDDM) like hyperglycaemic state. In this model, following STZ injection, transient hyperglycaemia rapidly develops, that lasts for 2-4 days, then plasma concentration remains at near normal levels until approximately 6 weeks of age, where upon frank chronic hyperglycaemia develops with plasma concentration ranging between 200 and 350 mg/dl [54].

3) Insulin deficiency due to insulin antibodies:
Guinea pig anti insulin serum given IV in the dose of 0.25-1ml to rats causes a dose dependent increase in blood glucose levels upto 300mg %. It is due to neutralization of endogenous insulin by the anti-insulin antibodies. Slow i.v injection or i.p injection prolongs the effect for more than a few hrs. However larger doses and prolonged administration are accompanied by ketosis, glycosuria and acidosis and are fatal to the animals [55].

4. Virus induced diabetes:
Viruses are thought to be one of the etiological agents for IDDM in both man and animals. Ex: Coxsackie B4, Mengo-2T, Reovirus, Rubella virus [56].
Viral agents cause diabetes by:
- Infecting and destroying beta cells in pancreas.
- By eliciting immune response auto reactivity to beta cells
- Viruses producing systemic effect not directly affecting beta cells.

Obesity
Obesity is defined as a condition of abnormal or excessive fat accumulation in adipose tissue to the extent that health may be impaired [57]. Obesity is a fast spreading epidemic, a major contributor to the global burden of chronic disease and disability.

History of obesity:
Human obesity is clearly depicted in Stone Age artefacts, notably numerous figurines that have been found within a 2000-kilometre band crossing Europe from South-Western France to Southern Russia. Palaeolithic (Old Stone Age) statuettes, produced some 23,000–25,000 years ago, were made of ivory, limestone or terracotta. Most famous is the ‘Venus of Willendorf’, an 11-centimetre figurine found in Austria. (Figure 5)
Figure 5: Venus of Willendorf, a Palaeolithic figurine carved out of fine-grained limestone, was found near Willendorf in the Wachau region of Lower Austria in 1908.

The Hindu physicians, Sushrut (Susrata) and Charak (500–400 B.C.) were the early recognizers of the sugary taste of diabetic urine, and also observed that the disease often affected indolent, overweight people who ate excessively, especially sweet and fatty foods.

The New Stone Age (Neolithic) period, between 8000 and 5500 B.C., was the first time in history that man began to own property. This era also produced numerous statuettes representing obesity, importantly the “Mother Goddess” artefacts found in modern Turkey (Anatolia).

Important landmarks in the history of obesity since the seventeenth century

1614 Santorio- Uses beam balance to measure metabolism
1628 Harvey- Discovers circulation of the blood
1679 Bonet- First dissections of obese cadavers
Eighteenth Century
1727 Short- First English language monograph on obesity
1760 Flemyng- Monograph on the treatment of obesity
1780 Cullen- Disease classification that includes obesity
1780s Lavoisier- First measurements of heat production by living animals; formulated the ‘oxygen theory’ (which replaced ‘phlogiston’ of the Ancients)
Nineteenth Century
1810 Wadd- Treatise on Corpulence
1826 Brillat-Savarin- Diet-based method for weight loss
1835 Quetelet- Obesity quantified as weight/(height squared)
1848 Helmholtz- Published Law of the Conservation of Energy (First Law of Thermodynamics)
1849 Hassall- Described structure and growth of fat cells
1863 W. Banting- Letter on Corpulence Addressed to the Public (first widely popular diet book)
1866 Russell- Sleep apnoea described as a complication of obesity
1879 Hoggan- Described growth of fat cells
1896 Atwater- First human calorimeter constructed
Twentieth Century
1900 Babinski and 1901 Fröhlich- Described syndrome of hypothalamic obesity
1912 Cushing- Described obesity caused by basophil pituitary tumour
1916 Cannon & Carlson- Proposed gastric mechanism for hunger
1921 F. Banting, Best, Macleod & Collip- Insulin isolated from pancreas and used to treat human diabetes
1927 Various- Dinitrophenol used to treat obesity (poor outcome)
1936 Himsworth- Insulin-insensitive diabetic patients identified
1937 Abramson- Amphetamine used to treat obesity
1944 Behnke- Underwater weighing used to estimate body density and composition
1947 Vague- ‘Android’ (central) obesity predisposes to diabetes and cardiovascular risk
1949 Fawcett- Described brown adipose tissue (BAT)
1954 Stellar- Formulated 'dual centre' hypothesis to explain control of feeding
1955 Lifson- Doubly-labelled water used to measure energy expenditure
1959 Berson and Yalow- Discovered radioimmunoassay technique to measure insulin concentrations
1962 Neel- ‘Thrifty gene’ hypothesis
1963 Randle- Glucose-fatty acid (Randle) cycle described
1967 Stewart- First use of behavioural therapy to treat obesity
1968 Various- Association for the Study of Obesity founded in UK
1968 Mason- Performed first gastric bypass operations to treat obesity
1973 Gibb- Cholecystokinin (CCK) found to induce satiety in rats
1979 DeFronzo- Insulin-glucose clamp developed to measure insulin sensitivity
1982 Nedergaard et al.- Thermogenin (later renamed UCP1) identified as source of heat production in BAT
1986 Various- International Association for the Study of Obesity founded
1988 Reaven- Described ‘Syndrome X’ (the insulin resistance or metabolic syndrome)
1989 Strosberg et al.- Identified β3-adrenoceptor
1994 Friedman et al.- Discovered leptin
1997 O’Rahilly et al- Described leptin and melanocortin 4 receptor mutations as causes of human obesity
2007 Sjöström et al.- Demonstrated that bariatric surgery prolongs life

In a study by Green MA et al., it identified that there are six different types of obese people [58]

- Obese but young, healthy females who have not yet developed obesity-related complications were more likely to take positive weight loss steps.
- Men who drink heavily (drinking at least 12 drinks per week) were less likely to manage their weight well.
- Unhappy, anxious middle-aged people, in particular women who have mental health issues, reported more depression, anxiety, and fatigue although they engaged in physical activity.
- Wealthy and healthy elderly people drink more alcohol and have higher blood pressure than average.
- Elderly individuals who are physically ill (suffering from chronic conditions like arthritis) but are happy and have good mental health reported a higher sense of well-being.
- People with the worst health, who have more chronic conditions than the other groups, were also more likely to be poorer. This category had the highest mean BMI and reported more levels of pain and fatigue.

**Measurement of obesity**

**Body fat can be measured in several ways:**

- The anthropometric methods like the body mass index (BMI), waist circumference (WC), waist-to-hip ratio (WHR), waist height ratio (WHtR), skinfold thicknesses, and
Bioelectrical impedance—are useful in clinics and community settings, as well as in large research studies.

- More sophisticated methods, such as nuclear magnetic resonance imaging, dual energy X-ray absorptiometry or computed tomography are so-called "reference measurements", techniques that are typically only used in research studies to confirm the accuracy of body measurement techniques.

**Body Mass Index (BMI)**

Body mass index (BMI) is a simple index of weight-for-height that is commonly used to classify overweight and obesity in adults. BMI is a number calculated by dividing a person’s weight in kilograms by his or her height in meters squared. Obesity is most commonly calculated using BMI. An adult with a BMI of 30 or greater is clinically obese. BMI is not used to determine a person’s actual percentage of body fat, but it is a good indicator to categorize weight in terms of what is healthy and unhealthy.

The WHO definition is:
- a BMI greater than or equal to 25 is overweight
- a BMI greater than or equal to 30 is obesity.

**Classification of obesity**

In the new graded classification system developed by the WHO, a BMI of 30 kg/m² or above denotes obesity (Table 4).

<table>
<thead>
<tr>
<th>Classification</th>
<th>BMI (kg/m²)</th>
<th>A risk of associated illness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Underweight</td>
<td>&lt;18.5</td>
<td>Low</td>
</tr>
<tr>
<td>Normal range</td>
<td>18.5 – 24.9</td>
<td>Average</td>
</tr>
<tr>
<td>Pre-obese/ overweight</td>
<td>25 – 29.9</td>
<td>Increased</td>
</tr>
<tr>
<td>Obese class I</td>
<td>30.0 – 34.9</td>
<td>Moderate</td>
</tr>
<tr>
<td>Obese class II</td>
<td>35.0 – 39.9</td>
<td>Severe</td>
</tr>
<tr>
<td>Obese class III</td>
<td>&gt;40.0</td>
<td>Very severe</td>
</tr>
</tbody>
</table>

**Obesity classification for Indians/Asians**

On the basis of the available data in Asia, the WHO expert consultation concluded that Asians generally have a higher percentage of body fat than white people of the same age, sex, and BMI. Also, the proportion of Asian people with risk factors for type 2 diabetes and cardiovascular disease is substantial even below the WHO BMI cut-off point of 25 kg/m². Thus, standard WHO cut-off points do not provide an adequate basis for taking action on risks related to overweight and obesity in many populations in Asia. What these populations have in common is that, in general, the mean or median BMI is lower than that observed for non-Asian populations (and hence the BMI distribution is shifted to the left), although the tendency towards abdominal obesity might be greater than in non-Asian populations. Such a trend leads to the concern that application of the standard WHO BMI cut-off points will underestimate obesity-related risks in these populations, thus a new consensus guidelines for classification of obesity in Asians and Indians have been formed [59] (Table 5).

<table>
<thead>
<tr>
<th>Classification</th>
<th>BMI (kg/m²)</th>
<th>A risk of associated illness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Underweight</td>
<td>&lt;18.5</td>
<td>Low</td>
</tr>
<tr>
<td>Normal range</td>
<td>18.5 – 23</td>
<td>Average</td>
</tr>
<tr>
<td>Overweight</td>
<td>23 – 27.5</td>
<td>Increased</td>
</tr>
<tr>
<td>Obesity</td>
<td>&gt;27.5</td>
<td>Severe</td>
</tr>
</tbody>
</table>
Prevalence of obesity in India
In India 270 million people live below the ‘poverty line’, still 30 million people are obese and carries high risk for obesity related diseases (Table 6). Number of overweight and obese people globally increased from 857million in 1980 to 2.1 billion in 2013. This is one-third of world’s population [60].

Table 6: Prevalence of obesity in India

<table>
<thead>
<tr>
<th>Age group</th>
<th>Overweight and obese</th>
<th>Obesity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boys &lt;20yrs</td>
<td>5·3 (4·3–6·4)</td>
<td>2·3 (1·8–2·8)</td>
</tr>
<tr>
<td>Men &gt;20yrs</td>
<td>19·5 (17·8–21·2)</td>
<td>3·7 (3·3–4·1)</td>
</tr>
<tr>
<td>Girls &lt;20yrs</td>
<td>5·2 (4·2–6·4)</td>
<td>2·5 (1·9–3·1)</td>
</tr>
<tr>
<td>Women &gt;20yrs</td>
<td>20·7 (18·9–22·5)</td>
<td>4·2 (3·8–4·8)</td>
</tr>
</tbody>
</table>

Central adiposity is a growing problem, particularly among Asian populations where individuals may exhibit a ‘normal’ BMI but have a disproportionately large WC. WHO recognizes that WC between 94.0–101.9cm in men and 80.0–87.9cm in women, and WHR more than 0.8 and 0.9 in women and men, respectively, correspond with the BMI overweight range of 25–29.9 kg/m². Although BMI has been the chosen by many researchers to measure body size in epidemiological studies, alternative measures, such as WC, WHR and WHtR which reflect central adiposity, have been suggested to be superior to BMI in predicting cardiovascular disease risk [61].

WHtR

Table 7: Categorisation of waist height ratio

<table>
<thead>
<tr>
<th>Children (up to 15)</th>
<th>Men</th>
<th>Women</th>
<th>Categorisation</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0.34</td>
<td>&lt;0.34</td>
<td>&lt;0.34</td>
<td>Extremely Slim</td>
</tr>
<tr>
<td>0.35 to 0.45</td>
<td>0.35 to 0.42</td>
<td>0.35 to 0.41</td>
<td>Healthy Slim</td>
</tr>
<tr>
<td>0.46 to 0.51</td>
<td>0.43 to 0.52</td>
<td>0.42 to 0.48</td>
<td>Healthy</td>
</tr>
<tr>
<td>0.52 to 0.63</td>
<td>0.53 to 0.57</td>
<td>0.49 to 0.53</td>
<td>Overweight</td>
</tr>
<tr>
<td>0.64 +</td>
<td>0.58 to 0.62</td>
<td>0.54 to 0.57</td>
<td>Very Overweight</td>
</tr>
<tr>
<td></td>
<td>0.63 +</td>
<td>0.58 +</td>
<td>Morbidly Obese</td>
</tr>
</tbody>
</table>

Causes for obesity
Obesity is due to an individual taking in more calories than they burn over an extended period of time. These “extra” calories are stored as fat. Although there are several factors that can lead to this energy imbalance in obese individuals, the main contributors are behavior, environment and genetics [62].

Behavior: In today’s fast-paced environment, it is easy to adopt unhealthy behaviors. Behavior, in the case of obesity, relates to food choices, amount of physical activity you get and the effort to maintain your health. Based on food choices, many people now select diets that are calorie-rich, but nutrient-poor. This behavioral problem also relates to the increase in meal quantity at home and when dining out.

Environment: Environment plays a key role in shaping an individual's habits and lifestyle. There are many environmental influences that can impact your health decisions. Today’s society has developed a more sedentary lifestyle. Walking has been replaced by driving cars, basic physical activity has been replaced by technology and nutrition has been overcome by fast foods.
Genetics: Science shows that genetics play a role in obesity. Genes can cause certain disorders which result in obesity. However, not all individuals who are predisposed to obesity become obese. Research is currently underway to determine which genes contribute most to obesity.

Social effects of obesity: Individuals affected by obesity often face obstacles far beyond health risks. Emotional suffering may be one of the most painful parts of obesity. Society often emphasizes the importance of physical appearance. As a result, people who are obese often face prejudice or discrimination in the job market, at school and in social situations.

Effects at Work: Due to the negative stigma associated with obesity, obese employees are often viewed as less competent, lazy and lacking in self-discipline by their co-workers and employers. Often times, discriminatory attitudes can negatively impact salary, promotions and employment status for obese employees. Finding a job can also be a difficult task for an obese individual. Studies show that obese applicants are less likely to be hired than thinner applicants, despite having identical job qualifications.

Effects at School: Educational settings also provide the possibility for discriminatory situations. Obese children face numerous obstacles, ranging from harassment, teasing and rejection from peers, to biased attitudes from teachers.

At a young age, children are exposed to obesity's negative stigma. Obese children are sometimes characterized as being unhappy, lazy, mean and not having many friends.

In Healthcare Settings: Negative attitudes about obese patients also exist in the healthcare setting. Obese patients are often reluctant to seek medical care, may be more likely to delay important preventative healthcare services and more frequently cancel medical appointments. Delaying medical attention can lead to delayed discovery or treatment of co-morbid conditions, such as diabetes and cardiovascular disease, while becoming more physically damaging.

The consequences of this discrimination can seriously impact an individual's quality of life and only further intensify the negative stigma associated with obesity.

Adipose Tissue
The adipose tissue or body fat is loose connective tissue composed mostly of adipocytes. Adipose tissue also contains the stromal vascular fraction (SVF) of cells including pre-adipocytes, fibroblasts, vascular endothelial cells and a variety of immune cells i.e., adipose tissue macrophages (ATMs). There are two types of adipose tissue. White adipose tissue stores energy, due to its capacity to store energy (around 200,000-300,000 Kcal in adults who are not obese) and provide it when necessary, it is the most important buffer system for energy balance. Brown adipose tissue was first identified by the Swiss naturalist Conrad Gessner in 1551 is practically absent in adult humans, but is found in foetuses and newborn infants, which possesses multilocular adipocytes with abundant mitochondria which express high amounts of uncoupling protein 1 (UCP-1), responsible for the thermogenic activity of this tissue [63].

Adipose tissue is not only a triglyceride (TG)-storage tissue; many studies have shown that white adipose tissue as a producer of certain substances with endocrine, paracrine, and autocrine action. These bioactive substances are denominated adipokines or adipocytokines, among which are found plasminogen activator inhibitor-1 (PAI-1), tumor necrosis factor-alpha (TNF-α), resistin, leptin, adiponectin, interleukin 6 (IL-6), monocyte chemotactic protein-1 (MCP-1) [64]. Dysregulated production of these adipocytokines participates in the pathogenesis of obesity-associated metabolic syndrome.
Adipokines and their role:
Although more than 50 adipokines have been identified, adiponectin and leptin have been studied most diversely for their functional role in obesity [65]. The view of adipose tissue has been changed from that of an “inert” storage tissue to an “active” endocrine organ able to secrete a number of adipokines, such as adiponectin, TNF-α, and leptin, which are reported to exert potent neuro-immunoendocrine effects [66].

Figure 6: Adipokines and their role in obesity

**Adiponectin:** Adiponectin (GBP-28, apM1, AdipoQ and Acrp30) is a protein encoded by the ADIPOQ gene which is involved in regulating glucose levels as well as fatty acid breakdown. Adiponectin was first characterised in 1995 in differentiating 3T3-L1 adipocytes and is located at chromosome 3q27. It is constituted of 244 amino acids, found in plasma at levels of 3-30 mg/ml, and forms three major oligomeric complexes with biological functions as trimer, hexamer, and high-molecular-mass form [67]. Adiponectin has high anti-inflammatory and anti-atherogenic activity since it inhibits the adhesion of monocytes to endothelial cells, the transformation of macrophages into foam cells and endothelial cell activation, inhibits TNF-α expression, decreases C-reactive protein (CRP) levels, and increases nitric oxide (NO) production in endothelial cells. Its globular isoform inhibits cell proliferation and production of ROS induced by low-density lipoprotein (LDL) oxidase during atheromatous plaque formation [64]. Adiponectin inhibits tumor necrosis factor α (TNF-α)-induced expression of adhesion molecules and the transformation of macrophages to foam cells, both of which are key components of atherogenesis. Adiponectin enhances insulin sensitivity in muscle and liver and increases free fatty acid (FFA) oxidation in several tissues, including muscle fibres also decreases serum FFA, glucose, and triacylglycerol concentrations. In contrast to other adipokines, adiponectin expression and plasma concentrations are not increased, but are rather decreased in a wide variety of diseases presenting insulin resistance and obesity [65]. Decrease in plasma adiponectin is the cause for insulin resistance and atherosclerosis in obesity [68].

**Leptin:** Leptin is a "satiety hormone," is a hormone produced by adipose cells that helps to regulate energy balance by inhibiting hunger. It was discovered in 1994 which is a 16-kd
protein encoded by the ob gene. It the first adipocyte hormone identified, which influences food intake through a direct effect on the hypothalamus [65]. Leptin regulates energy balance by two effects

1. In a population of parvocellular hypothalamic arcuate nucleus (ARC) neurons, it stimulates expression of neuropeptides, which induce inhibition of nutritional intake (proopiomelanocortin [POMC] and cocaine- and amphetamine-related transcript [CART]) and increase overall energy consumption; with this last involving a population of neurons similar to the paraventricular nucleus which promote an increase in sympathetic tone

2. In a population of ARC neurons, it inhibits expression of neuropeptide Y (NPY) and the agouti peptide, which are involved in increasing nutritional intake and reducing energy consumption [69].

Concentration of leptin circulation in plasma is proportional to total body adiposity and direct nutritional state. Deficiency of leptin is associated with increased appetite and manifest obesity in mice and humans, reduced inflammation in models of autoimmune disease, increased susceptibility to the toxicity of pro-inflammatory stimuli, such as endotoxin and TNF-α [70]. Leptin inhibits lipogenesis and stimulates lipolysis by reducing intracellular lipid levels in skeletal muscle, liver, and pancreatic beta cells, thus improving insulin sensitivity. The limbic system stimulates dopamine reuptake, by blocking the pleasure of eating and, through the locus coeruleus nucleus, activates the sympathetic nervous system, which will lead to increased resting energy expenditure [64]. NO mediated vasodilatation is increased by leptin. NO synthesis is decreased by inhibition of hypothalamic NPY in the brain. Nitric oxide activates cytoplasmic granulate cyclase and facilitates dilatation of vascular smooth muscle and the rate of blood stream increases NO synthesis in endothelial cells. This shear stress, increases both NO synthesis and secretion. And also NO is directly or indirectly related to food intake and supposed to be the central regulator in food consumption which linked to neuropeptides. NO is necessary for lipolytic activity and Nitric oxide synthase (NOS) inhibits lipolysis [71].

Tumor necrosis factor α: Tumor necrosis factor alpha (TNFα, cachexin, or cachectin) is a cell signaling protein (cytokine) involved in systemic inflammation and that make up the acute phase reaction which is produced chiefly by activated macrophages, although it can be produced by many other cell types such as CD4+ lymphocytes, NK cells, neutrophils, mast cells, eosinophils, and neurons. TNF is primarily produced as a 212-amino acid-long type II transmembrane protein arranged in stable homotrimers. The human TNF gene (TNFA) was cloned in 1985 [72]. The first indication for increased cytokine release in obesity was provided by the identification of increased expression of TNFα in the adipose tissue of obese mice in the early 1990s. The increased expression of TNFα in adipose tissue was considered to be responsible for the development of obesity or diabetes due to the induction of insulin resistance [73]. TNF α causes stimulation of the hypothalamic-pituitary-adrenal axis by stimulating the release of corticotropin releasing hormone (CRH), Suppress appetite. Increased level of TNF-α suppress adiponectin production which leads to a reduction in the release of anti-inflammatory cytokines such as Interleukin-1 (IL-1) and IL-10 — as a result adiponectin is unable to inhibit the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-Kb) pathway that prevents insulin signaling by phosphorylating the insulin receptor with serine via the NF-κB pathway activation and upregulates adhesion molecules on endothelial cells as a result more monocytes are recruited into the adipose tissue, further adding to the inflammation and TNF-α levels [74].

IL-6: IL-6 acts as both a pro-inflammatory cytokine and an anti-inflammatory myokine. In humans, it is encoded by the IL6 gene secreted by T cells, macrophages and adipocytes to stimulate immune response. IL-6 is an inflammatory cytokine that is correlated with
hyperglycemia, insulin resistance, and type 2 diabetes mellitus. IL-6 expression in adipose tissue from obese individuals is 10-fold more in adipose tissue from lean individuals if normalized for the number of adipocytes present. IL-6 increases lipolysis and fat oxidation in humans and plasma IL-6 concentrations correlate with insulin resistance [65].

**PAI-1:** PAI-1 also known as endothelial plasminogen activator inhibitor or serpin E1 is a protein that in humans is encoded by the SERPINE1 gene, located on chromosome 7. PAI-1 is mainly produced by the endothelium (cells lining blood vessels), but is also secreted by other tissue types, such as adipose tissue. PAI-1 plays an important role in the regulation of adipose tissue blood supply and the flow of fatty acids from it. Plasma levels of PAI-1 are regulated by the accumulation of visceral fat, and a high concentration of PAI-1 is associated with insulin resistance and pro-inflammatory cytokines [64].

**Lipotoxicity:** Lipotoxicity is a metabolic syndrome which results from the accumulation of lipid intermediates in non-adipose tissue, leading to cellular dysfunction and death. The tissues affected include the kidneys, liver, heart and skeletal muscle. The tissue disease which occurs when fatty acid spillover in excess of the oxidative needs of those tissues enhances metabolic flux into harmful pathways of non-oxidative metabolism includes congenital generalized lipodystrophy, obesity due to mutations in the leptin or leptin receptor genes, diet induced obesity, and aging [75]. Adipocytes have a lower insulin receptor density and a higher density of beta-3 adrenergic receptors in obese individuals, thus increasing the lipolysis rate with release of FFA, that leads to several metabolic consequences which include, increase in the production of oxygen-derived free radicals, induction of insulin resistance, synergism in the action of IL-6 and TNF-α, and induction of apoptosis in pancreatic beta cells; all these effects together called as lipotoxicity [64].

**Oxidative stress, antioxidants and obesity**

The imbalance between antioxidant defence mechanism and free radical formation is known as oxidative stress which leads to a serious tissue damage. In obese individuals there is increase in free radicals and reactive oxygen species as a result of which oxidative stress is observed [71]. The mechanism contributing to the obesity-associated oxidant stress include increased oxygen consumption and subsequent radical production via mitochondrial respiration, increased fat deposition and cell injury causing increased rates of radical formation. Cells have developed an enzymatic pathway against reactive oxygen species; the dismutation of superoxide anion (O$_2^-$) to hydrogen peroxide (H$_2$O$_2$) catalyzed by superoxide dismutase (SOD); and the conversion of H$_2$O$_2$ to H$_2$O by glutathione peroxidase (GPx) or catalase (CAT). This activity must be balanced in order to prevent oxidative damage in cells [76]. Oxidative stress in adipose tissue is one of the early step in the development of metabolic syndrome in obesity. In a study by Furukawa S et al. showed that oxidative stress in accumulated fat mediates the obesity-associated development of metabolic syndrome by the following mechanisms: (a) increased oxidative stress in accumulated fat leads to dysregulated production of adipocytokines, and (b) the selective increase in reactive oxygen species (ROS) production in accumulated fat leads to elevation of systemic oxidative stress [68]. Oxidative stress leads to insulin resistance within adipose tissue as well as in peripheral tissues which is one of the hallmarks of obesity and its comorbidities. NADPH oxidase is a major contributor to oxidative stress in many tissues, including adipose tissue and the vasculature [77]. Increased oxidative stress in accumulated fat is an early instigator of metabolic syndrome and that the redox state in adipose tissue is a potentially useful therapeutic target for obesity-associated metabolic syndrome [68]. The negative correlation between plasma insulin levels and plasma α-tocopherol concentrations suggested that decreased antioxidant vitamin levels and reduced antioxidant capacity might be a characteristic feature of obese children with multi-metabolic syndrome (MMS) [78]. During persistence of obesity for a longer duration antioxidant sources will be depleted, decreasing the activity of enzymes such as SOD and CAT. In recent years, antioxidants have been used
extensively to overcome the effects of excess reactive oxygen species. The common antioxidants include vitamin A, E and C, coenzyme Q, α-lipoic acid, lycopenes, and polyphenols. In a huge review by Abdali D et al. showed that different antioxidants are effect against oxidative stress and its complications [74].

**Obesity and inflammation**

Obesity is a low-grade chronic inflammatory disease that may contribute to the development of insulin resistance. Recent clinical studies have suggested that mediators of low-grade chronic inflammation, such as adipokines, cytokines, ghrelin, and acute phase reactants, are involved in the development of co-morbid conditions [66]. One of the possible explanations for adipose tissue producing adipokines and acute phase proteins is hypoxia which is produced during the overgrowth of adipose tissue during obesity [64]. The small adipocytes in lean individuals promote metabolic homeostasis whereas the enlarged adipocytes in obese individuals recruit macrophages and promote inflammation and predispose to insulin resistance [65]. Inflammation is a source of oxidative stress resulting in increased production of reactive oxygen species which enhance the inflammatory response by activating redox-sensitive nuclear transcription factors such as Adaptor protein-1 (AP-1) and NF-ƙB which are essential for the inducible expression of genes associated with immune and inflammatory responses, including cytokines, cell adhesion molecules, and inducible NO synthase. Thus, the pro-inflammatory and pro-oxidant effects of increased adiposity represent a potential link between cardiovascular disease and obesity [79].

**Health risks associated with obesity**

The Ancient Greek physician Hippocrates who related the health hazards to obesity stated that “sudden death is more common in those who are naturally fat than in the lean”... "Corpulence is not only a disease itself, but the harbinger of others". The ancient clinical observations, suggest that obesity is associated with both diabetes and sudden death, although the significance of the morbidity and excess mortality due to overweight and obesity has only been fully appreciated more recently.

There are more than 30 medical conditions that are associated with obesity. Individuals who are obese are at risk of developing one or more of these serious medical conditions, causing poor health or, in severe cases, early death. In fact, more than 112,000 annual deaths in the USA are attributable to obesity [80]. The most prevalent obesity-related diseases include:

- Diabetes
- Hypertension
- Dyslipidemia
- Ischemic heart diseases
- Stroke
- Gallbladder stone disease
- Non-alcoholic fatty liver disease (steatosis), steatohepatitis and cirrhosis
- Gastroesophageal Reflux Disease
- Severe pancreatitis
- Osteoarthritis
- Sleep apnea and respiratory problems
- Polycystic ovarian disease
- Some cancers like breast, uterus, cervix, colon, esophagus, pancreas, kidney and prostate
Adipogenesis
Adipogenesis is the process of cell differentiation by which pre-adipocytes become adipocytes [81]. Adipose derived pluripotent mesenchymal stem cells (MSCs) can be isolated from the bone marrow of rats, pigs and humans and then can be cultured in vitro. These MSCs have the ability to differentiate into several types of tissue, includes adipocytes (adipose), osteoblasts (bone), chondrocytes (cartilage) and myocytes (muscle) which occurs in a series of stages.

In humans, pre-adipocytes begin to differentiate into adipose tissue during late embryonic development and in rat and mouse pre-adipocytes begin conversion into adipose tissue after the birth. All the species have the ability to differentiate into pre-adipocytes throughout their life spans in response to the body’s fat storage demands [82].

Figure 7: Development of adipocytes from mesenchymal stem cells.

Stages of adipocyte differentiation [83]
Determination phase: The conversion of the stem cell to a pre-adipocyte, which cannot be distinguished morphologically from its precursor cell.
Terminal differentiation phase: The pre-adipocyte takes on the characteristics of the mature adipocyte.

To study the molecular pathways of adipogenesis and adipocyte function in vitro variety of cellular model systems are used to study the. These models are classified into two classes. The first group includes pluripotent fibroblasts that have the ability to differentiate into several cell types like myocytes, chondrocytes, and adipocytes which include the 10T1/2, BALB/c-3T3, RCJ3.1, and CHEF/18 fibroblast cell lines. The second groups of model systems are fibroblast like pre-adipocytes that are committed to differentiating into adipocytes which includes 3T3-L1,3T3-F422A, 1246, Ob1771, TA1, and 30A5 pre-adipocytes. The second groups of cells are used most frequently to study adipogenesis. 3T3-F442A and 3T3-L1 are the two most extensively characterized and used pre-adipocyte cell lines. Both of these lines were derived from disaggregated 17- to 19- day-old Swiss 3T3 mouse embryos [84].

The differentiation of pre-adipocytes into mature insulin sensitive adipocytes, which produce adiponectin, is regulated by two families of transcription factors, peroxisome proliferator-activated receptors (PPARs) and CCAAT/enhancer binding proteins (C/EBPs) [85]. The
other positive regulators include sterol-regulatory element-binding protein (SREBP), Krox20, signal transducer and activator of transcription 5, and members of the Klf family. The combined actions of these transcription factors result in a complex transcriptional cascade that causes adipocyte differentiation and tissue formation [86].

PPARs are ligand activated transcription factors which are the members of the nuclear receptor family and also includes the estrogen receptor and the thyroid hormone receptor [87]. They play essential for the regulation of cellular differentiation, development, and metabolism of carbohydrate, lipid, and protein, and tumorigenesis [88].

Types of PPARs [87,89]

1. α (alpha) - It is a master regulator of fatty acid oxidation. Its activation increases the expression of lipoprotein lipase, which hydrolyzes fatty acids from triglycerides, represses the expression of apolipoprotein (Apo) C3, the endogenous lipoprotein lipase repressor, increases expression of fatty acid repressors such as CD36 and increases the multiple β-oxidation enzymes. It is expressed in liver, kidney, heart, muscle, adipose tissue, and others.

2. β/δ (beta/delta) or NUC-1 or FAAR - activation can increase fatty acid oxidation, repress inflammation, and increase ApoA1 and high density lipoprotein (HDL) levels like and expressed in many tissues but markedly in brain, adipose tissue, and skin

3. γ (gamma) – It is a master regulator of adipogenesis and in vivo, as an insulin sensitizer It has three isoforms:
   - γ1 - expressed in virtually all tissue including heart, muscle, colon, kidney, pancreas, and spleen
   - γ2 - expressed mainly in adipose tissue (30 amino acids longer)
   - γ3 - expressed in macrophages, large intestine, white adipose tissue.

PPAR γ: PPAR γ is the master regulator of adipogenesis, also called as glitazone receptor or NR1C3 (nuclear receptor subfamily 1, group C, member 3) is a type II nuclear receptor, encoded by the PPARG gene [90]. PPARγ must heterodimerize with another nuclear hormone receptor like the retinoid X receptor, or RXR to bind DNA and be active transcriptionally [85].
Functions of PPARγ:

Role of PPARγ in Adipogenesis
The adipogenic regulatory protein peroxisome proliferator-activated receptor (PPAR)γ is the most critical of the regulators because its absence precludes formation of all adipose tissue [86]. Ablation of PPARγ in embryonic stem (ES) cells leads to embryonic lethality due to a defect in placentation as a result of PPARγ's participation in formation of the trophoblast [91]. The tetraploid embryo strategy of E.D. Rosen et al, generated only one mouse, which died soon after birth, but allowed these investigators to observe that PPARγ deficiency in these animals resulted in failure to form adipose tissue [92]. PPARγ2 profoundly affects adipogenesis and that increases of only PPARγ2 were observed in adipocytes of morbidly obese individuals [93].

In a study by the LeBlanc SE et al. showed that presence of Prmt5 promoted the binding of ATP-dependent chromatin-remodeling enzymes which was required for the binding of PPARγ2 at PPARγ2-regulated promoters and also indicate that Prmt5 acts as a coactivator for the activation of adipogenic gene expression and promotes adipogenic differentiation [86]. In a study by Foryst-Ludwig A et al. showed the novel mechanism of PPARγ function in the development of insulin resistance (IR), acting as a potent anti-inflammatory factor on T-lymphocyte activation and infiltration into fat tissue, and contributing to the attenuation of the systemic development of IR by the intervention with PPARγ agonists [94]. Choi JH et al. showed that phosphorylation on serine 273 (Ser273) by cyclin-dependent kinase 5 (Cdk5) selectively decreases expression of a of PPARγ target genes in adipocytes. Ser273 phosphorylation can be inhibited by the compound MRL24 which appears to be sufficient to confer the insulin sensitizing effects observed with PPARγ activation by thiazolidinedione drugs. Ser273 phosphorylation did not appear to affect the regulation of adipogenesis by PPARγ, demonstrating that the anti-diabetic and pro-adipogenic roles of PPARγ can be
independently manipulated pharmacologically [95]. In a study by Medina-Gomez Get al. showed that the PPARγ2 isoform prevents lipotoxicity by promoting adipose tissue expansion, increasing the lipid-buffering capacity of peripheral organs, and facilitating the adaptive proliferative response of β cells to insulin resistance [8]. Effect of MC extract supplementation was also compared with effect of thiazolidinedione (TZD) on high fat diet supplemented rats. The adipose tissue mass, TAG content and glycerol-3-phosphate dehydrogenase activity of the MC extract supplemented rats were significantly lower than those of the TZD supplemented rats, implying that MC might reduce lipogenesis in adipose tissue. Also MC extract supplemented rats showed lower levels of lipogenic gene expression, like lower mRNA levels of fatty acid synthase, acetyl-CoA carboxylase-1, lipoprotein lipase and adipocyte fatty acid-binding protein. These results indicate MC extract can reduce insulin resistance as effective as the anti-diabetic drug TZD. Furthermore, MC extract can suppress the visceral fat accumulation and inhibit adipocyte hypertrophy, which may be associated with markedly down regulated expressions of lipogenic genes and their transcriptional regulator, PPARγ and adipocyte determination and differentiation factor 1/sterol regulatory element-binding protein-1c (ADD1/SREBP-1c) in the adipose [17].

C/EBP: It belongs to a family of transcription factors composed of six members, C/EBP α, β, γ, δ, ε and ζ. They are characterized by a highly conserved basic-leucine zipper (bZIP) domain at the C-terminus. This domain is involved in dimerization and DNA binding, as are other transcription factors of the leucine zipper domain-containing family (c-Fos and c-jun). The bZIP domain structure of C/EBPs is composed of a α-helix that forms a "coiled coil" structure when it dimerizes. C/EBP β and δ are transiently induced during the early stages of adipocyte differentiation (adipogenesis), while C/EBPα is upregulated during the terminal stages of adipogenesis [96]. Cell culture studies show that C/EBPα is sufficient to trigger differentiation of pre-adipocytes into mature adipocytes. C/EBPα is therefore considered a master regulator of adipose tissue development. Ablation of C/EBPα in all tissues except the liver showed that C/EBPα is required for formation of white adipose tissue and also C/EBPα is not required for the formation of brown adipose tissue [97]. C/EBPα is most abundantly expressed in adipose tissue, placenta, and liver, also detectable in a variety of other organs, such as lung, kidney, small intestine, brain, and hematopoietic cells.

Functions of C/EBP family: Cellular differentiation, inflammatory response, liver regeneration, control of metabolism and numerous other cellular responses.

PPARγ can induce adipogenesis in C/EBPα-deficiency, whereas C/EBPα is incapable of driving the adipogenic program in the absence of PPARγ [98]. This observation suggests that C/EBPα and PPARγ participate in a single pathway of adipose development, which shows that PPARγ is a dominant factor in regulating adipogenesis. C/EBPα does provide a critical function during terminal stages of adipogenesis, failure to express C/EBPα results in insulin resistance in cell culture models and an inability to develop white adipose tissue in vivo [99].

Sterol regulatory element-binding proteins (SREBP)

Sterol regulatory element-binding proteins (SREBPs) are transcription factors that bind to the sterol regulatory element DNA sequence TCACNCCAC. Mammalian SREBPs are encoded by the genes SREBF1 and SREBF2. SREBPs belong to the basic-helix-loop-helix leucine zipper class of transcription factors [100].

Inactivated SREBPs are attached to the nuclear envelope and endoplasmic reticulum membranes. In cells with low levels of sterols, SREBPs are cleaved to a water-soluble N-terminal domain that is translocated to the nucleus. These activated SREBPs then bind to specific sterol regulatory element DNA sequences, thus upregulating the synthesis of enzymes involved in sterol biosynthesis. Sterol in turn inhibit the cleavage of SREBPs and
therefore synthesis of additional sterols is reduced through a negative feedback loop [101,102].

**Isoforms**

Mammalian genomes have two separate SREBP genes (SREBF1 and SREBF2):

- SREBP-1 expression produces two different isoforms, SREBP-1a and -1c. These isoforms differ in their first exons owing to the use of different transcriptional start sites for the SREBP-1 gene. SREBP-1c is responsible for regulating the genes required for de novo lipogenesis.
- SREBP-2 regulates the genes of cholesterol metabolism.

SREB proteins are indirectly required for cholesterol biosynthesis, for uptake and fatty acid biosynthesis [103].

Animal cells maintain proper levels of intracellular lipids under widely varying circumstances. A principal step in this response is to make more of the mRNA transcripts that direct the synthesis of these enzymes. Conversely, when there is enough cholesterol around, the cell stops making those mRNAs and the level of the enzymes falls. As a result, the cell quits making cholesterol once it has enough [104-106].

The feature of SREBP pathway is the proteolytic release of a membrane-bound transcription factor, SREBP. Proteolytic cleavage frees it to move through the cytoplasm to the nucleus. Once in the nucleus, SREBP binds to specific DNA sequences (the sterol regulatory elements or SREs) that are found in the control regions of the genes that encode enzymes required to synthesis lipids.

Insulin, cholesterol derivatives, T3 and other endogenous molecules have been demonstrated to regulate the SREBP1c expression, particularly in rodents.

Serial deletion and mutation assays reveal that both SREBP (SRE) and LXR (LXRE) response elements are involved in SREBP-1c transcription regulation mediated by insulin and cholesterol derivatives. Peroxisome proliferation-activated receptor alpha (PPARα) agonists enhance the activity of the SREBP-1c promoter via a DR1 element at -453 in the human promoter. PPARα agonists act in cooperation with LXR or insulin to induce lipogenesis [107].

SREBP-1c has also been shown to upregulate in a tissue specific manner the expression of PGC1alpha expression in brown adipose tissue [108].

**Adipolysis**

Adipolysis is the breakdown of lipids and involves hydrolysis of triglycerides into glycerol and free fatty acids (FFA). The hormones which induce lipolysis are epinephrine, norepinephrine, ghrelin, growth hormone, testosterone, and cortisol. These trigger 7TM receptors (G protein-coupled receptors), which activate adenylate cyclase which results in increased production of cyclic adenylate monophosphate (cAMP) and activates protein kinase A, which subsequently activates lipases found in adipose tissue [109]. The white adipocytes stores excess energy as TG, and play a key role in energy metabolism by providing FFA and glycerol through the hydrolysis of TG. The induction of lipolysis and inhibition of TG synthesis in white adipocytes, has been considered a target of therapy for the prevention and improvement of obesity and its related disorders [110].

The cytokine TNF-α, an important mediator of lipid metabolism, plays a role in inducing lipolysis. TNF-α can perturb the normal regulation of energy metabolism, and enhanced TNF-α expression could be a cause of the decrease of lipidic depots in white adipose
tissue, the inhibition of insulin action, and the promotion of apoptosis. In a study by Dave S et al. evaluated that the lipolytic response of stem bromelain (SBM) on 3T3L1 by measuring the expression levels of perilipin, hormone sensitive lipase (HSL), and TNF-α during adipocyte differentiation. SBM downregulated perilipin while upregulating TNF-α and no appreciable changes for HSL expression. PPARγ is known to upregulate perilipin expression and TNF-α promote phosphorylation and downregulation of perilipin. The TNF-α induction and PPARγ repression caused by SBM can explained SBM treatment causes perilipin downregulation. PPARγ impact on glucose uptake, metabolism and lipogenesis repression by SBM may contribute to reduced lipogenesis while enhancing lipolysis. TNF-α-induced lipolysis also downregulate anti-lipolytic genes (phosphodiesterases-3B) PDE3B and Gα1. Thus TNFα leads to both physiological apoptosis and lipolysis as which is evident from target gene expression [111]. In a study by Zimmermann R et al. showed that the enzyme ‘adipose triglyceride lipase’ (ATGL), is expressed predominantly in white adipose tissue which is localized to the adipocyte lipid droplet and specifically initiates triacylglycerol hydrolysis resulting in the generation of diacylglycerols and FFAs. ATGL helps in the mobilization of FFAs from mammalian triacylglycerol stores which includes the overexpression of ATGL enhanced basal and isoproterenol-stimulated lipolysis in 3T3-L1 adipocytes, the inhibition of ATGL by antisense technologies reduced basal and isoproterenol-stimulated lipolysis in 3T3-L1 adipocytes and the antibody-directed inhibition of ATGL in murine fat pads decreased triacylglycerol lipase activity in murine adipose tissue of wild-type mice as much as 70%, and led to an almost complete loss of triacylglycerol hydrolase activity in white adipose tissue of HSL-knockout mice [112]. In a study by Arsenijevic D et al. showed that uninephrectomy in a rat induces adipose tissue lipolysis in response to increased levels of a subset of lipolytic cytokines like interferon-gamma and granulocyte macrophage–colony stimulating factor of splenic origin [113].

Natural compounds have been used to develop drugs to treat many health related problems for many decades. The study of natural products is called phytochemistry. The non-nutrient plant chemical compounds or bioactive components are known as phytochemicals (‘phyto’ from Greek meaning ‘plant’) or phytoconstituents which plays a crucial role in many health related problems. Vast diversities and minimum side effects have made natural compounds a good source for drug development. Hippocrates, one of the ancient authors, first extracted aspirin from the willow tree in the fifth century BC; its uses include relief of fever and pain, and in childbirth and he listed approximately 400 different plant species for medicinal purposes [114]. According to World Health Organization, a medicinal plant is one which contains substances that can be used for therapeutic purposes which may be present in plant parts like leaves, roots, rhizomes, stems, barks, flowers, fruits, grains or seeds. Due to the increasing inefficacy or side effects of modern drugs, medicinal plants are increasingly gaining acceptance recently.

Momordica charantia

Momordica charantia (MC) or bitter melon (BM) has been used traditionally as an antidiabetic agent [16], the fruit of which is known as karela. Bitter gourd or BM is a common edible vegetable in Asia [17]. The Latin name Momordica means “to bite” (referring to the jagged edges of the leaf, which appear as if they have been bitten). Ben Cao Gang Mu (Li Shizhen, 1578), a famous ancient Chinese pharmacopoeia, published during the Ming Dynasty (1368-1644), described that MC fruit can “Remove hot and dry, relieve weary, clean the dust on heart and brighten eyes”. Unripe fruit, seed and aerial parts of MC have been used in the folk medicine as anti-diabetic remedy in China, India, Sri Lanka and the West Indies. MC has been the subject of about more than four hundred scientific articles describing its pharmacological or phytochemical properties.
Vernacular names of *Momordica charantia* [115,116]
- Sanskrit - Sushavi, Karavella
- English - Bitter gourd, Balsam pear, Balsam apple.
- Hindi - Karela, Kardi
- Bengali - Karela, Uchchhe, Kerula
- Tamil - Pakal, Pavaka, Chedi, Paharkai
- Kannada - Hagal
- Malayalam - Kaipp, Kaippavlli, Paval
- Guajarati - Karela
- Bombay - Kurela, Jangro
- Telgu - Koekara, Kaaya
- Arab - Quisaul – barri
- Urdu - Karela
- Oria - Kalara, Salara
- Assam - Kakiral, Kakral

Botanically it belongs to the family of Cucurbitaceae, of the genus: *Momordica* and is a member of the same family of squash, watermelon, cantaloupes, cucumber, etc.

**Scientific classification:**
- Scientific name: *Momordica charantia*
- Kingdom: Plantae
- Division: Magnoliophyta
- Family: Cucurbitaceae
- Order: curcurbetales
- Genus: Momordica
- Species: Charantia

**Distribution**
Bitter melon are perennial climbers cultivated throughout India and in Southern China and is now found naturalized in almost all tropical and subtropical regions. It is an edible vegetable in India, Sri Lanka, Vietnam, Thailand, Malaysia, the Philippines, Southern China and also in small scale in tropical America. It is mainly grown for the production of immature fruit, although the young leaves are edible as a vegetable [117].

**Description**
It is an herbaceous, tendril-bearing vine growing up to 5 m in length. It has simple, alternate leaves, with 3-7 deeply separated lobes. Each plant bears separate yellow male (long) and female (short) flowers [118].

The bitter melon fruit is cylindrical shaped and 4 to 12 inches in length and 1 and a half to 3 inches in diameter, oblong, pendulous, fusiform, usually pointed or beaked, ribbed and bearing numerous triangular tubercles.

The young fruit is emerald green, but turns to orange-yellow when ripe. At maturity, the fruit splits into irregular valves that curl backwards and release numerous brown or white seeds encased in scarlet arils. In cross section the fruit is hollow with thin layer of flesh surrounding a central cavity filled with large flat.

Staminate flowers are yellow, solitary on a bracteates scape, hypanthium shallow, calyx 5 lobed and petals 5.

Seeds are few to numerous, 8 to 13mm, ovate, long compressed, usually sculptured [119]. All parts of the plant, including the fruit, taste bitter.

**Biochemical components** [120-123]:
The main biochemical components responsible for medicinal effects include alkaloids, momordicin, charantin, charine, cryptoxanthin, cucurbitins, cucurbitacins, cucurbitanes, cycloartenols, diosgenin, elaeeostearic acids, erythrodial, flavonoids, galacturonic acids, gentisic acid, goyaglycosides, goyasaponins, guanylate cyclase inhibitors, gypsogenin,
hydroxytryptamines, karounidiols, lanosterol, lauric acid, linoleic acid, linolenic acid, momordicharases, momorcharins, momordinol, momordicillin, momordicine, momordicosides, momordin, momordolo, multiflorenol, myristic acid, nerolidol, oleanolic acid, oleic acid, oxalic acid, pentadecanes, peptides (MCh-1 and MCh-2), petroselinic acid, polypeptides, proteins, ribosome-inactivating proteins, rosmanin acid, rubixanthin, spinasterol, steroidal glycosides, polypeptide"p"or plant insulin, stigmastadiols, stigmasterol, taraxerol, trehalose, trypsin inhibitors, uracil, vaccine, v-insuline, verbascoside, vicine, zeatin, zeatin riboside, zeaxanthin, zeinoxanthin, aspartic acid, serine, glutamic acid, thscinne, alanine, gaminobutyric acid and picolic acid, ascorbigen, bisstosterol-d-glucicide, citruline, elasterol, flavochrome, lutein, lycopene, picolic acid, phenolic compounds like, gallic acid, tannic acid, caffeic acid, p-coumaric, gentisic acid, chlorogenic acid, and epicatechin.

**Structure of few phytochemicals:**

![Structure of phytochemicals](image)

**Figure 10: Structure of phytochemicals present in *Momordica charantia* **

**Cucurbitane type triterpenoids:**
The cucurbitane type triterpenoids found in plants and which belongs to the cucumber family (Cucurbitaceae) are the main chemical constituents of M. charantia. Many cucurbitane type triterpenoids are isolated from different part of M. charantia. [124] Charantin, a mixture of two compounds, namely, sitosteryl glucoside and stigmasteryl glucoside [125]; kuguacins A-S; 3β, 25-dihydroxy-7β-methoxy cucurbita-5, 23(E)-diene, 3β-hydroxy-7β, 25-dimethoxy cucurbita-5, 23(E)-diene, 3β, 7β, 25-trihydroxy cucurbita-5, 23(E)-diene-19-al, 5β, 19-epoxycucurbita-6, 23(E)-diene-3β, 19, 25-triol, 5β, 19-epoxy-19-methoxycucurbita-6,23(E)-diene-3β, 25-diol [126,127]; 3β, 25-dihydroxy-5β,19-epoxycucurbita-6, 23(E)-diene 12; momordicine I, II and III [128]; karavilagenin A, B, C, D and E; 19(R)-methoxy-5β,19-

[C129-131]

Cucurbitane type triterpene glycoside:

Oleanane type triterpene saponins:
The goyasaponins I, II and III are the Oleanane type triterpene saponins present in the fresh fruit of M. charantia. [121]

Phenolic and flavonoids compound:
Phenolics, phenols or polyphenolics (or polyphenol extracts) are chemical components that occur ubiquitously as natural colour pigments responsible for the colour of fruits of plants. Phenolics in plants are mostly synthesized from phenylalanine via the action of phenylalanine ammonia lyase (PAL). They are very important to plants and have multiple functions. The most important role may be in plant defence against pathogens and herbivore predators, and thus are applied in the control of human pathogenic infections (Puupponen-Pimiä et al., 2008). They are classified into (i) phenolic acids and (ii) flavonoid polyphenolics (flavonones, flavones, xanthones and catechins) and (iii) non-flavonoid polyphenolics. Caffeic acid is regarded as the most common of phenolic compounds distributed in the plant flora followed by chlorogenic acid known to cause allergic dermatitis among humans (Kar, 2007). Phenolics essentially represent a host of natural antioxidants, used as nutraceuticals, and for their enormous ability to combat cancer and are also thought to prevent heart ailments to an appreciable degree and sometimes are anti-inflammatory agents. Other examples include flavones, rutin, naringin, hesperidin and chlorogenic.

HPLC analysis has been proved that leaf, stem, fruit (green and ripe) contains Phenolic compounds like Gallic acid, tannic acid, (+)-catechin, Caffeic acid, p-coumaric, gentisic acid, chlorogenic acid and epicatechin [135].
Biological activities [116]
Root - Acrid, astringent, bitter.
Leaf - Antipyretic, bitter, emetic, purgative.
Fruits - Acrid, anthelmintic, anti-diabetic, anti-inflammatory, appetizer, bitter, depurative, digestive, purgative, stimulant, stomachic, thermogenic.
In ayurveda it is said that bitter melon contain:
Rasa - Tikt, katu
Guna - Laghu
Virya - Usna
Vipaka - Katu
Karma - Vatahara, Kaphaharr, Raktadosahara, Dipana, Hrdya, Bhedi

Nutritional value of MC per 100 g (3.5 oz)
- Energy 79 kJ (19 kcal)
- Carbohydrates 4.32 g
- Sugars 1.95 g
- Dietary fiber 2 g
- Fat 0.18 g
- Protein 0.84 g
- Vitamin A equiv. 6 μg (1%)
- beta-carotene (1%) 68 μg
- Lutein xanthin 1323 μg
- Thiamine (B1) (4%) 0.051 mg
- Riboflavin (B2) (4%) 0.053 mg
- Niacin (B3) (2%) 0.28 mg
- Pantothenic acid (B5) (4%) 0.193 mg
- Vitamin (B6) (3%) 0.041 mg
- Folate (B9) (13%) 51 μg
- Vitamin C (40%) 33 mg
- Vitamin E (1%) 0.14 mg
- Vitamin K (5%) 4.8 μg
- Calcium (1%) 9 mg
- Iron (3%) 0.38 mg
- Magnesium (5%) 16 mg
- Manganese (4%) 0.086 mg
- Phosphorus (5%) 36 mg
- Potassium (7%) 319 mg
- Sodium (0%) 6 mg
- Zinc (8%) 0.77 mg
- Water 93.95 g

Bakare RI et al. have showed the nutritional and chemical composition in different parts of MC using standard analytical methods which showed the calorific values for leaf, fruit and seed were 213.26, 241.66 and 176.61 Kcal/100 g respectively. The elemental analysis of M. charantia leaf revealed the presence of potassium (413 ppm), sodium (2200 ppm), calcium (20510 ppm), zinc (120 ppm), magnesium, iron, manganese and copper. Vitamins like, Vitamin A (0.03 ppm), vitamin E (600 ppm), folic acid (20600 ppm), cyanocobalamin (5355 ppm) ascorbic acid (66000 ppm), niacin (B3), pyridoxine (B6), cholecalciferol (Vitamin D) and phyloquinone (Vitamin K) were present in the methanolic and pet-ether leaf extract of MC. They also showed that phytochemicals like alkaloids, tannins, flavonoids, saponins and glycosides were present [136]. Chungath TT et al. have showed that uncovered (47.9%) and covered (65.2%) boiling of MC leads to high loss of charantin when compared to other types
of cooking like microwave (91.7%), blanching (88.3%) and steaming (86.6%). The total amount of charantin was taken as 100% and the analysis was done using soxhlet extract of unripe MC and charantin was estimated by high performance liquid chromatography [137].

**Traditional use of different parts of the plant [14,117,138]**

**Fruit**: used in treatment of diabetes, obesity, cancer, tumors, asthma, burning sensation, colic, constipation, cough, malaria, gout, helminthiases, inflammation, leprosy, skin diseases, ulcer, wound, pyorrhea, blood purification and prevention of liver damage

Leaves: are used in treatment of diabetes, menstrual troubles, burning sensation, constipation, malaria, colic, worms and parasites infection, measles, hepatitis, helminthiases, and to induce abortion.

Seeds: are used in the treatment of ulcers, liver and spleen problems, diabetes, intestinal parasites, high cholesterol, wounds and stomachache

Roots: are used in the treatment of syphilis, rheumatism, boils, ulcer, septic swellings, opthalmia, and in Prolapsus vaginæ

**Hypoglycaemic effect:**
The biochemical components like Polypeptide -p, a plant insulin, charantin, vicine, glycosides present in bitter melon regulate blood sugar levels by their hypoglycaemic effects via different physiological and biochemical processes Polypeptide -p, a plant insulin closely resembles bovine insulin with the exception of one extra amino acid, methionine [139]. These include insulin, secretagogue like effect, stimulation of skeletal muscle and peripheral cell glucose utilization, inhibition of intestinal glucose uptake, inhibition of hexokinase activity, suppression of key gluconeogenic enzymes, stimulation of key enzymes of hexose monophosphate (HMP) pathway, preservation of pancreatic islet cells and their functions and inhibition of adipocyte differentiation [12,14,140-142]. A novel protein termed as ADMc1 from the seed extract of M. charantia showed significant antihyperglycemic activity in type 1 diabetic rats and in non-obese diabetic mice. The amino acid sequence of the protein ADMc1 isolated from the seed extract of M.charantia used for comparative sequence analysis and homology modeling and the results showed common disulfide linkage pattern and structural conservation with 2S albumin family proteins. The protein ADMc1 was identified as a novel small protein of two chains consisting of four disulfide bonds. The proteins of this family primarily act as storage proteins and also diverse functions have been reported for many proteins of this family [143]. Oral administration of aqueous extract of MC fruit protect the cardiac remodeling in type 1 diabetic animal model by increasing the antioxidant level, reducing the collagen deposition and specific collagen expressions which shows that it contains antihyperglycemic, antioxidative and cardioprotective properties which may be beneficial in the treatment of diabetic cardiac fibrosis [144]. The nanofraction extracts of M. charantia and its adsorption on polyethylene glycol (PEG) microspheres represent an alternative for the control and treatment of blood disorders in diabetic patients. The nanoparticles of MC extracts lowered blood viscosity at equivalent rates in normo and hyperglycemic individuals. PEG microspheres alone did not reduce blood viscosity in hyperglycemic individuals. However, PEG microspheres adsorbed with nanofraction extracts of M. charantia reduced blood viscosity [145]. The methanolic fruit extract of MC exhibited anti-hyperglycaemic effects comparable to those of metformin, in alloxan-induced diabetic rats. Significant reduction in fasting blood sugar (FBS) occurred at 2 hours for 125mg/kg of extract (-3.2%), 375mg/kg of extract (-3.9%) and metformin (-2.6%) when compared to normal saline. The maximum percentage reduction in FBG occurred between 3 and 12 hours post dose. The anti-hyperglycaemic activity increased with an increase in dose of extract [146]. One of the study showed that MC has a weaker hypoglycemic effect but
ameliorates the diabetes associated cardiovascular risk factors more effectively than glibenclamide [147].

**Anti-obesity effect:**
The biochemical components in bitter melon improve lipid profiles by different mechanism. Bitter melon decreases the body weight gain by increasing the hepatic and muscle mitochondrial carnitine palmitoyl transferase-I (CPT-1) and acyl-CoA dehydrogenase enzyme [148]. Carnitine palmitoyltransferase (CPT) system is the predominant system for transporting the fatty acid to mitochondrial matrix. Adipocyte hypertrophy is inhibited by lowering mRNA levels of fatty acid synthase, acetyl-CoA carboxylase-1, lipoprotein lipase, and adipocyte fatty acid-binding protein, downregulating lipogenic genes in adipose tissues [17]. The adipocyte death is facilitated by Camp activated protein kinase (PKA) mediated apoptosis in white adipose tissues. Adipocyte differentiation is inhibited by reducing PPARγ, SREBP, and perilipin mRNA gene expression and by increasing lipolysis in primary human adipocyte [142]. MC increase the activity of adenosine 5 monophosphate kinase (AMPK), an enzyme that facilitates cellular glucose uptake and fatty acid oxidation. It reduce liver secretion of apolipoprotein B (Apo B) – the primary lipoprotein of low-density "bad" cholesterol reduce apolipoprotein C-III expression, the protein found in very-low density cholesterol which turns into LDL/Bad Cholesterol and increases the expression of apolipoprotein A-I (ApoA1) the major protein component of high density "good" cholesterol [20]. Bitter melon also improve obesity-associated peripheral inflammation and neuroinflammation by increased Sirt3 mRNA expression, normalized Sirt1 protein levels and oxidative stress, suggesting antioxidant effect of BM. Further reduction and normalization of ionized calcium-binding adapter molecule 1(lba1), Glial fibrillary acidic protein (GFAP), NF-kB1, IL-16 and IL-22 mRNA expression suggests that BM inhibits microglial cells activation possibly by Sirt-NF-kB interactions. BM not only prevent neuroinflammation characterized by blood brain barrier (BBB) disruption, lymphocyte transmigration across BBB and microglial cells activation, but also prevent systemic stress and inflammation [149].

**Anti-cancer effect:**
The anti-tumor activity of bitter melon has recently begun to emerge. Many researchers have found that treatment of bitter-melon-related products in a number of cancer cell lines induces cell cycle arrest and apoptosis without affecting normal cell growth. MC derivative Alpha-eleostearic acid act by decreasing cell proliferation, G(2)-M block in the cell cycle, increase apoptosis on human breast cancer cell lines [150] and increase apoptosis in luekaemia and colon cancer cell lines [132]. Oral administration of BME inhibits prostate cancer progression in TRAMP mice by interfering cell cycle progression and proliferation as evidenced by reduced expression of Proliferating cell nuclear antigen (PCNA), and PARP cleavage [151]. MC treatment in Head and neck squamous cell carcinoma (HNSCC) cell lines Cal27, JHU-29 and JHU-22 cells, showed reduced phosphoStat3, c-myc and Mcl-1 expression, downstream signaling molecules of c-Met, modulated the expression of key cell cycle progression molecules leading to halted cell growth and also bitter melon feeding in mice bearing HNSCC xenograft tumor showed inhibition of tumor growth and c-Met expression [152]. Kwatera D et al. determined that Momordica Charantia extract (MCE) enhanced the effect of doxorubicin (DOX) on colon cancer cell lines, HT-29 cells and Madin-Darby canine kidney (MDCK) cell proliferation and sensitized the cells towards DOX upon pretreatment and reduction in the expression of Multidrug resistance conferring proteins (MDRCP) P-glycoprotein (P-gp), multidrug resistance associated protein 2(MRP-2) and breast cancer resistance protein (BCRP). MCE suppressed pregnane X receptor (PXR) promoter activity thereby suppressing its expression and also MCE use different pathways other than AMPK pathway for the anticancer and MDR modulating activities. All this suggest that MCE can enhance the bioavailability and efficacy of conventional chemotherapy [153]. Weng JR et al.determined that the bioactive constituent 3β,7β-dihydroxy-25-methoxycurcurbita-5,23-diene-19-al (DMC), a cucurbitane-type triterpene isolated from a crude extract of wild bitter
gourd, induced apoptotic death in breast cancer cells, Michigan Cancer Foundation-7 (MCF-7) and MDA-MB-231, through PPARγ activation which provides a mechanistic basis to account for the antitumor activity of wild bitter gourd [154]. Konishi T et al. demonstrated that the MCE containing 1-monopalmitin is most potent than soybean, dokudami and welsh onion in inhibiting the P-gp activity in Caco-2 intestinal cells [155]. Ray RB et al. showed that MCE treatment on human breast cancer cells, MCF-7 and MDA-MB-231 and primary human mammary epithelial cells resulted in a significant decrease in cell proliferation and induced apoptotic cell death accompanied by increased poly (ADP-ribose) polymerase cleavage and caspase activation. The study also showed that MCE treatment of breast cancer cells inhibited survivin and claspin expression and enhanced p53, p21, and pChk1/2 and inhibited cyclin B1 and cyclin D1 expression, suggesting an additional mechanism involving cell cycle regulation [156]. Deshmukh et al. showed that the treatment of crude fruit and endophyte extracts of MC on HeLa cell lines were shown the highest antiproliferative activity which was ranged from 70 to 96% [157]. Fongmoon D et al. showed that cytotoxicity effect of MCE on cervical cancer cell line i.e, 0, 51 and 98% at concentrations of 80, S100 and 120 μg/ml for Henrietta Lacks (HeLa) cells was 0, 30 and 70% at concentrations of 140, 160 and 180 μg/ml for Siha cells [158].

**Side effects:**
The recommended dose of Charantia is 3 grams per day. Alkaloid substances like quinine, resins and saponic glycosides may be intolerable by few people. A potentially fatal hypoglycemic coma was reported in two children. Individuals with glucose-6-phosphate deficiency are at risk of developing favism [16]. Toxic symptoms include excessive salivation, facial redness, dimness of vision, stomach pain, nausea, vomiting, diarrhea, muscular weakness. Bitterness and toxicity can be reduced by parboiling and soaking it in salt water for 10 minutes. The plant should be avoided in pregnancy [159].

**Storage of MC juice:**
It was showed that potassium metabisulphite is a better preservative than Na-benzoate and their combination for the stability of physicochemical and phytochemical components and maintaining the antioxidant activity of the MC juice in a study of 6 months duration [160].

Because of adverse effect of modern drugs and growing burden of obesity and its complications, the natural products are gaining its importance among the researchers to find the natural compound which work without any adverse effect or with less side effect and much attention has been directed towards the development of ethno-medicines with strong antioxidant and anti-inflammatory properties which can be used to treat obesity and its complications.

**Research Question**
- Although preliminary experimental animal model studies have implicated the influence of MC on glucose-insulin metabolism in T2DM, knowledge gaps still exist in the action of MC on lipid metabolism
- Therefore we planned an experimental study to elucidate the effects of MC on the expression of adipogenic factors (PPAR & SREBP) in cell lines and animal models