Chapter 1
Introduction

Obesity

- Serum lipids/Blood glucose: \( \uparrow \uparrow \)
- Proinflammatory adipokines: \( \uparrow \uparrow \uparrow \)
- Anti-inflammatory adipokines: \( \downarrow \downarrow \)
- Insulin resistance: \( \uparrow \)
- Hypoxia: \( \uparrow \)

- Chronic low-grade Inflammation
- Proliferative pathways
- Anti-apoptotic pathways

Cancer

- Progression
- Occurrence
- Metastasis
- Resistance to chemotherapy
- Cancer-related mortalities
1.1. Metabolic diseases

Metabolism is a biochemical process involving complex set of biochemical reactions, which are used to generate energy from food at cellular level in an organism. Food consists of proteins, carbohydrates and fats. Various enzymes and other factors in the body break down food into simpler substances like sugars and acids which are utilized by the body as fuel. Organisms either use these substances directly/immediately, or store them as energy in the body tissues (organ), such as liver, muscles in the form of glucose or in the adipose tissue as fat.

Metabolic diseases are any of the diseases or disorders those disrupt normal metabolism. Metabolic diseases occur if the metabolic processes fail and cause the body to have either too much or too little of the essential substances which are vital for normal health and homeostasis. Thousands of enzymes participating in a number of interdependent metabolic pathways accomplish these processes. Metabolic diseases affect the ability of the cell to perform critical biochemical reactions which involve the processing or transport of proteins (amino acids), carbohydrates (sugars and starches), or lipids (fatty acids).

Our bodies are very sensitive to errors in metabolism. The body must have amino acids and many types of proteins to perform all of its functions. For example, the brain needs calcium, potassium and sodium to generate electrical impulses, and lipids (fats and oils) to maintain a healthy nervous system.

Metabolic diseases could be in many forms, for instance:

1. A missing enzyme or vitamin which is vital for the important biochemical reactions;

2. Abnormal chemical reactions those hamper metabolic processes;

3. Disease in the liver, pancreas, endocrine glands or other vital organs involved in metabolism;

Metabolic diseases are typically hereditary, yet most persons affected by them may appear healthy for days, months, or even years. The onset of symptoms usually occurs when the body’s metabolism comes under stress such as after prolonged fasting or during a febrile illness. Metabolic diseases involve any alteration in the normal metabolism of carbohydrates, lipids, proteins and nucleic acids, evidenced by various syndromes and health complications. These are associated with either a deficiency or an excess of a particular metabolic component resulting in an imbalance in corresponding metabolic pathway.

All metabolic diseases have a genetic background, and some of them are expressed as specific genetic diseases. Other factors affecting metabolism include internal control mechanisms those are superimposed on the genetic background. One of the most important mechanisms is the hormonal control system, which consists of the endocrine, paracrine and autocrine systems. The second control system that has a significant effect on metabolism is the neural control system. The third control system is the immune control system, which relates to both, the endocrine and neural systems. Genetic background, environmental factors, and the three major control mechanisms, in conjunction with age and sex, bring about profound changes in metabolism, which ultimately result in structural and functional alterations.

1.1.1. Diseases of amino acid metabolism

These include abnormalities related to increased (hyperproteinemia) or decreased (hypoproteinemia) production of proteins, production of abnormal proteins, and excretion of unusual amounts of amino acids. Hypoproteinemia can result from lack of amino acids, a metabolic block, or other disturbances with normal protein synthesis. Albuminuria, which is manifested as loss of albumin in the urine is common cause of hypoproteinemia. Kwashiorkor is another condition of hypoproteinemia resulting from dietary deficiency. Amino acids are the building blocks of proteins. Hereditary disorders of amino acid
processing can result from defects either in the breakdown of amino acids or in their absorption. Diseases those affect the metabolism of amino acids include phenylketonuria, tyrosinemia, homocystinuria, non-ketotic hyperglycinemia and maple syrup urine disease. These disorders are autosomal recessive, and all may be diagnosed by analyzing amino acid concentrations in body fluids.

1.1.2. Diseases of carbohydrate metabolism

The metabolism of the carbohydrates galactose, fructose, and glucose is intricately linked through interactions between different enzymatic pathways, and disorders affecting these pathways may have symptoms ranging from mild to severe or even life-threatening. Clinical features include various combinations of hypoglycemia (low blood sugar), liver enlargement, and muscle pain. Most of these disorders can be treated, or at least controlled, with specific dietary interventions. Cluster of diseases caused due to abnormal carbohydrate metabolism include mainly glycogen storage diseases (GSD) which are caused by defects in the enzymes involved in the metabolism of glycogen, resulting in growth abnormalities, weakness, and confusion. The main GSDs include Pompe disease, von Gierke disease, Cori disease, Forbe disease and McArdle disease. Other carbohydrate metabolism-related diseases include galactosemia, hereditary fructose intolerance, fructose 1,6-diphosphatase deficiency, congenital disorders of glycosylation and disease of pyruvate metabolism. However, the most important disease associated with carbohydrate metabolism is diabetes mellitus (especially type-2), which is characterized by increased circulatory levels of glucose, which in turn, adversely affects whole-body physiology.

1.1.3. Diseases of nucleic acid metabolism

Purines and pyrimidines are essential building blocks of DNA, RNA, and compounds involved in cellular energy transfer and biosynthetic reactions (e.g., adenosine triphosphate, ATP). Purine and pyrimidine disorders have a wide spectrum of signs and symptoms, including autism, kidney stones,
susceptibility to infections, and severe mental retardation. Symptoms may present from infancy to old age. Most metabolic screening tests do not detect disorders of purine or pyrimidine metabolism; hence, they must be specifically sought out by having specialized analyses performed. The main nucleotide metabolism-related diseases include adenosine deaminase (ADA) deficiency, Lesch-Nyhan syndrome and lupus erythematosus.

1.1.4. Diseases of lipid metabolism

Although lipid stores are considered as secondary energy reserve in starvation, the breakdown of lipids associated with diabetes and starvation leads to the production of ketone bodies in the urine resulting in serum acidosis (ketosis). Alteration in lipid metabolism causes hyperlipemia, an excess of lipid in the blood which is often secondary to uncontrollable diabetes, hypothyroidism, biliary cirrhosis, and lipoid nephrosis. A condition of excess proliferation of fat cells is known as a lipoma, which occasionally becomes malignant, producing a liposarcoma. Diseases associated with lipid metabolism include Gaucher's disease, Tay-Sachs disease, Niemann-Pick disease Fabry's disease, familial hypercholesterolemia, familial dysbetalipoproteinemia, abetalipoproteinemia, sudden infant death syndrome (SIDS), medium-chain acyl-CoA dehydrogenase deficiency (MCAD), long-chain 3-hydroxy-acyl-CoA dehydrogenase (LCHAD) deficiency. However, due to sedentary lifestyle, consumption of fat-rich diet and reduced physical activity, of most concern in the modern world is the developing trend of increase in fat deposits leading to overweight and obesity.

1.2. Obesity

Obesity is a chronic metabolic state characterized by an excess accumulation of body fat. It is a complex disease of appetite regulation and energy metabolism controlled by specific biological factors. Obesity is defined as an excessively high amount of body fat in relation to lean body mass. Obesity is characterized by measuring body mass index (BMI) (Kopelman, 2000). BMI is expressed in mathematical formula in which a person's body weight in
kilograms is divided by the square of his or her height in meters \[
\text{Body weight (kg)/Height}^2 \ (\text{m})
\]. How obesity related multiple defects are associated with cancer is a major focus of current research efforts. Individuals with a BMI of 25 to 29.9 are classified as overweight, while individuals with a BMI of 30 or more are considered obese (Kopelman, 2000; Aronne, 2002).

The obesity epidemic has been recognized by the World Health Organization (WHO) as one of the top 10 global health problems. WHO has also recognized that the prevalence of obesity is rapidly rising to epidemic proportions around the world. The WHO has reported that the fundamental causes of this epidemic are sedentary lifestyles, high-fat/energy-dense diets, booming economy and increased urbanization (James et al., 2001; Caballero, 2007). Obesity causes devastating and costly health problems, reduces life expectancy, and is associated with stigma and discrimination. Several other diseases have been linked to obesity (Figure 1), including diabetes, heart diseases, high blood pressure, stroke and certain types of cancer (Aronne, 2002).

About 2.1 billion adults are overweight all over the world, among which approximately 700 million are obese (Ng et al., 2014). Data from the National Health and Nutrition Examination Survey (NHANES) revealed dramatic increase in the prevalence of overweight and obesity in the adult population in US (Flegal et al., 2002; Ogden et al., 2002; Hedley et al., 2004). Worldwide, there has been an astonishing increase in rates of obesity and overweight in both adults (28% increase) in the past 33 years (1980-2013), with the number of overweight and obese people rising from 857 million in 1980 to 2.1 billion. This increase in weight is not limited to adults; a similar trend is evident in children. The percentage of overweight in children has increased by 47% between 1980 and 2013 in the world. In India, around 41 million people are obese. About 19% male and 21% female are overweight in India. Approximately 20% Indian school children are overweight which is an alarming situation.
Excess fat typically locates readily to either the upper abdominal part of the body, or to lower sites around the hips and thighs. Abdominal fat stores are classified as superficially subcutaneous, deep subcutaneous and visceral components, which differ in their metabolic activity and contribute in varying degrees to the hormonal milieu. The visceral adipose tissue depots are located in the greater and lesser omenta and the mesenteric fat plus some retroperitoneal deposits. The ratio of the waist-to-hip circumference measurement has been the most frequently used method to assess body fat distribution in epidemiological studies. The upper body or central obesity is represented by a high ratio. However, the waist circumference alone is a somewhat better indicator of the visceral adipose tissue than the waist-to-hip ratio (Vona Davis et al., 2007). Besides its public health implications, the economic burden associated with obesity is staggering. The excess body fat predisposes an obese individual to chronic diseases, such as coronary heart disease, type 2 diabetes and diseases of the gall bladder and several types of cancer.
1.2.1. Classification of obesity

Proper identification and classification of obesity through determination of BMI and waist circumference together with identification of specific clinical risk factors are the most important steps to be taken before beginning weight loss treatment. Individuals with a BMI of 25 to 29.9 are considered overweight, while individuals with a BMI of 30 or more are considered obese. Additionally, people who carry fat mainly around the waist are more likely to develop obesity-related health problems (Aronne, 2002; Kopelman, 2000). The international classification of obesity on the basis of BMI is shown in Table 1.

Table 1 – The International Classification of adult underweight, overweight and obesity according to BMI (Taken from WHO webpage)

<table>
<thead>
<tr>
<th>Classification</th>
<th>Principal cut-off points (BMI(kg/m²))</th>
<th>Additional cut-off points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Underweight</td>
<td>&lt;18.50</td>
<td>&lt;18.50</td>
</tr>
<tr>
<td>Severe thinness</td>
<td>&lt;16.00</td>
<td>&lt;16.00</td>
</tr>
<tr>
<td>Moderate thinness</td>
<td>16.00 - 16.99</td>
<td>16.00 - 16.99</td>
</tr>
<tr>
<td>Mild thinness</td>
<td>17.00 - 18.49</td>
<td>17.00 - 18.49</td>
</tr>
<tr>
<td>Normal range</td>
<td>18.50 - 24.99</td>
<td>18.50 - 22.99</td>
</tr>
<tr>
<td>Overweight</td>
<td>≥25.00</td>
<td>≥25.00</td>
</tr>
<tr>
<td>Pre-obese</td>
<td>25.00 - 29.99</td>
<td>25.00 - 27.49</td>
</tr>
<tr>
<td>Obese</td>
<td>≥30.00</td>
<td>≥30.00</td>
</tr>
<tr>
<td>Obese class I</td>
<td>30.00 - 34.99</td>
<td>30.00 - 32.49</td>
</tr>
<tr>
<td>Obese class II</td>
<td>35.00 - 39.99</td>
<td>35.00 - 37.49</td>
</tr>
<tr>
<td>Obese class III</td>
<td>≥40.00</td>
<td>≥40.00</td>
</tr>
</tbody>
</table>

On the basis of fat accumulation in the body, obesity is classified as “apple-shaped” obesity and “pear-shaped” obesity. In “apple-shaped” obesity, bodies of people are heavier in the middle and have their body fat accumulated around the waist, closer to the heart, and carry a lot of weight around their abdomens putting them at a higher risk for metabolic syndrome and abdominal obesity, which in turn, promotes number of diseases, including
type II diabetes, cardiovascular disease and certain cancers. In “pear-shaped” obesity, excess fat is deposited in the lower body especially on the hips and buttocks. These individuals have a narrower waist, and are at a relatively lower risk of developing diabetes, heart disease and other complications of metabolic syndrome. Besides its public health implications, the economic burden associated with obesity is overwhelming.

1.2.2. Management of obesity

Obesity is a chronic, stigmatized and economically-costly disease. The potencies of treatment options for the management of obesity—including behavior/lifestyle interventions, pharmacotherapy, and bariatric surgery—generally increase in proportion to their risk and cost. Because obesity can rarely be cured, treatment strategies are effective only as long as they are used especially in combination with dietary control.

1.2.2.1. Lifestyle modification

Many studies demonstrate that obese adults can lose about 0.5 kg per week by decreasing their daily intake to 500 to 1000 kcal below the caloric intake required for the maintenance of their current weight (Wadden and Foster, 2000). Over a period of six months, persons who combine caloric restriction and exercise may lose about 5 to 10 percent of body weight. It also helps long-term maintenance of a reduced weight (Blackburn, 1999; Smyth and Heron, 2006). In the later stages, pharmacological intervention will become more prevalent.

Lifestyle modification—a combination of diet, physical activity, and behavioral strategies such as keeping records or setting goals—is central to weight management (Wadden et al., 2013). Current guidelines suggest the use of comprehensive lifestyle modification as the first-line treatment for obesity, with a recommended initial weight loss of 5%–10% of body weight within the first 6 months (Jensen et al., 2014). It is recommended that patients attempt to achieve an energy deduction of ≥500 kcal/day by means of physical activity, reduction in caloric intake, or both based on patients' preferences and health
status (Jensen et al., 2014). Pharmacological and surgical approaches to weight loss are recommended to be used as adjuncts to lifestyle modification in appropriate patients (Apovian et al., 2015).

1.2.2.2. Current pharmacotherapies

Present management of obesity essentially includes, exercise and diet modification, surgical intervention, treatment with approved drugs, plant extracts and plant derived compounds. Currently approved drugs for long-term treatment of obesity include orlistat, phentermine/topiramate, Lorcaserin, naltrexone/bupropion and Liraglutide (Patel, 2015). Drugs like diethylproprion, phendimetrazine and phentermine have been approved by the food and drug administration (FDA), USA for short-term use. The detailed list of the FDA approved drugs for long-term treatment of obesity is given in Table 2.

Table 2 – FDA-approved anti-obesity drugs for long term use (Adapted from Patel, Metabolism. 2015)

<table>
<thead>
<tr>
<th>Generic name</th>
<th>Trade name</th>
<th>Year of approval</th>
<th>MOA</th>
<th>Dosage</th>
<th>Clinical trial(s)</th>
<th>Weight loss relative to baseline weight, drug vs. placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orlistat</td>
<td>Xenical®</td>
<td>1999</td>
<td>GI lipase inhibitor</td>
<td>120 mg TID</td>
<td>XENDOS</td>
<td>−5.8 vs. −3.0; 4 years</td>
</tr>
<tr>
<td>Lorcaserin</td>
<td>Belviq®</td>
<td>2012</td>
<td>Serotonin 3C receptor agonist</td>
<td>10 mg BID</td>
<td>BLOOM</td>
<td>−5.8 vs. −2.2; 1 year</td>
</tr>
<tr>
<td>Phentermine/topiramate ER (extended release)</td>
<td>Qsymia®</td>
<td>2012</td>
<td>Sympathomimetic amine with anorectic effect/mechanism unknown</td>
<td>3.75 mg/23 mg (41 days) 7.5 mg/46 mg (thereafter)</td>
<td>CONQUER*</td>
<td>−10.2 vs. −1.4; 1 year</td>
</tr>
<tr>
<td>Naltrexone/bupropion</td>
<td>Contrave®</td>
<td>2014</td>
<td>Opioïd receptor antagonist/ aminoketone antidepressant</td>
<td>32 mg/360 mg (achieved after 4 weeks)</td>
<td>COR-I</td>
<td>−6.1 vs. −1.4; 1 year</td>
</tr>
<tr>
<td>Liraglutide</td>
<td>Saxenda®</td>
<td>2014</td>
<td>GLP-1 receptor agonist</td>
<td>3 mg</td>
<td>SCALE Maintenance</td>
<td>−6.0 vs. −0.1; 1 year</td>
</tr>
</tbody>
</table>

BID = twice daily; GLP-1 = glucagon-like peptide-1; MOA = mechanism of action; TID = three times daily.

* The numbers reported here are for the higher dose of phentermine 15 mg/topiramate 92 mg and placebo.
* Data not yet published.
**Current options for long-term pharmacotherapy**

Obesity and its comorbidities have been established as chronic conditions requiring long-term management. This has spurred the development of many long-term anti-obesity pharmacotherapies, which are either in the market or in the pipeline (Kim et al., 2013; Yanovski et al., 2014).

**Orlistat:** Orlistat is approved for long-term treatment of obesity in conjunction with a reduced-calorie diet. It is an inhibitor of pancreatic lipase that prevents the hydrolysis and absorption of triglycerides in the intestine (Genentech USA, Inc., 2013; Bray et al., 2014), leading to the excretion of nearly 30% of ingested fat (Yanovski et al., 2014). It is available in 2 versions: 120 mg dose by prescription, marketed under the name Xenical® approved for adults and adolescents aged 12–16 years (Yanovski et al., 2014), and an over-the-counter 60 mg dose marketed under the name Alli® for adults (Greenway et al., 2009). Major side effects of orlistat include gassiness, cramps, and oily stools.

Lorcaserin, marketed as Belviq®, is approved for chronic weight management as an adjunct to a reduced-calorie diet and increased physical activity in adults. Lorcaserin is prescribed at 10 mg twice daily (BID) to be taken orally (Woodcliff Lake, NJ: Eisai Inc.; 2014). It is a selective serotonin 2C (5-HT2C) receptor agonist that is thought to regulate energy balance and satiety by activating 5-HT2C receptors in the hypothalamus (Heisler et al., 2006; Thomsen et al., 2008; Woodcliff Lake, NJ: Eisai Inc., 2014). Consistent with lorcaserin’s proposed mechanism of action, mice lacking the 5-HT2C receptor are hyperphagic, develop obesity, and do not show anorectic responses to serotonergic agents that suppress appetite; these effects are attenuated in mice expressing 5-HT2C receptors in proopiomelanocortin (POMC) neurons (Xu et al., 2008; Berglund et al., 2013)

**Phentermine/Topiramate:** Phentermine/topiramate extended release (ER), marketed as Qsymia®, is a combination therapy approved for chronic weight management as an adjunct to a reduced-calorie diet and increased physical
activity in adults. The starting dose is phentermine 3.75 mg/topiramate 23 mg for 14 days. After 14 days, it is recommended to increase the dose to phentermine 7.5 mg/topiramate 46 mg. If the drug does not induce ≥3% initial body weight loss by 12 weeks, treatment with phentermine/topiramate is to be discontinued (Mountain View, CA: VIVUS, Inc.; 2014). Phentermine/topiramate was approved with a risk evaluation and mitigation strategy (REMS) to address the risk of orofacial clefts in infants, the importance of pregnancy prevention during its use, and the need to discontinue use if pregnancy occurs (http://www.qsymiarems.com/index.html). Phentermine is a sympathomimetic amine that acts to increase concentrations of norepinephrine in the central nervous system, thereby suppressing appetite (Jordan et al., 2014). Topiramate is a γ-aminobutyric acid receptor modulator. However, the mechanism by which topiramate regulates weight is not well understood (Bray et al., 2014).

**Naltrexone/Bupropion:** A combination of naltrexone/bupropion was approved by the FDA in September 2014. Naltrexone/bupropion (32 mg/360 mg), marketed as Contrave®, is a combination therapy approved for chronic weight management as an adjunct to a reduced-calorie diet and increased physical activity in adults (Takeda Pharmaceuticals America, Inc.; 2014). Bupropion is a dopamine and norepinephrine reuptake inhibitor, and its anorexigenic effect is likely due to combined dopaminergic and noradrenergic effects on POMC signaling (Greenway et al., 2009). Naltrexone is an opioid receptor antagonist that has weight-loss effects (Greenway et al., 2009).

**Liraglutide:** It is a medication that was previously approved for treatment of type 2 diabetes and has been recently approved in a higher dose as an obesity medication for patients with or without diabetes. In December 2014, the U.S. Food and Drug Administration approved Saxenda (liraglutide [rDNA origin] injection) as a treatment option for chronic weight management in addition to a reduced-calorie diet and physical activity (Novo Nordisk Inc.; 2014). Saxenda is a glucagon-like peptide-1 (GLP-1) receptor agonist and should not be used in combination with any other drug belonging to this class,
including Victoza, a treatment for type 2 diabetes. Saxenda and Victoza contain the same active ingredient (liraglutide) at different doses (3 mg and 1.8 mg, respectively). However, Saxenda is not indicated for the treatment of type 2 diabetes, as the safety and efficacy of Saxenda for the treatment of diabetes has not been established.

Current options for short-term pharmacotherapy

The short-term, anti-obesity pharmacotherapies were all approved before it became apparent that tackling obesity required a long-term approach. There are currently four FDA-approved noradrenergic agents indicated for short-term use (usually considered ≤12 weeks) in managing obesity. None of these drugs are required by the FDA to meet the current standard of inducing mean weight loss of ≥5% more than placebo (Yanovski et al., 2014).

**Phentermine:** Phentermine was approved by the FDA for short-term use (12 weeks) to treat obesity, but it is often prescribed, off-label, for long-term use. It is marketed as Adipex-P®, Ionamin, Obepe, Obermine, Phentrol, and Phenterex in the US (Yanovski et al., 2014). Phentermine is the most commonly prescribed medication for obesity in the US and is indicated for short-term use as an adjunctive therapy in patients with BMI ≥30 kg/m2 or BMI ≥27 kg/m2 with obesity-related comorbidities (Cosentino et al., 2013). It is a sympathomimetic that acts as an appetite suppressant by inhibiting adrenaline reuptake (Zhang et al., 2012). Phentermine is administered orally once or twice daily with dosing ranging from 15 to 37.5 mg daily (Cosentino et al., 2013).

**Diethylpropion, Phendimetrazine, and Benzphetamine:** These 3 drugs are also FDA approved for short-term adjunctive treatment of obesity, but are prescribed far less often than phentermine (Yanovski et al., 2014). All of these three drugs act primarily as appetite suppressants.

**Sibutramine:** It inhibits the reuptake of both serotonin and noradrenaline, produces a feeling of satiety or fullness, and therefore decreases food intake. It is indicated for the management of obesity, including weight loss and weight
maintenance when used in conjunction with a reduced-calorie diet. The principal side effects include dry mouth, insomnia and asthenia (Seagle et al., 1998). In 2010, it was withdrawn from the market due to increase blood pressure and heart rate in patients.

1.2.2.3. Surgical interventions

Expensive surgical intervention is the only proven way to achieve long-term weight control for the severely obese person with a BMI of 40 or greater or a BMI of 35 to 40 with other health problems (Yanovski and Yanovski, 2002). Obesity surgery involves creating a small pouch in the stomach and a small outlet at the bottom of the pouch (bariatric surgery) that limits ingestion of food before one feel full. Some patients develop complications following surgery. These include wound infections, leaks or narrowing at connection to the pouch, ulcers, hernias, breathing problems and blood clots.

1.2.2.4. Herbal medicine for the management of obesity

In addition to fenugreek, only limited numbers of plants have been investigated for the application in the management of obesity. Randomized, controlled trials have shown that intake of vegetables 400 g/day and fruit 300 g/day contribute to weight reduction in obese patients (Svendsen et al., 2007). Green tea polyphenol, epigallocatechin gallate can act directly to inhibit differentiation of preadipocytes and to induce apoptosis of mature adipocytes is proposed as an important adjunct in the treatment of obesity (Lin et al., 2005). Extract of Panax ginseng berry is shown to decrease body weight of ob/ob mice (Attele et al., 2002). Moderate use of grape juice and white wine are also known to decrease body fat, waist circumference, blood pressure, blood glucose, insulin, TG and cholesterol (Flechtner-Mors et al., 2004). Since Garcinia extract inhibits lipid droplet accumulation without affecting adipose conversion in 3T3-L1 cells, it is proposed to be useful in preventing obesity (Hasegawa, 2001). Our group has reported that a novel thermostable extract of fenugreek seeds (TEFS) shows hypolipidemic activity through inhibition of fat accumulation and upregulation of LDL receptor (Vijayakumar et al., 2010).
1.2.3. Established cell lines

Mouse preadipocyte 3T3-L1 cell line is one of the well characterized models for studying the conversion of preadipocytes into adipocytes (Green and Kehinde, 1975). 3T3-L1 preadipocytes spontaneously differentiate over a period of several weeks into fat cells when maintained in culture with fetal calf serum. This can be potentiated by adipogenic cocktail containing dexamethasone (DEX), a glucocorticoid agonist and isobutylmethylxanthine (IBMX), a cAMP-phosphodiesterase inhibitor. Insulin has been used in combination with these inducing agents. Within 1 h after the addition of differentiation inducing cocktail, enhanced expression of c-fos, c-jun, junB, c-myc and CCAAT/enhancer binding proteins (C/EBP) β and δ is observed (Cornelius et al., 1994). The activity of C/EBPs then leads to the expression of PPAR-γ. Once activated, PPAR-γ and C/EBP-α cross-regulate each other and sustain their gene expression (Shao and Lazar, 1997). Exposure of preadipocytes to hormonal agents also leads to an early upregulation of ADD1/SREBP-1c gene expression (Ericsson et al., 1997). ADD1/SREBP-1c enhances the expression of endogenous PPAR-γ ligands and lipogenic enzymes. Differentiated 3T3-L1 cells are visually distinct from the original cells by the presence of oil droplets in the cytoplasm (Pandey et al., 2009).

1.2.4. Animal models of obesity

C57BL/6J-ob/ob mouse, db/db mouse, KK mouse and its yellow mouse variants (KKAy), obese Zucker (fa/fa) rat, and Zucker diabetic fatty (ZDF/drt-fa) rat have been produced which exhibit obese phenotype. C57BL/6J mice exposed to HFD are the commonly employed model to study weight lowering effects of drugs and plant products.

1.3. Adipose tissue

Adipose tissue is the loose connective tissue wherein the energy is stored in the form of fat. There are two types of adipose tissue, brown and white adipose tissue. Brown adipocytes function to dissipate energy in the form of
heat. A defect in brown adipose machinery advances to obesity (Bray and York, 1979). White adipocytes are a dedicated cell type that synthesize and store TG in the time of nutritional excess and can hydrolyze and liberate non-esterified fatty acids on requirement. The number of adipocytes in an organism is not fixed categorically. Therefore, the expansion of adipose cell number can occur any time in reaction to nutritional excess (Ailhaud et al., 1992). This provides a foundation for understanding the physiological and pathophysiological mechanisms that trigger adipose tissue formation and for the development of novel and sound therapeutic approaches to treat lipid related diseases. In addition to proteins involved in lipid and lipoprotein metabolism (cytokines, and growth factors) adipocytes also synthesize factors involved in the regulation of food intake and energy homeostasis (Scherer, 2006). Adipocyte-derived factors include leptin, adipsin, acylation stimulation protein, agouti, angiotensin II, prostaglandins, Acrp30, resistin, TNF-α, IL-6 macrophage migration inhibitory factor, secreted protein (Trujillo and Scherer, 2006). Generally, these adipose tissue derived factors are referred to as adipokines that interact with a large number of different organ systems (Figure 2).

![Factors Secreted by Adipose Tissue](http://www.medscape.org)

**Figure 2 – Factors secreted by white adipose tissue** (Adapted from http://www.medscape.org).
In obese state, the secretory profile of adipose tissue is altered. Figure 3 elaborates the alteration in secretory profile of adipose tissue in obesity, which, in turn, leads to many obesity-associated health complications.

**Figure 3 – Alteration in secretory profile of adipose tissue in obesity** (Adapted from Khandekar et al., 2011).

### 1.4. Adipokines

The discovery of adipokines or adipocytokines unveils novel mechanisms by which obesity, inflammation and immunity might be connected to each other. Emerging evidence suggests that the adipokines, such as leptin, resistin and adiponectin, which are synthesized mainly in the adipose tissue function in a hormone-like manner and have much in common to classical cytokines. These two mediators have recently dominated the field of adipokine research,
and it is fairly evident that these are involved in many diseases beyond obesity (Tilg and Moschen, 2008).

1.4.1. Leptin

Leptin was first detected and cloned as the monogenic mutation responsible for the morbidly obese phenotype observed in the *obese* (*ob/ob*) mouse (Zhang et al., 1994). Leptin is a highly conserved 16-kDa hormone secreted mainly by adipose tissue and is present in circulation and cerebrospinal fluid. Serum leptin levels are positively correlated with body mass index (BMI) with concentrations in human serum at approximately 1–10 ng/ml (Friedman, 2002). Leptin acts on the region which controls regulation of food uptake in hypothalamus. Centrally, it is capable of altering food intake, body weight, energy expenditure, and neuroendocrine functions, whereas it also has peripheral effects on skeletal muscle, liver, pancreas, adipose tissue, and numerous other cell types (Niswender and Schwartz, 2003). Figure 4 shows the role of leptin receptor on cellular signaling.

![Figure 4 – Leptin mediated signaling pathways](Adapted from Ando & Catalano, Nat. Rev. Endocrinol., 2011).
As shown in figure leptin receptor interacts with several important signaling intermediates at membrane level which is further propagated at the nuclear level, thereby causing several changes in cellular systems.

1.4.2. Adiponectin

Adiponectin is a 30-kDa adipose-specific secreted protein (Kadowaki and Yamauchi, 2005). Adiponectin levels in human serum range from 5–30 nm, with circulating levels approximately two to three times higher in females (Scherer et al., 1995; Combs et al., 2003). The mature protein consists of an amino-terminal collagen-like domain and a carboxy-terminal head domain has structural similarities to complement factor C1q. Serum adiponectin (FL-Ad) is found as a low-molecular-weight complex consisting of a dimer of trimers as well as a high-molecular-weight complex consisting of up to six trimers (Pajvani et al., 2003). A third form, generated by cleavage of the collagenous stalk region that results in globular trimer (gAd), has not conclusively been shown to exist as a physiological intermediate but has potent pharmacological activity (Fruébis et al., 2001). Plasma levels of adiponectin are decreased in response to several metabolic impairments, including T2DM, dyslipidemia and extreme obesity. This obesity-related decrease can be partially reversed by weight loss. Figure 5 shows the signaling modules affected by adiponectin and functions attributed to them.
1.4.3. Resistin

Resistin is a name derived from “resistance to insulin”. It is a hormone secreted by adipocytes and is known to predispose the individuals to diabetes and thus provides an important link between obesity and type 2 diabetes. Resistin also known as adipose tissue-specific secretory factor (ADSF) or C/EBP-epsilon-regulated myeloid-specific secreted cysteine-rich protein (XCP1) is a cysteine-rich adipose-derived peptide hormone that in humans is encoded by the RETN gene. Resistin was discovered in 2001 by the group of Dr. Mitchell A. Lazar from the University of Pennsylvania School of Medicine.

In primates, pigs, and dogs, resistin is secreted by immune and epithelial cells, while, in rodents, it is secreted by adipose tissue. The length of the resistin pre-peptide in human is 108 amino acid (aa) residues and in the mouse and rat it is 114 aa; the molecular weight is ~12.5 kDa. Resistin is an adipose-derived hormone (similar to a cytokine) whose physiologic role has
been the subject of much controversy regarding its involvement with obesity and type II diabetes mellitus (T2DM). Figure 6 illustrates the signaling mediated by resistin in cells.

Figure 6 – Resistin signaling in cells (Adapted from Booth et al., Horm Mol Biol Clin Investig, 2015).

1.4.4. Other newly discovered adipokines

PBEF/visfatin is a 52 kDa secreted molecule. Intracellular PBEF/visfatin acts as a dimeric type II phosphoribosyltransferase (NAD biosynthesis) (Martin et al., 2001; Rongvaux et al., 2002; Wang et al., 2006). Visfatin works synergistically with insulin to enhance glucose uptake and metabolism in muscle and to block gluconeogenesis in liver. A new adipocytokine termed vaspin (visceral adipose tissue- derived serine protease inhibitor) has been recently identified (Hida et al., 2005) and is known to improve insulin sensitivity. Preliminary studies indicate that it suppresses the production of TNF-α, leptin and resistin (Hida et al., 2005). Omentin, another new adipocytokine, is a protein expressed and secreted from visceral adipose
tissue selectively that increases insulin sensitivity in human adipocytes (de Souza Batista et al., 2007).

1.5. Cancer

Cancer is a group of diseases characterized by the uncontrolled growth and spread of abnormal cells. If the spread is not controlled, it can result in death. Cancer is caused by external factors, such as tobacco, infectious organisms, and an unhealthy diet, and internal factors, such as inherited genetic mutations, hormones, and immune conditions. These factors may act together or in sequence to cause cancer. Ten or more years often pass between exposure to external factors and detectable cancer. Treatments include surgery, radiation, chemotherapy, hormone therapy, immune therapy, and targeted therapy (drugs that specifically interfere with cancer cell growth). Cancer cells exhibit uncontrolled growth, invasion and sometimes metastasis. These three malignant properties of cancers differentiate them from benign tumors, which are self-limited, and do not invade or metastasize. Most cancers form a tumor but some, like leukemia, do not. The difference between dividing capacity of normal (non-cancerous) and cancer cells is shown in Figure 7.

Tumor cells display a characteristic set of features those distinguish them from normal cells. These traits allow the individual cells to form a tumor mass and eventually to metastasize to other parts of the body. A wide range of changes occur during the transformation of a normal cell to a cell capable of forming a cancerous growth. All cancer cells acquire the ability to grow and divide in the absence of appropriate signals and/or in the presence of inhibitory signals. There are also detectable changes in the physical properties of the cells. These changes include cytoskeletal changes, cell adhesion and motility and nuclear changes and also secretion of enzymes that enable them to invade neighboring tissues. Data from United States of America suggests the overall estimate of approximately 1,658,370 new cases is the equivalent of more than 4,500 new cancer diagnoses each day. This
estimate does not include carcinoma in situ (noninvasive cancer) of any site except urinary bladder, nor does it include basal cell or squamous cell skin cancers. In 2015, about 589,430 Americans are expected to die of cancer, or about 1,620 people per day (Cancer Facts and Figures 2015; Siegel et al., 2015). Slightly more than 1 million new cases of cancer are diagnosed every year in India. Estimated 600,000–700,000 cancer-related deaths have been documented in 2012 which represents about 8% of all estimated global cancer deaths and about 6% of all deaths in India. Many cancer cases in India are associated with tobacco use, infections, and other avoidable causes (Mallath et al., 2014). Also, in a population-based study in Pune, India, the percentage occurrence of liver, prostate, pancreatic and lung cancer in diabetics is much higher, as compared to their occurrence in non-diabetics (Sinha et al., 2013).

Figure 7 – Normal versus cancer cells (Taken from www.images.flatworldknowledge.com).
1.5.1. Cancer therapy

Surgery is the conventional treatment which includes either tumor removal or removal of tumor containing organ if it’s non-fatal. However, surgical intervention is difficult for vital organs such as brain, heart liver and lung cancers. Another mode of treatment is radiation therapy which involves destruction of cells with X-rays or gamma rays containing high energy and penetrating capabilities are used in a controlled way. Yet another mode of therapy which has developed recently is biologic therapy wherein substances made by the body or made in a laboratory are used to boost, direct, or restore the body’s natural defenses against cancer. An example of this type is vaccine therapy. Chemotherapy is vital for eliminating cancer/cancer cells. The term chemotherapy refers to a wide range of drugs used to treat cancer. These drugs generally work by killing dividing cells. Since cancer cells have lost many of the regulatory functions present in normal cells, they will continue to attempt to divide when other cells do not. This feature makes cancer cells susceptible to a wide range of cellular poisons. There are drugs developed to cause DNA damage, microtubule stabilization and destabilization in proliferating cells (Lowe and Lin, 2000).

Classification of chemotherapeutic agents used in cancer treatment

**Antimetabolites:** Agents which interfere with the formation of key biomolecules in the cells such as nucleotides, the building blocks of DNA. These drugs eventually restrict with DNA replication and thereby cell division. Examples: 5-fluorouracil, methotrexate.

**Alkylating agents:** These are the drugs which act by chemically altering the cellular DNA by adding alkyl groups to the electronegative groups. Example: carboplatin, cisplatin.

**Plant alkaloids and terpenoids:** These plant-based chemicals block cell division by inhibiting microtubule function. Spindle fibres, made of microtubules, help to separate the chromatids during cell division. Examples: vincristine and vinblastine.
Podophyllotoxins: These plant-derived compounds are primarily extracted from *Podophyllum peltatum* (American mayapple). They prevent the cells from entering the G1 phase and also affect DNA synthesis. Examples: etoposide, tenoposide.

Taxanes: Taxanes are plant-based compounds that increase the stability of microtubules thereby preventing the separation of chromatids during mitotic anaphase. Examples: paclitaxel, docetaxol.

Topoisomerase inhibitors: Topoisomerases are enzymes that are essential to maintain the topology of DNA. Interfering with these enzymes prevents the normal functions of the DNA, such as transcription, replication and repair. Examples: topotecan, etoposide.

Antitumor antibiotics: There are many differing antitumor antibiotics, but generally they prevent cell division by two ways: (1) binding to DNA and making it unable to separate; (2) inhibiting ribonucleic acid (RNA), preventing enzyme synthesis. Examples: mitomycin C, doxorubicin.

Hormones: Prednisone and dexamethasone are examples of hormones which, in high doses, can damage lymphoma or lymphocytic leukemia cells.

Monoclonal antibodies: Monoclonal antibodies attach themselves to tumor-specific antigens, thereby increasing immune response to tumor cell. Examples- rituximab, cetuximab and trastuzumab.

1.6. Melanoma

Melanoma is a cancerous growth of melanocytes and most frequently develops in the skin. Melanoma may also develop in other parts of the body that contain melanocytes including the meninges, the digestive tract, the eyes and lymph nodes. Melanomas are categorized based on their appearance, either with the naked eye or microscopically.

Superficial spreading melanoma is the most frequently observed melanoma. This may develop in any region of the skin. Lesions are usually raised around
the edges and a brown color with hints of pink, white, gray and blue. Second type of melanomas characterized by nodular type lesions arises on all regions of the body and is typically black or brown in color. Third are acral lentiginous lesions characterized by flat, brown or black tumors that often develop on the hands and feet. Fourth type is lentigo maligna melanoma which develops on an individual's face and is distinguished by its irregular border and tan to brown color.

1.6.1. Epidemiology

Melanoma is the least common but the most deadly skin cancer, accounting for only about 2% of all cancers, but contributes to the vast majority of skin cancer death. Melanoma accounts for only 3% of skin cancer cases but it causes approximately 80% of skin cancer-related deaths. Melanoma is the fifth most common cancer among males and sixth most common cancer in females. While melanoma is uncommon in African Americans, Latinos and Asians, it is frequently fatal to these populations. Melanomas in African Americans, Asians, Filipinos, Indonesians, and native Hawaiians most often occur on non exposed skin with less pigment, with upto 60-75% of tumors arising on the palms, soles, mucous membranes and nail regions (Cancer Facts and Figures, 2015). According to the International Agency for Research on Cancer (IARC) Report, worldwide 2,50,178 new cases of melanoma have been reported in 2015. Although melanoma cases are uncommon (less than 0.5% of all cancers) in India, the incidences are increasing every year. In 2015, 2,272 new cases of melanoma have been documented in India.

1.6.2. Risk factors

1.6.2.1. Ultraviolet light exposure: Ultraviolet (UV) radiation is a major risk factor for most melanomas. Sunlight is the main source of UV radiation. Tanning lamps and booths are also the sources of UV radiation. People with high levels of exposure to light from these sources are at greater risk for skin cancer, including melanoma.
1.6.2. Moles/Nevus: A person who has many moles is at increased risk for development of melanoma. Although mostly dysplastic nevi (atypical moles which are not present at birth) never become cancerous however, they may turn into melanoma in certain cases. Lifetime melanoma risk may be 10% higher in those who have dysplastic nevi. Congenital myelocytic nevi (moles present since birth) cause a 0-10% increase in the risk for melanoma development depending on the size of nevus.

1.6.2.3. Fair skin, freckles and light hair: Melanoma risk for fair people is 10 times higher than people who have dark complexion. Skin pigment (melanin) has a protective role against harmful radiations such as UV rays. Red haired people have the highest risk.

1.6.2.4. Family history of melanoma: Around 10% of people suffering from melanoma have a history of melanoma in their family. Having melanoma cases in the first degree relatives enhances the risk greatly. A person who had melanoma once has a higher risk of developing it again.

1.6.2.5. Xeroderma pigmentosum: It is an autosomal recessive genetic disorder caused by defects in the DNA repair machinery. Thus the DNA damage caused by UV radiations cannot be repaired leading to increased susceptibility to skin cancer.

1.6.3. Treatment options for melanoma

Early-stage melanomas can often be treated effectively with surgery alone, but more advanced cancers usually require other treatments. Sometimes more than one type of treatment can be used.

Five types of standard treatment are used:

- Surgery
- Chemotherapy
- Immunotherapy
• Targeted therapy
• Radiation therapy

1.6.4. Chemotherapy

Chemotherapy is be used to treat advanced melanoma, but it is not often used as the first treatment since newer forms of immunotherapy and targeted drugs have become available. Chemo is usually not as effective in melanoma as it is in some other types of cancer, but it may relieve symptoms or extend survival for some patients.

Several chemotherapeutic drugs are used to treat melanoma. These include Dacarbazine (also called DTIC), Temozolomide, Nab-paclitaxel, Paclitaxel, Carmustine (also known as BCNU), Cisplatin, Carboplatin, and Vinblastine. Among these, DTIC is used as the first line of chemotherapy. Some of these drugs are given alone, while others are often combined with other drugs. It’s not clear if using combinations of drugs is more helpful than using a single drug, but it can add to the side effects.

Some studies suggest that combining chemotherapeutic drugs with immunotherapy drugs such as interferon-alpha and/or interleukin-2. This type of treatment is also called biochemotherapy or chemoimmunotherapy.

On 28 October 2015, the U.S. Food and Drug Administration expanded the approved use of Yervoy (ipilimumab) to include a new use as adjuvant therapy for patients with stage III melanoma, to lower the risk that the melanoma will relapse following surgery (www.fda.gov).

1.6.4.1. Dacarbazine

Dacarbazine [5-(3,3-Dimethyl-1-triazenyl)imidazole-4-carboxamide], CAS Number: 4342-03-4, (abbreviated as DTIC). DTIC works by methylating guanine at the O-6 and N-7 positions. Guanine is one of the four nucleotides that make up DNA. The alkylated DNA strands stick together such that cell division becomes impossible. This affects cancer cells more than healthy cells.
because cancer cells divide faster. Unfortunately, however, some of the healthy cells are also damaged. The chemical structure of DTIC is shown in Figure 8.

![Chemical structure of dacarbazine](Taken from webpage of Sigma, USA).

1.7. Interrelation of obesity with cancers

A number of studies have recently emerged providing plausible evidence for the role of obesity, an indispensable component of metabolic syndrome and a severe metabolic disorder in pathogenesis and progression of cancer. Excess body weight has been implicated in 20% of all cancers deaths in women and 14% in men and an estimated 90,000 cancer deaths yearly in the US (Calle et al., 2003). There is strong evidence to support the role of obesity in cancer risk and mortality. In a cohort study of 900,000 individuals, those men and women with a body mass index (BMI; body weight measured kilograms divided by the square of height measured in meters) of >40 had death rates from all cancers that were 52 and 62% greater than men and women in the normal range.

Obese individuals have large amount of adipose tissue that secretes various growth factors and cytokines which plays a role in the low-grade, chronic inflammatory state, linking obesity and subsequent cancer risk (Calle et al., 2003). Several hormones serve as intermediate and long-term communicators of nutritional state and have been implicated in both energy balance and carcinogenesis. These hormones include insulin-like growth factor-1 (IGF-1),
insulin and leptin, which play interactive roles in endocrine, paracrine and autocrine signaling networks controlling body composition, energy metabolism and cancer cell growth (Hursting et al., 2007). Obesity causes increased secretion of hormones like leptin and adiponectin in addition to several inflammatory cytokines which facilitate the progression and metastasis of various cancers. Reports which have come up recently suggest a positive link between factors associated with obesity and melanoma occurrence as well as its progression (Brandon et al., 2009, Gogas et al., 2008). The mechanisms regulating energy balance and adiposity, and the relationship of these factors to cancer are not completely understood. The cancers associated with overweight and obesity are listed in Table 3.

Table 3 – List of cancers associated with obesity (Adapted from Renehan et al., Nat Rev Cancer. 2015)

<table>
<thead>
<tr>
<th>Cancer type</th>
<th>Cancer subtype</th>
<th>Number of cohorts</th>
<th>Summary risk estimate (95% CI)</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colon</td>
<td>Adenocarcinoma</td>
<td>29</td>
<td>1.24 (1.20, 1.28)</td>
<td>1.09 (1.05, 1.13)</td>
<td></td>
</tr>
<tr>
<td>Rectum</td>
<td>Adenocarcinoma</td>
<td>29</td>
<td>1.09 (1.06, 1.12)</td>
<td>1.02 (1.00, 1.05)</td>
<td></td>
</tr>
<tr>
<td>Oesophageal</td>
<td>Adenocarcinoma</td>
<td>5</td>
<td>1.52 (1.33, 1.74)</td>
<td>1.51 (1.31, 1.74)</td>
<td></td>
</tr>
<tr>
<td>Oesophageal</td>
<td>SCC</td>
<td>5</td>
<td>0.71 (0.60, 0.85)</td>
<td>0.57 (0.47, 0.69)</td>
<td></td>
</tr>
<tr>
<td>Gastric</td>
<td>Adenocarcinoma</td>
<td>8</td>
<td>0.97 (0.88, 1.06)</td>
<td>1.04 (0.90, 1.20)</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>HCC</td>
<td>9</td>
<td>1.19 (1.09, 1.29)</td>
<td>1.12 (1.03, 1.22)</td>
<td></td>
</tr>
<tr>
<td>Gallbladder</td>
<td>Adenocarcinoma</td>
<td>4</td>
<td>1.09 (0.99, 1.21)</td>
<td>1.59 (1.02, 2.47)</td>
<td></td>
</tr>
<tr>
<td>Pancreatic</td>
<td>Adenocarcinoma</td>
<td>23</td>
<td>1.13 (1.04, 1.22)</td>
<td>1.10 (1.04, 1.16)</td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>Not investigated</td>
<td>13</td>
<td>0.76 (0.70, 0.83)</td>
<td>0.80 (0.66, 0.97)</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>Not investigated</td>
<td>12</td>
<td>1.24 (1.15, 1.34)</td>
<td>1.34 (1.25, 1.43)</td>
<td></td>
</tr>
<tr>
<td>Advanced-stage prostate*</td>
<td>Not investigated</td>
<td>23</td>
<td>1.08 (1.04, 1.12)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Postmenopausal breast</td>
<td>Not investigated</td>
<td>34</td>
<td>NA</td>
<td>1.12 (1.08, 1.16)</td>
<td></td>
</tr>
<tr>
<td>Premenopausal breast</td>
<td>Not investigated</td>
<td>34</td>
<td>NA</td>
<td>0.92 (0.88, 0.97)</td>
<td></td>
</tr>
<tr>
<td>Endometrial</td>
<td>Not investigated</td>
<td>19</td>
<td>NA</td>
<td>1.59 (1.50, 1.68)</td>
<td></td>
</tr>
<tr>
<td>Ovarian</td>
<td>Not investigated</td>
<td>34</td>
<td>NA</td>
<td>1.06 (1.00, 1.12)</td>
<td></td>
</tr>
</tbody>
</table>

BMI, body mass index; CI, confidence interval; HCC, hepatocellular carcinoma; NA, not applicable; SCC, squamous cell carcinoma.
*Defined differently across studies but including: American Joint Committee on Cancer stages 3 and 4; metastatic cancer; Whitmore–Jewett stages C and D; high grade; and Gleason grade 7.
1.7.1. Factors regulating the association of obesity with cancer

Obesity is a chronic state of increased body fat mass. This is associated with many physiological and biological changes. The mechanisms and pathways involved in obesity-related carcinogenicity are multifaceted and difficult to understand. As shown in Figure 9, there are several changes associated with obesity which may lead to increased occurrence or progression of cancer. The central player in this is the altered endocrine functions of adipose tissue. Following are some of obesity related causes which may facilitate occurrence and progression of cancers in obese individuals. Figure 9 shows the proposed role of obesity-associated factors in cancer progression.

Figure 9 – Propose role of obesity-associated factors in cancer development and progression (Adapted from Khandekar et al., 2011).
1.7.1.1. Increased energy intake

Dietary factors account for about 30% of cancers in the Western countries (Doll and Peto, 1981). Thus, diet is second only to tobacco as a modifiable cause of cancer. Total caloric intake along with the composition of consumed food can affect carcinogenesis. Majority of case control and prospective studies imply a causal relationship between increased caloric intake and breast, colon and prostate cancer (Pan et al., 2009). Obesity virtually by definition is a result of overeating energy-rich compounds such as fat and also modern food including complex proteins, toxins and artificial food additives whose metabolic derivatives (for example, toluols, nitrites) may contribute to large bowel carcinogenesis and other malignancies (Key et al., 2002).

1.7.1.2. Decreased energy output

Reduced adipose tissue mass and its hazardous metabolic and endocrine consequences especially reduced pro-inflammatory cytokine expression and physical activity, is independently associated with reduced mortality from cancer. Potential mechanisms include the reduction of oxidative stress and increase of protective cytokines (Thompson, 2006).

1.7.1.3. Increased adipose tissue mass

The endocrine function of adipose tissue is altered when it becomes hypertrophic or hyperplastic. Both conditions result in significant changes in hormone and growth factor secretion. This has complex consequences on molecular pathways involved in inflammation, cell proliferation, oxidative stress and carcinogenesis (Percik and Stumvoll, 2009).

1.7.1.4. Insulin and IGF-1 pathways

Chronic hyperinsulinemia and insulin resistance increases risk for several malignancies (Lukanova et al., 2004; Calle et al., 2004; Yakar et al., 2005; Shaw and Cantley, 2006). The mechanisms are not fully understood but may involve direct growth promoting effects of insulin or indirect effects via
stimulation of the IGF-1 receptor or modulation of the release of other hormones. Both insulin and IGF-1 act in vitro as growth factors to promote cancer cell proliferation and decrease apoptosis (Yakar et al., 2005).

1.7.1.5. Inflammation

Adipose tissue has been shown to release inflammatory adipokines including IL-1, TNF-α and plasminogen- activator inhibitor – 1 (PAI-1) (Trayhurn et al., 2004) which affects cell growth, survival, proliferation and angiogenesis.

1.7.1.6. Sex hormones

Adipose tissue aromatase converts androgenic precursors produced in adrenals and gonads to estrogens. Increased insulin and bioactive IGF-1 levels typically accompanying obesity down-regulate levels of sex hormone binding globulin (SHBG), resulting in an increased fraction of bioavailable estradiol and testosterone. The epidemiologic literature suggests this increased bioavailability of sex hormones is strongly associated with risk of endometrial and postmenopausal breast cancers (Kaaks et al., 2002).

1.7.1.7. Adipokines and cancer

It is evident from the literature that conditioned medium from adipocytes promotes tumorigenesis, including increased cell proliferation, invasive potential, survival, and angiogenesis (Iyengar et al., 2003). Sufficient literature has emerged which attributes these effects to various adipocytokines specifically released by adipose tissue. One of the important factors is leptin which acts as a mitogen for various cell types, including myelocytic and primitive hematopoietic progenitor cells (Bennett et al., 1996), normal and transformed breast epithelial cells (Hu et al., 2002; Somasundar et al., 2003), and vascular endothelial cells (Park et al., 2001; Artwohl et al., 2002). Leptin also exerts antiapoptotic effects (Artwohl et al., 2002; Rouet-Benzineb et al., 2004), and promotes cellular proliferation. By a series of transfection experiments it has been demonstrated that long isoform of leptin receptor can activate signal transducer and activator of transcription (STAT) factors 1, 3,
and 6, mitogen-activated protein kinase (MAPK) protein (Baumann et al., 1996; Yamashita et al., 1998), and c-fos gene transcription (Bjorbaek et al., 1997); whereas the short isoform mainly activates MAPK protein, and has little effect on STAT activation (Bjorbaek et al., 1997; Yamashita et al., 1998). The link between adiponectin and cancer risk is not well-characterized, although there is a report demonstrating that adiponectin infusion inhibits endothelial proliferation as well as growth of transplanted fibrosarcoma (Brakenhielm et al., 2004). Recent findings suggest that leptin and adiponectin interact antagonistically to influence carcinogenesis (Ray et al., 2007; Grossman et al., 2008), although this interaction has not been well established. Accumulating evidence suggests that adiponectin is an important regulator of cell proliferation and it may act either directly on cancer cells or indirectly by regulating whole-body insulin sensitivity.

1.7.1.8. Adipose tissue as a reservoir for lipophilic chemical carcinogens

A new concept has recently emerged which states that there is storage of lipophilic exogenous chemical carcinogens in adipocytes. These can be permanently released in the blood and consequently target peripheral tissues at convenient doses for carcinogenesis (Irigaray et al., 2007). Many environmental chemical carcinogens such as dioxins are indeed lipophilic, so they can bioaccumulate in adipocytes (Irigaray et al., 2005) and these carcinogens may be permanently released from the adipocytes during lipolysis. Likewise, it has been shown that the adipose tissue can store many liposoluble molecules including organochlorinated pesticides (Ohmiya and Nakai, 1977; Chevrier et al., 1998), polychlorinated biphenyls (PCBs), dioxin-like polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/PCDF), e all of which being organochlorines (Payne et al., 2001) and also some polybrominated flame retardants (BFRs) and other pollutants, such as phthalates esters (Mes et al., 1974; Mullerova and Kopesky, 2007).
1.8. Cellular signaling: crosstalk associating obesity with cancer

Cellular signaling consists of an intricate network of innumerable pathways involving infinite signaling intermediates. Obesity provides stimulus for activation or inhibition of many signaling intermediates those affect the occurrence and progression of tumors. These can result in alteration in signaling modules such as JAK-STAT, MAPK and metabolic components as fatty acid synthase (FASN) additionally serve as signaling intermediates in cancer cell physiology and proliferation. The molecules whose expression is altered by the metabolic changes can present important checkpoints for intervention in suppression of carcinogenesis facilitated by obesity.

1.8.1. Akt kinase

Since the discovery that the Akt/protein kinase B (PKB) serine/threonine protein kinase is a target of phosphoinositide 3-kinase (Franke et al., 1995; Toker and Yoeli-Lerner, 2006) and Akt increases cell survival in a PI3K-dependent manner (Dudek et al., 1997), evidences suggest that Akt plays an important role in the pathogenesis of degenerative diseases and cancer (Franke et al., 1995). Akt controls a variety of cellular responses, and that the three Akt isoforms, Akt1 (PKBa), Akt2 (PKBh), and Akt3 (PKBg), are ubiquitously expressed in all cell types and tissues. Akt regulates both growth and survival mechanisms in normal as well as cancer cells by phosphorylating a large number of substrates (Marte and Downward, 1997). Further the downstream targets of Akt also include eNOS (Nitric Oxide Synthase), mTOR (Mammalian Target of Rapamycin), IKK (I-KappaB Kinase), NF-KappaB (Nuclear Factor-KappaB), MDM2 (Mouse Double Minute-2), p21(CIP1) (Cyclin Dependent Kinase Inhibitor-p21), p27(KIP1) (Cyclin Dependent Kinase Inhibitor-p27), Chk1 (Cell Cycle Checkpoint Kinase-1), Raf1 (v-Raf1 Murine Leukemia Viral Oncogene Homolog-1) (Zhou et al., 2001; Toker and Yoeli-Lerner, 2006). Akt has been shown to be involved in differentiation of adipocytes. Histological analysis of the brown adipose tissue of
PKBα−/−PKBβ−/− neonates revealed very thin dorsal pads which contained no visible lipid droplets in the cells (Peng et al., 2003; Baudry et al., 2006). There is inhibition of differentiation of 3T3-L1 cells into adipocytes when PKBα expression is down-regulated by RNAi (Xu and Liao, 2004). Additionally, the contributions of the different PKB isoforms to insulin signaling and maintenance of glucose homoeostasis have been studied extensively using knockout mouse models and RNAi knockdown approaches. Of the three mammalian PKB isoforms, PKBβ correlates with the regulation of glucose homoeostasis and is the predominant PKB isoform expressed in insulin-responsive tissues. Targeted disruption of the PKB-β locus in mice leads to defective insulin signaling as evident by impaired insulin stimulated glucose uptake in muscle and adipocytes, and failure to suppress hepatic glucose output (Cho et al., 2001).

1.8.2. Fatty acid synthase (FASN)

Fatty acid synthase (FASN) is a cytoplasmic enzyme of 270 kDa. This complex enzyme has seven catalytic domains and is multifunctional. Its functional units are thioesterase (TE), malonyl/acetyltransferase (MAT), β-ketoacyl synthase (KS), dehydrogenase (DH), acyl carrier protein (ACP), enoyl reductase (ER) and β-ketoacyl reductase (KR) in which substrates are handed from one functional domain to the next resulting in the synthesis of palmitate (C16:0, a long-chain saturated fatty acid) from acetyl-CoA and malonyl-CoA, in the presence of NADPH. Fatty acids are essential building blocks of cell membrane. These serve as an anchor for membrane proteins and intracellular second messengers and at the same time provide vital substance for energy metabolism. There are two types of fatty acids, one that is endogenously-synthesized and the other derived from exogenously eaten food. The former is synthesized by FASN. Generally, FASN activity and its expression are minimal because FASN expression is controlled by signals derived from hormones (Kuhajda et al., 2000). Increased expression of FASN is found in various tumors including stomach, breast, bladder, prostate, lung, and many other types of cancers (Kuhajda et al., 2000; Kuhajda et al., 2006;
Lupu and Menendez, 2006, Menendez and Lupu, 2007). Various reports suggest that FASN expression and its activity are strongly associated with poor prognosis and aggressiveness of tumors (Kuhajda et al., 2000; Kuhajda et al., 2006; Menendez and Lupu, 2007; Pandey et al., 2012). Inhibitors of FASN such as Orlistat and C75 or siRNA effectively and selectively kill cancer cells (Pizer et al., 2000; Zhou et al., 2003; Wang et al., 2005). With the development of FASN inhibitors, targeting of FASN has opened up an option to treat cancer (Kuhajda et al., 2000; Kridel et al., 2004; Alli et al., 2005; Chiang et al., 2007). The FDA approved drug, orlistat and other naturally occurring flavonoids including C71 and C93 may serve as novel agents to treat tumors (McNeely and Benfield, 1998; Kuhajda et al., 2000; Kridel et al., 2004).

1.8.3. Caveolin (Cav)-1

Plasma membrane is the most complex component of cellular signaling because it being the first part of cell to encounter the changes in cellular microenvironment. It plays important role in drug influx or efflux and in regulation of different cellular signaling, important in drug trafficking. Small caves of 50-100 nm size present in the plasma membrane are known as Caveolae, and have been proposed as targets for cancer chemotherapy. Cav-1 is a structural protein present in plasma membrane and is a 21-24 kDa protein that serves as an inhibitory clamp. It entraps various important signaling molecules through its scaffolding binding domain and regulates their signaling pathways. Cav-1 is reported to be a tumor suppressor gene in breast, oral and in cervical cancers and is found mutated in a very high percentage of breast cancer patients (Hayashi et. al., 2001; Chan et. al., 2003). On the other hand, it appears to be associated with progression of prostate, colon cancer and melanoma. Moreover, some reports also claim its involvement in metastasis of lung, pancreatic, prostate and esophageal cancers (Yang et. al., 1999; Davidson et. al., 2001, Tahir et. al., 2001; Tahir et al., 2008). However, role of Cav-1 in cancer chemotherapy is unexplored and is not very clear. It is known to regulate MDR (Lavie et. al., 1998; Lavie and
Liscovitch, 2000; Lavie et al., 2001; Cai and Chen et al., 2004) but it can also
initiate drug mediated cell death in some cancer cells (Belanger et. al., 2005,
Couet et. al., 2001; Shajahan et. al., 2007). Its expression levels act as a
novel diagnostic marker associated with cisplatin sensitivity in certain cancers
cells (Nakatani et. al., 2005). Collectively, all these reports highlight the
importance of Cav-1 as an important molecule that is associated with different
aspects of cancer cell signaling and potentially has a role to play in cancer
chemotherapy, which is as yet to be explored.

1.8.4. P-glycoprotein (P-gp)

P-glycoprotein (permeability glycoprotein, abbreviated as P-gp) also known as
multidrug resistance protein 1 (MDR1) or ATP-binding cassette sub-family B
member 1 (ABCB1) or cluster of differentiation 243 (CD243) is an important
protein of the cell membrane that pumps many foreign substances out of
cells. More formally, it is an ATP-dependent efflux pump with broad substrate
specificity. It exists in animals, fungi and bacteria and is likely evolved as a
defense mechanism against harmful substances. Drug resistant cancer cells
also express higher level of P-gp, which renders these cancers multi-drug
resistant. P-gp is a glycoprotein that in humans is encoded by the ABCB1
gene. P-gp is a well-characterized ABC-transporter (which transports a wide
variety of substrates across extra- and intracellular membranes) of the
MDR/TAP subfamily. P-gp was discovered in 1971 by Victor Ling. P-gp is a
170 kDa transmembrane glycoprotein, which includes 10-15 kDa of N-
terminus glycosylation. It was shown to be responsible for conferring multidrug
resistance upon mutant cultured cancer cells that had developed resistance to
cytotoxic drugs.

1.8.5. Heat shock proteins (Hsps)

Cells from various ranges of tumors express abnormal levels of one or more
heat shock proteins (Hsps) (Jolly and Morimoto, 2000). Hsps perform various
functions including folding and unfolding of other proteins, regulation of cell
cycle and modulation of p53 function. Hsps are also associated with the
development of cancer drug resistance (Sharma et al., 2010). The main Hsps include Hsp27, Hsp40, Hsp60, Hsp70, and Hsp90. These are associated with signaling proteins, including ligand-independent transcription factors, for example, MyoD (Shaknovich et al., 1992), tyrosine kinases like v-Src (Hartson and Matts, 1994) ligand-dependent transcription factors, for example steroid receptor (Nathan and Lindquist, 1995), and serine/threonine kinases such as Raf-1 (Wartmann and Davis, 1994). Hsp90 is involved in signal-transducing kinase activity and promote conformational maturation of these receptors. Hsp90 is associated with protecting cancer cells from apoptosis.

1.9. Adipokines and cancer progression

The secretory profile of adipokines is altered in obese state. Under obese state, usually, the circulating levels of proinflammatory adipokines are increased while decreased levels of anti-inflammatory adipokines are observed in obesity. This alteration in the levels of adipokines can lead to different aspects of tumorigenesis. Adipokines act through binding to their respective receptors present on the cells and transmit their signals by modulating many important pathways involved in vital functions in the cells. Since cancer cells express receptors for most of the adipokines, there is a cross talk between various molecules and pathways involved in tumorigenesis upon interaction between adipokines and their receptors.

Cancer cells rely on signals transmitted by cytokines for their growth and survival. Therefore, alterations in systemic levels of adipokine provide favorable conditions to cancer cells to grow readily in obese state. The crucial pathways modulated by adipokines include PI3K/Akt, JAK/STAT, MAPK and NF-κB signaling. Once activated, these pathways support cancer cells in terms of survival, growth, invasion, migration and metastasis.

Leptin, resistin, IL-6, and TNF-α are some of the crucial pro-inflammatory adipokines, and provide tumor favoring microenvironment to the cancer cells. Studies suggest that these adipokines are implicated in many obesity-associated cancers including those of breast, liver and prostate. Adiponectin
and SFRP5 are the important anti-inflammatory adipokines which can negatively affect cancer cells with respect to growth and survival. Reports suggest that adiponectin and SFRP5 reduce the cancer risk by suppressing pathways those are vital for carcinogenesis. The role of adipokines with respect to cancer progression, and the outcome of cancer therapy have not been well understood. Therefore, the involvement of adipokines in these aspects needs to be investigated, in order to get better clinical outcome and for preventing rapid cancer progression.

1.10. Obesity and cancer chemotherapy

In addition to being an influential tumor promoting factor, obesity has also been reported to have a critical impact on the outcome of therapeutic responses in obesity-associated cancers resulting in increased cancer-related deaths despite therapeutic regimens. Although the early responses to chemotherapeutic regimens in majority of cancers are promising, the development of chemoresistant phenotypic tumors is an unfortunate common outcome. To investigate the limitations of current therapeutic approaches, researchers and clinicians have commenced evaluating influential host-related factors those implicated in clinical outcomes, including overweight or obesity.

Under obese state, numerous key inflammatory and metabolic factors, and their pathways are hypothesized to mediate the obesity-associated impairment of chemotherapeutic responses (Lashinger et al., 2014). The purported mechanisms underlying increased cancer risk in the obese relate to multiple molecular and metabolic changes arising primarily as a consequence of adipose tissue expansion. The obesity-related changes include elevated hormones and growth factors such as insulin, insulin-like growth factor (IGF)-1, and sex steroid hormones; adipokine imbalances; and a chronic state of low-grade inflammation. The altered systemic and local environment that occurs as a consequence of the obese state not only increases the likelihood of tumor development and progression but also creates the potential for
unfavorable responses to chemotherapeutic regimens. The mechanistic studies on the impact of obesity on the outcome of cancer therapy are lacking. There is a need to re-consider the dosing pattern of chemotherapeutic drugs with concomitant implication of interventions which curtail adiposity.

1.11. Obesity and melanoma

Population-based and meta-analysis of epidemiological studies suggest that obesity is associated with increased risk of melanoma. Both genetic defects and environmental risk factors are involved in the carcinogenesis of melanoma. Activation of multiple signal pathways such as the PI3K/Akt and MAPK pathways are necessary for the initiation of melanoma. Activation of the MAPK pathway as a result of activating mutations in BRAF is commonly seen in melanoma though it alone is not sufficient to cause malignant transformation of melanocytes.

Since it lies in close proximity to subcutaneous adipose tissue, melanoma is a very relevant model to study obesity-cancer connection. Obesity can result in the activation of many signal pathways including PI3K/Akt, MAPK and STAT3 which could be mediated by factors associated with obesity. Activation of these pathways may have a synergistic effect with the genetic defects thereby increasing the incidence of melanoma.

The mechanism-based studies on interrelation between obesity and melanoma are poorly understood. In addition, the role of adipokines in melanoma growth and progression has not been studied very well. More importantly, the impact of obesity and weight control interventions on chemotherapeutic outcome in cancers has not been evaluated so far. Therefore, investigating these aspects are critical to the prevention, better chemotherapeutic outcome would be beneficial in the management of melanoma and possibly other obesity-promoted malignancies.
1.12. Objectives of the study

A number of clinical, epidemiological population-based as well as meta-analysis studies strongly underline relationship between obesity and cancer. However, the association between these two diseases at cellular or molecular level remains to be investigated. The consequences of obesity and obesity-related factors on the outcome of growth, proliferation and survival of solid tumors remain obscure. Most importantly, no study has been done on how controlling obesity alters the risk of developing variety of solid tumors. Being the deadliest form of skin cancer, melanoma is of one of the obesity-promoted malignances, and it lies in close proximity to the subcutaneous adipose tissue. Present study is aimed to address these questions by employing appropriate \textit{in vitro} as well as \textit{in vivo} models of obesity in melanoma with the following objectives:

1. To investigate the impact of controlling obesity on cancer progression.

2. To decipher the role of obesity in the outcome of cancer chemotherapy and the underlying mechanism(s).

3. To study the effect of obesity-related factors or adipokines on cancer progression and elucidating the molecular pathways involved.