3.1. **Type 2 diabetes: an overview**

Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects of insulin secretion, insulin action, or both [American Diabetes Association, 2005]. Some 4.6 million people between 20-79 years of age died from diabetes in 2011 [International Diabetes Federation, 2011]. Forty-eight percent of deaths due to diabetes are in people under the age of 60. The highest number of deaths due to diabetes occurred in countries like India, China, United States of America, and the Russian Federation. The number of people living with diabetes is expected to rise from 366 million in 2011 to 552 million by 2030. At present China is the diabetes capital of the world and India comes second in position. The number of people suffering from diabetes in India is 61.3 million which is expected to rise to 101.1 million in 2030 [International Diabetes Federation, 2011]. There are mainly two types of diabetes: type 1 and type 2. More than 90% of diabetes mellitus cases belong to type 2 diabetes [Jarret, 1991;
Mohan et al., 2007]. Type 2 diabetes mellitus is emerging worldwide as a major health and socio-economic problem [Sreekumar & Nair, 2007; Toye & Gauguier, 2003]. Besides type 1 and type 2 diabetes, there are other specific types of diabetes [Fig. 1] [Fernandez-Mejia, 2006].

Type 2 diabetes is a multifactorial disorder with both genetic and environmental components [Almind et al., 2001; Gerich, 1998; Hamman, 1992; Knowler et al., 1990; Sladek et al., 2007]. The pathogenesis of type 2 diabetes includes relative insulin deficiency, which occurs as a result of insulin resistance coupled with progressive failure of β cells to secrete insulin in response to glucose [Brownlee, 2003; Kasuga, 2006; Saad et al., 1991]. The relative insulin deficiency in type 2 diabetes is manifested in the

Fig. 1: Etiologic classification of diabetes mellitus [Fernandez-Mejia, 2006].
form of altered glucose and fatty acid metabolism in different tissues especially liver, muscle and adipose tissue leading to persistent hyperglycemia [Anderwald & Roden, 2004; Khan, 2003; Skulachev, 1999], increased plasma fatty acid levels, increased accumulation of triacylglycerol and other lipid moieties in non adipose tissue such as β cell, heart, liver and skeletal muscle as well as dysregulation of lipid metabolism during fasting and fed state [Lewis et al., 2002; Peterson et al., 2003]. Profile of serum adipokines or proinflammatory cytokines may alter or several serious complications like stroke, renal failure, blindness, damage to feet and legs, and ischemic heart diseases can occur [Hajer et al., 2008; Taylor, 2004].

3.2. Molecular mechanisms affected in type 2 diabetes:

It is now well accepted that defective post receptor insulin signaling plays a central role in insulin resistance of type 2 diabetes. Insulin signaling is impaired by several mechanisms such as tyrosine dephosphorylation, imbalance of serine/threonine phosphorylation, or insulin receptor internalization [Zick, 2004]. Adipose tissue dysfunction plays a crucial role in the development of insulin resistance in type 2 diabetes [Hajer et al., 2008]. Evidence suggests that molecules such as free fatty acids, tumour necrosis factor α (TNFα), interleukin 6 (IL6), released from adipocytes inhibit insulin signaling and induce insulin resistance, and activate serine/threonine kinases that phosphorylate the insulin receptor substrate (IRS) proteins and inhibit their function [Rajala & Scherer, 2003; Schinner et al., 2005]. At molecular level, elevated free fatty acids (FFAs) causes a
reduction in insulin-stimulated IRS-1 phosphorylation and IRS-1 associated phosphatidylinositol 3 kinase (PI3K) activity [Saltiel & Kahn, 2001]. FFAs by increasing cellular diacylglycerol concentrations activate cellular kinases, including atypical protein kinase C isoforms which in turn activate the inflammatory kinase inhibitor kB (IKK) and c-Jun N-terminal kinases, thus increasing serine/threonine phosphorylation of IRS-1 and eventually reducing downstream IRS-1 signalling [Stumvoll et al., 2005]. Insulin resistant states is often associated with a two-to three fold elevation of circulating IL6 levels. IL6 causes a reduction in tyrosine phosphorylation of the IRS-1, and association of the p85 subunit of PI3K with IRS-1 in response to physiologic insulin levels. In addition, IL6 inhibits insulin dependent activation of Akt [Senn et al., 2002]. These events are mediated through increased expression of suppressor of cytokine signaling-3 (SOCS-3) protein [Senn et al., 2003]. A recent study has shown that IL6 treatment also downregulates expression of IRS-1 and glucose transporter 4 (GLUT 4) [Rotter et al., 2003]. Adipocytes also produce TNFa which is implicated widely in obesity associated insulin resistance and the pathogenesis of type 2 diabetes [Hotamisligil, 1999]. Several mechanisms have been suggested to account for the metabolic effects of TNFa which includes induction of elevated FFAs via stimulation of lipolysis, downregulation of genes required for normal insulin action, such as GLUT4, direct effects on insulin signaling, and negative regulation of peroxisome proliferator-activated receptor γ (PPARγ) [Hotamisligil, 1999; Moller, 2000]. PPAR's are nuclear receptors which by heterodimerizing with the retinoic X receptor regulates transcription of a number of genes. Many genes involved in insulin action,
such as, Sterol Regulatory Element-Binding Protein-1c (SREBP-1c) and Phosphoenolpyruvate carboxykinase (PEPCK) are regulated by PPAR γ [Auwerx, 1999; Tontonoz et al., 1995]. Activation of PPARγ results in inhibition of expression of TNFα, which, in, turn, is an inhibitor of PPARγ gene expression [Remels et al., 2008; Xing et al., 1997]. The notion that PPARγ plays an important role in type 2 diabetes is supported by the recent evidence where families having mutations in PPARγ suffered from severe insulin resistance, diabetes and hypertension [Barroso et al., 1999]. Several investigations have suggested that mitochondrial function might also play a pivotal role in the pathogenesis of insulin resistance and type 2 diabetes [Abdul-Ghani & Defronzo, 2008; Hojlund et al., 2008].

3.3. Obesity: an Overview

Obesity may be considered as the result of positive energy balance in the conditions of energy excess. Economic, social and lifestyle changes are responsible for this common condition of different populations living in environments characterized by abundant calorie rich food and low physical activity [Balistreri et al., 2010; Frayn et al., 1995; Gregoire et al., 1998]. The number of obese individuals world-wide is increasing at an alarming rate, leading to an explosion of obesity related health problems associated with increased morbidity and mortality [Balistreri et al., 2010; McTeman et al., 2005]. More than two thirds of American population is overweight which is common feature for other western population [Balistreri et al., 2010; Ogden et al., 2006]. The highest frequency in obesity is observed in United States, Europe [Pengelly & Morris, 2009]. Obesity predisposes to a variety of
diseases, which includes insulin resistance, type 2 diabetes, hypertension, dyslipidemia, atherosclerosis and its complications, fatty liver diseases, osteoarthritis, rheumatoid arthritis, and cancer [Garg & Misra, 2002; Reaven, 1996; Schelbert, 2000].

Adipose tissue can vary from 2-3% in athletes to 60-70% of body mass in the morbidly obese. There are two patterns of body fat distribution based on somatotypes, i.e android, or male pattern where fat is stored centrally and gynoid, or female pattern where fat is stored on hips and thighs [Arner, 1997]. In comparison with the gynoid obesity, android obesity is more frequently at a risk of diseases like diabetes mellitus, coronary artery disease, gout, and uric acid renal stones [Vague, 1956]. The adipose tissue mass depends upon either the number of adipocytes present or adipocyte volume or both [McTernan et al., 2005]. In case of increased energy intake hypertrophic (increased cell volume) and hyperplastic (increase in adipocyte number) growth can occur. This activity is regulated at the hormonal and genetic level with the help of key genes responsible for lipogenesis or lipolysis [Gregoire et al., 1998; McTernan et al., 2005]. Usually, adipose tissue contains a mixed group of adipocytes of different cell size. As fat mass increases, the adipose tissue loses its heterogeneity with more mature adipocytes having increased cell size [McTernan et al., 2005].

There is a great controversy regarding the relative importance of subcutaneous versus visceral fat in the pathogenesis of metabolic syndrome. Many in vitro and in vivo studies have suggested that visceral fat is responsible for the health risks associated with central obesity [McTernan et
al., 2005]. Visceral fat which constitutes 6-20% of the total body fat volume is metabolically more active and the smaller visceral adipocytes are more responsive to the lipolytic effects of the catecholamines and less responsive to the anabolic effects of insulin [Engfeldt & Arner, 1998; Eriksson et al., 2000; Ostman et al., 1979]. Increased lipolysis results in greater non-esterified fatty acid (NEFA) release, which is implicated in development of insulin resistance [McTernan et al., 2005]. Studies in normal weight subjects, where insulin resistance is induced by lipid infusion have shown that excess NEFAs results in reduction in glucose uptake; glucose oxidation and glycogen synthesis [Ferrannini et al., 1983]. However, many studies contradict this and say that subcutaneous fat which constitutes 80% of the total body fat volume plays a significant role in the pathogenesis of central obesity associated disease [Arner et al., 1991; McTernan et al., 2005]. Evidence suggest that lipolysis within abdominal depots of subcutaneous adipose tissue is twice as high as the gluteofemoral adipose tissue in males [Arner et al., 1991].

3.4. Adipose tissue as an endocrine organ

Adipocytes and adipose tissue are known to express and secrete a wide range of hormones and cytokines, which may act at both the local (autocrine and/or paracrine) and systemic (endocrine) level [Laclaustra et al., 2007]. They are involved in glucose metabolism (e.g. adiponectin, resistin), lipid metabolism (e.g. cholesteryl ester transfer protein, CETP), inflammation (e.g. TNFα, IL6), coagulation (PAI-1), blood pressure (e.g. angiotensinogen, angiotensin II), and feeding behaviour (leptin) thus affecting metabolism and
function of many organs and tissues including muscle, liver, vasculature, and brain [Table 1] [Hajer et al., 2008]. There has been a great interest in the possibility that adipose tissue derived factors can contribute to the metabolic and hemodynamic disturbances observed in obesity related insulin resistance and type 2 diabetes [Hajer et al., 2008]. Plasma adipocytokine levels rise with an increase in adipose tissue and adipocyte volume, except for plasma adiponectin which decreases in obesity [Hajer et al., 2008; Kershaw & Flier, 2004; Skurk et al., 2007].

**Table 1: Adipokines secreted by adipose tissue** [Hajer et al., 2008].

<table>
<thead>
<tr>
<th>Adipocytokine</th>
<th>Full name</th>
<th>Effects on</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin</td>
<td>Leptin</td>
<td>Food intake, fat mass</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>Adiponectin</td>
<td>Insulin resistance, inflammation</td>
</tr>
<tr>
<td>Resistin</td>
<td>Resistin</td>
<td>Insulin resistance, inflammation</td>
</tr>
<tr>
<td>Visfatin</td>
<td>Visfatin</td>
<td>Insulin resistance</td>
</tr>
<tr>
<td>Omentin</td>
<td>Omentin</td>
<td>Insulin resistance</td>
</tr>
<tr>
<td>Vaspin</td>
<td>Visceral adipose tissue-derived serpin</td>
<td>Insulin resistance</td>
</tr>
<tr>
<td>Apelin</td>
<td>Apelin</td>
<td>Vasodilation</td>
</tr>
<tr>
<td>CETP</td>
<td>Cholesteryl ester transfer protein</td>
<td>Lipid metabolism</td>
</tr>
<tr>
<td>LPL</td>
<td>Lipoprotein lipase</td>
<td>Lipid metabolism</td>
</tr>
<tr>
<td>HSL</td>
<td>Hormone sensitive lipase</td>
<td>Lipid metabolism</td>
</tr>
<tr>
<td>A-FABP 4 (aP2)</td>
<td>Adipocyte fatty acid-binding protein 4</td>
<td>Lipid metabolism</td>
</tr>
<tr>
<td>Pinolipin</td>
<td>Pinolipin</td>
<td>Lipid metabolism</td>
</tr>
<tr>
<td>RBP4</td>
<td>Retinol-binding protein 4</td>
<td>Lipid metabolism</td>
</tr>
<tr>
<td>ASP</td>
<td>Acylation stimulating protein</td>
<td>Lipid metabolism</td>
</tr>
<tr>
<td>AT II</td>
<td>Angiotensin II</td>
<td>Blood pressure</td>
</tr>
<tr>
<td>ACE</td>
<td>Angiotensin converting enzyme</td>
<td>Blood pressure</td>
</tr>
<tr>
<td>AGT</td>
<td>Angiotensinogenes</td>
<td>Blood pressure</td>
</tr>
<tr>
<td>TNF-a</td>
<td>Tumour necrosis factor-a</td>
<td>Inflammation</td>
</tr>
<tr>
<td>IL-6</td>
<td>Interleukin-6</td>
<td>Inflammation</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
<td>Inflammation</td>
</tr>
<tr>
<td>Adipsin</td>
<td>Adipocyte triglyceride complement factor D</td>
<td>Inflammation</td>
</tr>
<tr>
<td>MCR-1</td>
<td>Macrophage chemo attractant protein-1</td>
<td>Macrophage attraction</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>Interleukin adhesion molecule-1</td>
<td>Macrophage activation</td>
</tr>
<tr>
<td>PAI-1</td>
<td>Plasminogen activator inhibitor-1</td>
<td>Fibrinolysis</td>
</tr>
</tbody>
</table>
3.4.1. Leptin

Leptin production is prominently augmented in large adipocytes [Considine et al., 1996; Hajer et al., 2008], is induced by eating-related hormones, energy status, sex hormones (being inhibited by testosterone and increased by ovarian sex steroids) and several proinflammatory mediators [Hajer et al., 2008; Saladin et al., 1995; Zhang et al., 2000]. Leptin acts via melanocortin system in the hypothalamus [Cheung et al., 1997; Schwartz et al., 2000]. It stimulates lipolysis, inhibits lipogenesis, improves insulin sensitivity, increases glucose metabolism, and stimulates fatty acid oxidation [Balistreri et al., 2010] and thus leptin is considered as a signalling molecule which relates the long-term nutritional and fat mass status to the brain [hypothalamus] [Cheung et al., 1997; Schwartz et al., 2000] as shown in Fig. 2 [Kershaw & Flier, 2004]. It is also known to be involved in other processes, such as the proliferation of lymphocytes (particularly CD4+) and induction of Th1 response, cytokine production, phagocytosis, and regulation of hypothalamic-pituitary-adrenal-axis, reproduction, angiogenesis, and oxidative stress [Lago et al., 2007; Lago et al., 2009]. Leptin increases with obesity [Van Hermelen et al., 1998; Zimmet et al., 1996] and is associated in insulin resistance in type 2 diabetes and obesity through its regulation of the deposition of fat in insulin responsive tissues, rather than through its effect on insulin signaling [Clarke & Mohamed-Ali, 2005]. Leptin is found more abundantly in subcutaneous than in visceral fat [Kershaw & Flier, 2004]. Leptin levels decrease rapidly with caloric restriction and weight loss [Kershaw & Flier, 2004].
3.4.2 Adiponectin

Adiponectin is exclusively produced by adipocytes. It circulates in plasma in three different full-length isoforms (trimer, hexamer, and multimers) and as globular form [Hajer et al., 2008; Kershaw & Flier, 2004]. Adiponectin increases fatty acid oxidation in both muscle and liver with reduction in plasma fatty acid levels, decreases plasma glucose levels, increases insulin sensitivity through inhibition of hepatic glucose production as shown in Fig. 3. Adiponectin also shows anti-inflammatory, antioxidant, antiatherogenic and anticancer properties through the inhibition TNF-alpha-mediated NF-κB pathway [Balistreri et al., 2010].
Adiponectin level is found to be reduced in obesity, insulin resistance, metabolic syndrome, and type 2 diabetes [Chandran et al., 2003; Diez & Iglesias, 2003; Hotta et al., 2001]. Adiponectin expression is higher in subcutaneous adipose tissue than in visceral adipose tissue [Fain et al., 2004; Kershaw & Flier, 2004]. Men have lower plasma adiponectin levels than women [Hajer et al., 2008]. Several polymorphisms in the adiponectin gene are associated with obesity and insulin resistance [Chandran et al., 2003; Diez & Iglesias, 2003]. Adiponectin level increases when insulin sensitivity improves as well as after weight reduction or treatment with insulin sensitizing drugs [Chandran et al., 2003; Diez & Iglesias, 2003]. It has recently been shown that adiponectin expression and mitochondrial content in adipose tissue are reduced in obese db/db mice, and these
changes are reversed by treatment with rosiglitazone. In cultured adipocytes, induction of increased mitochondrial biogenesis (via adenoviral overexpression of nuclear respiratory factor-1) increases adiponectin synthesis, whereas mitochondrial functional impairment decreases it [Koh et al., 2007].

3.4.3 Interleukin 6

IL6 is found to be associated with obesity and insulin resistance [Fernandez-Real & Ricart, 2003] and circulates in multiple glycosylated forms ranging from 22 to 27 kDa in size. IL6 is one of the crucial pro-inflammatory mediator, secreted by several body's cell types like monocytes, adipocytes, endothelial cells, fibroblasts, etc. [Cancello & Clément, 2006; Juge-Aubry et al., 2005]. Expression and secretion of IL6 are 2 to 3 times greater in visceral adipose tissue compared to subcutaneous adipose tissue [Fain et al., 2004; Wajchenberg, 2000]. One third of circulating IL6 originates from adipose tissue [Fernandez-Real & Ricart, 2003]. IL6 has varied effects on muscle, adipose tissue, liver and brain [Fig. 4] [Kershaw & Flier, 2004].

Adipose tissue IL6 expression as well as circulating IL6 concentrations are positively correlated with obesity, impaired glucose tolerance, and insulin resistance [Fernandez-Real & Ricart, 2003]. Both expression and circulating levels of IL6 have been found to decrease with weight loss [Fernandez-Real & Ricart, 2003]. Furthermore, genetic polymorphisms of the IL6 locus have been linked to obesity, energy expenditure, insulin sensitivity, and type 2 diabetes [Fernandez-Real & Ricart, 2003]. Peripheral administration of IL6

Fig. 4: Functions of IL6 [Kershaw & Flier, 2004].

IL6 plays a complex role in energy homeostasis and in the CNS are found to be negatively correlated with fat mass in overweight humans, suggesting central IL6 deficiency in obesity. Furthermore, central administration of IL6 increases energy expenditure and decreases body fat in rodents [Kershaw & Flier, 2004].
3.4.4. Tumour Necrosis Factor α

TNFα is another important proinflammatory cytokine and is the first adipokine to be directly associated with insulin resistance [Pekala et al., 1983]. Associations between the expression of TNFα in adipose tissue and obesity and insulin resistance has been reported in both humans and animals [Hotamisligil et al., 1993; Hotamisligil et al., 1995]. There is accumulating evidence showing its capacity to modulate components of the insulin signaling cascade, its effect on fat oxidation and adipocyte apoptosis and the expression and activity of the other adipokines [Clarke & Mohamed-Ali, 2005]. The role of TNFα has been discussed earlier.

3.5. Type 2 diabetes and obesity: metabolic dysfunction

Obesity is considered as one of the most important risk factor for type 2 diabetes [Chan et al., 1994; Reaven, 1988]. Obesity, body fat distribution and weight gain throughout adulthood are important predictors of diabetes [Chan et al., 1994; Goossens, 2008; Hans et al., 1998]. The cause and effect relationship between obesity and insulin resistance is well known [Bak et al., 1992; Friedman et al., 1992]. Adipose tissue dysfunction plays a pivotal role in the etiopathogenesis of obesity-related insulin resistance and type 2 diabetes. Enlarged adipocytes, ectopic fat storage, impaired adipose tissue blood flow, local inflammation, macrophage infiltration seems to be interrelated and lead to the development and/or progression of insulin resistance and ultimately to type 2 diabetes [Goossens, 2008].
3.5.1. Ectopic fat storage syndrome

Many recent studies have suggested that NEFAs induce different isoforms of protein kinase C which interfere with the intracellular signaling pathway of insulin and ultimately block glucose transport activity [Griffin et al., 1999]. Adipose tissue helps in buffering the daily influx of dietary fat entering the circulation by suppressing the release of NEFAs into the circulation and by increasing the clearance of triacylglycerol (TAG) [Goossens, 2008]. In obesity, adipose tissue becomes overloaded with TAG and the buffering capacity of adipocytes is reduced in the postprandial state [Frayn, 2001, Goossens, 2008]. This causes an influx of TAG and fatty acids which results in the storage of triacylglycerol and other lipid moieties in non-adipose tissues like skeletal muscle [Forouhi et al., 1999; Jacob et al., 1999; Pan et
al., 1997], liver [Banerji et al., 1995; Bjorntorp et al., 1995], and pancreatic β cells [Koyama et al., 1997]. Ectopic fat storage plays an important role in the development of insulin resistance and/or impaired insulin secretion [Goossens, 2008] [Fig. 5].

3.5.2 Adipose tissue blood flow in lipid metabolism

Blood flow helps in regulating metabolism in both muscle and adipose tissue [Baron & Clark, 1997; Bulow & Madsen, 1981; Galitzky et al., 1993; Samra et al., 1996]. Evidence suggests that disturbances in adipose tissue blood flow (ATBF) may affect adipose tissue lipid handling. This results in increased lipid supply to non-adipose tissues, which in turn may lead to ectopic fat deposition as discussed earlier [Ardilouze et al., 2004; Bulow et al., 1987; Coppack et al., 1992; Evans et al., 1999]. It has been suggested that in obesity, both fasting ATBF and ATBF responsiveness to nutrients are reduced [Blaak et al., 1995; Goossens et al., 2007; Jansson et al., 1998; Summers et al., 1996; Virtanen et al., 2002]. An impaired postprandial ATBF seems to be associated with insulin resistance [Jansson et al., 1998; Karpe et al., 2002]. It has been reported that in obese compared to lean subjects, plasma TAG extraction in adipose tissue is decreased in the fasting and postprandial state [Potts et al., 1995].

3.5.3 Adipose tissue inflammation and macrophage infiltration

Adipose tissue is a heterogeneous tissue and consists of different types of cells, including mature adipocytes, preadipocytes, endothelial cells, vascular smooth muscle cells, leukocytes, monocytes and macrophages [Goossens,
Macrophages are non-adipocyte cells which contribute to adipose tissue production of inflammatory cytokines except for adiponectin and leptin which are primarily secreted by adipocytes [Fain, 2006; Goossens, 2008]. As obesity develops, adipose tissue gets infiltrated by more and more macrophages [Weisberg et al., 2003; Xu et al., 2003] and this infiltration may play an integral role in the inflammatory response of adipose tissue. Macrophage secreted products on adipocytes may contribute significantly to the systemic inflammation and insulin resistance associated with obesity [Permana et al., 2006].

3.6. Mitochondria and its functions: an overview

Mitochondria perform several important cellular functions, including essential pathways of oxidative phosphorylation, intermediating metabolism, amino acid biosynthesis, fatty acid oxidation, steroid metabolism and apoptosis and also regulates cellular calcium homeostasis [Patti & Corvera, 2010]. Oxidative phosphorylation generates most of the cell’s ATP. Any impairment of the organelle’s ability to produce energy can have catastrophic consequences, not only due to the primary loss of ATP, but also due to indirect impairment of ‘downstream’ functions, such as the maintenance of organelles and cellular calcium and other ionic homeostasis, activities of different enzymes, apoptotic signals etc [Patti & Corvera, 2010]. Moreover, deficient mitochondrial metabolism may generate ROS which can damage the tissue compartments or alters the cell signalling pattern.
Mitochondrion appears to be a discrete, small, double membraned organelle with outer and inner membranes composed of phospholipid bilayers studded with proteins. The outer membrane is envelops the inner membrane, which extends into the matrix of the organelle as invaginations called cristae that contain all the transmembrane proteins of the electron transport chain [Fig. 6]. The outer mitochondrial membrane, which encloses the entire organelle, contains numerous integral proteins called porins which contains internal channel (about 2-3 nm) that is permeable to all molecules of 5000 daltons or less [Patti & Corvera, 2010]. The outer mitochondrial membrane is composed of about 50% phospholipids by weight and contains an array of enzymes which are involved in activities such as the elongation of fatty acids, oxidation of epinephrine (adrenaline), and the degradation of tryptophan. The cristae expand the surface area of the inner mitochondrial membrane, enhancing its ability to generate ATP [Patti & Corvera, 2010].
Phosphatidyl inositol is found exclusively in the outer membrane, whereas cardiolipin occurs almost exclusively in the inner membrane. Cardiolipin (diphosphatidyl glycerol) serves as an insulator, forms super molecular assemblies with respiratory chain complexes which is an important regulator of mitochondrial ETC activity and oxidation phosphorylation coupling [Paradies et al., 2000; Pfeiffer et al., 2003; Schlame et al., 2000]. It also play an pivotal role in controlling inner mitochondrial membrane permeability to small molecules, establishes mitochondrial proton gradient, facilitates the activities of key mitochondrial inner membrane enzymes and affects the activities of the adenine nucleotide transporter, cytochrome c oxidase, and other respiratory enzymes [Paradies et al., 1998]. Unlike the outer membrane, the inner membrane does not contain porins, and is highly impermeable. Almost all ions and molecules require special membrane transporters to enter in to or exit out of the matrix. The matrix is the space enclosed by the inner membrane. The matrix contains mitochondrial ribosomes, tRNA, and several copies of the mitochondrial DNA genome along with hundreds of enzymes [Patti & Corvera, 2010]. Recent studies based on light microscopy in live cells have revealed that mitochondria exist as a reticulum that is in continuous communication through dynamic fusion and fission events, moving actively to different regions of the cell through interactions with the cytoskeleton [Patti & Corvera, 2010].

Mitochondria are under the dual genetic control of nuclear DNA and mitochondrial genome. The human mtDNA is a super-coiled, double stranded, closed circular molecule of approximately 16.5 kilo bases (kb) [Fig.
There are several copies of mtDNA per mitochondrion and many hundred mitochondria per cell. The mtDNA encodes 37 genes: 2 rRNAs (12 S and 16 S rRNAs), 22 tRNAs and 13 polypeptides, encoding subunits of the OXPHOS system as shown in table 2. 

Fig. 7: Organisation of human mitochondrial DNA. [Nadege et al., 2009]
Table 2: Respiratory chain subunits encoded by mitochondrial genome

<table>
<thead>
<tr>
<th>Complex</th>
<th>Enzyme</th>
<th>Total number of subunits</th>
<th>mtDNA encoded subunits</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>NADH ubiquinone reductase</td>
<td>43</td>
<td>7 (ND1, 2, 3, 4L, 5, 6)</td>
</tr>
<tr>
<td>II</td>
<td>Succinate ubiquinone reductase</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>Ubiquinol cytochrome c reductase</td>
<td>11</td>
<td>1 (cytochrome b)</td>
</tr>
<tr>
<td>IV</td>
<td>Cytochrome c oxidase (COX)</td>
<td>13</td>
<td>3 (COX1, COX2, COX3)</td>
</tr>
<tr>
<td>V</td>
<td>ATP synthase</td>
<td>17</td>
<td>2 (ATPase 6, ATPase 8)</td>
</tr>
</tbody>
</table>

According to their G+T bases composition, the strands are denominated heavy (H) and light (L). One polypeptide (ND6) and eight tRNA genes are encoded in the L-strand while the rest are encoded in the H-strand. The genetic organization of the mtDNA is extremely compact. There are no intronic sequences and almost no non-coding nucleotides between genes. A non-coding region (control region) of approximately 1.1 kb is located between the tRNA Phe and tRNA Pro. It contains the origin of replication for the heavy strand, the transcription promoters (LSP and HSP, light and heavy strand promoters, respectively) and the regulatory elements for the mtDNA expression [Montoya et al., 2006]. MtDNA transcription requires an organelle specific RNA polymerase (mtRPOL) and transcription factors like Tfam (mitochondrial transcription factor A) and either TFB1M or TFB2M (mitochondrial transcription factor B1 and B2) [Montoya et al, 2006]. Mitochondria require about 850 polypeptides, mostly encoded by nuclear DNA of which 75 are required for structural components of the respiratory
complexes and at least another 20 are required to assemble and maintain them in working order.

Fig. 8: The mitochondrial electron transport chain and ATP synthesis.

The five complexes of the respiratory chain system – complex I (NADH ubiquinone oxidoreductase), complex II (succinate ubiquinone oxidoreductase), complex III (ubiquinone-cytochrome c reductase), complex IV (cytochrome c oxidase), and complex V (ATP synthase) all are located in inner mitochondrial membrane [Fig. 8] [Hatefi, 1986; Chance & Williams, 1955]. There are also two electron carriers, ubiquinone (also called coenzyme Q) and cytochrome c which connect the different complexes so that the electron can pass from one complex to another [Fig. 8]. Oxidative phosphorylation is a process through which the NADH and FADH$_2$ produced by nutrient oxidation are oxidized with the concomitant formation of ATP.
The electron liberated by the oxidation of NADH and FADH₂ pass through four protein complexes, the electron transport chain, with the coupled synthesis of ATP [Mitchell, 1961]. Complex I, III, IV participate in the oxidation of NADH producing three ATPs per NADH. In case of FADH₂ oxidation, which involves complex II, III, IV, producing only two ATPs per FADH₂. The proton gradient generates a high electronegative potential which couples the oxidation with ATP synthesis through F₀F₁ ATP synthesis and also helps Ca²⁺ to enter into the mitochondrial matrix thus buffering its concentration in the cytoplasm.

Complex I is the biggest and most complicated enzyme complex of the OXPHOS system. Complex I is composed of 42–43 different polypeptides, including a FMN-containing flavoprotein and 6 iron-sulfur centers [Hatefi Y 1985; Hinchliffe & Sazanov, 2005]. Complex I has an L-shaped form, with the long arm as a hydrophobic integral membrane protein and the short arm extending into the matrix with the hydrophilic part that contains the FMN and the NADH active center. The two arms of the L-shaped complex I have separated genetic origin and independent assembly. Ubiquinone is a lipid soluble benzoquinone with a long isoprenoid side chain that is laterally diffusible in each of the two layers of the phospholipid bilayer of the inner membrane and adapted to shuttle electrons between membrane proteins. [Hinchliffe & Sazanov, 2005].

Complex II is the membrane-bound component of the citric acid cycle that also functions as a component of the mitochondrial respiratory chain. The integral protein has a covalently bound FAD and iron-sulfur centers in the
membrane extrinsic domain that catalyze electron transfer to ubiquinone and b heme in the hydrophobic membrane domain [Cecchini, 2003].

Complex III is composed of 9–10 polypeptides, 3 of which are associated with redox centers. These centers are b562, b566, and c1 hemes and a [2Fe-2S] cluster [Hatefi Y, 1985]. In addition, two ubisemiquinone bind to two separate domains of complex III. Cytochrome c is a peripheral protein facing the intermembrane space, easily solubilized by salt treatments, that transfers electrons from complex III to complex IV.

Complex IV (cytochrome c oxidase, cytochrome oxidase; cytochrome c-O2 oxidoreductase) is the final catalyst of the respiratory chain. Complex IV passes electrons through CuA, cytochrome a, CuB, and cytochrome a3 to O2 which, in a four-electron process, is reduced to H2O [Fig. 8].

Mitochondrial ATP synthase (complex V) is a F-type ATPase consisting of F1, a peripheral membrane protein, and F0, which is integral to the membrane [Boyer, 1998; Walker et al., 1995]. The F1 catalytic domain is a globular assembly of 5 proteins α, β, γ, δ and ε with the stoichiometry 3:3:1:1:1. The γ, δ and ε- subunits form a central stalk linking the (α β)3 subcomplex of the F1 domain to F0. The (α β)3 subcomplex and the F0 domain are linked by a peripheral stalk. The γ–subunit protrudes from (α β)3 subcomplex and the δ and ε - subunits are associated with its foot. The movement of the subunits of ATP synthase is critical to its function, with the central stalk rotating at 50–100 times/s. The rotation is produced in F0, which is in contact with the foot of the central stalk and is fuelled by the H+ flow [Walker et al., 1995].
3.6.1. Oxidation and electrochemical potentials in the mitochondrial respiratory chain

Complexes I, III, and IV function as H+ pumps, acting in series with respect to electron flux and in parallel with respect to the H+ circuit. The chemical free energy of the fall in redox potential of the electrons passing through the respiratory complexes is used to generate a H+ electrochemical potential gradient, \( \Delta \mu_H \), expressed in electric potential units as the proton-motive force (\( \Delta p \)) [Mitchell & Moyle, 1965] as, \( \Delta p (\text{mV}) = \Delta \Psi_m - (2.3 \, R \, T / F) \Delta \text{pH} \), that at 37°C, results: \( \Delta p = \Delta \Psi_m - 60 \Delta \text{pH} \). In the equation, \( \Delta \Psi_m \) is the electric potential across the inner mitochondrial membrane, \( \Delta \text{pH} \) is the pH gradient across the inner membrane, and \( R \), \( T \), and \( F \) refer to the gas constant, the absolute temperature, and the Faraday constant, respectively.

Under most conditions, \( \Delta \Psi_m \) is the dominant component of \( \Delta p \), accounting for 150–180 mV of a \( \Delta p \) of 200–220 mV [Mitchell & Moyle, 1969]. The \( \Delta p \) drives ADP phosphorylation and stops electron flow in the controlled metabolic condition of absence of ADP.

The energy stored in the electrochemical proton gradient is utilized by proton-translocating ATP synthase (proton pumping ATPase, F1F0-ATPase) in the synthesis of ATP by coupling this process to the exergonic transport of H+ back into the mitochondrial matrix [Walker et al., 1995]. The rate of mitochondrial respiration depends on ADP availability to F1-ATP synthase. The gradients of ADP and ATP across the inner membrane are equilibrated by the adenine nucleotide translocase activity, which is functional to provide ADP to the matrix and ATP for energy-dependent processes to the cytosol.
3.7. **ROS production in mitochondria**

ROS can be generated from different enzymatic reactions such as xanthine oxidase, monoamine oxidase, peroxisomal fatty acyl CoA dehydrogenase, NADPH oxidase, cytochrome P450 dependent enzymes etc. and non-enzymatic reactions e.g. autoxidation of hemoglobin or catecholamines but mitochondria are considered the major intracellular source of ROS [Fig. 9] [Halliwell & Guttridge, 1999; Murphy, 2009]. Thermodynamically favorable leakage of electrons from reduced or partially reduced redox-proteins or other redox molecules of the respiratory chain to molecular oxygen results in the formation of superoxide radicals (O$_2^-$) which either undergoes dismutation reaction spontaneously or gets catalyzed by mitochondrial manganese superoxide dismutase (Mn-SOD) to produce H$_2$O$_2$ which gets decomposed further by transition metals (Fenton’s reaction) to give rise to highly reactive hydroxyl radicals [Andreyev et al., 2005; Murphy, 2009]. The O$_2^-$ radicals can also react with mitochondria derived NO to produce toxic peroxynitrite radicals. Intra-mitochondrial environment is highly reducing, and various respiratory components, including flavoproteins, iron-sulfur clusters and semiubiquinone, are thermodynamically capable of transferring one electron to oxygen constituting the primary source of O$_2$ •⁻ in most tissues. Most steps in the respiratory chain involve single-electron reaction which again favors the monovalent reduction of oxygen. However, the relative contribution of every site to the overall O$_2$ •⁻ production varies from organ to organ and depends on whether mitochondria are actively respiring (state3) or the respiratory chain is highly reduced (state 4) [Barja, 1999].
Fig. 9: Mitochondrial ROS production and defense. Superoxide (O₂⁻) generated by the respiratory chain is mostly released to the matrix at complex I and the IMS at complex III (indicated by stars). O₂⁻ can naturally dismutate to hydrogen peroxide (H₂O₂) or is enzymatically dismuted by matrix Mn-SOD (1) or Cu/ZnSOD (2) in the IMS or cytosol. H₂O₂ is detoxified in the matrix by catalase (3), the thioredoxin/thioredoxin peroxidase system (4), or the glutathione/glutathione peroxidase system (5). Alternately, H₂O₂ can react with metal ions to generate the highly reactive hydroxyl radical (·OH) via Fenton chemistry (6). O₂⁻ is not membrane permeable but can pass through ion channels (solid lines), whereas H₂O₂ can pass freely through membranes (dashed lines). IMM, inner mitochondrial membrane; IMS, intermembrane space; OMM, outer mitochondrial membrane; Mn-SOD, manganese superoxide dismutase; Cu/ZnSOD, copper/zinc superoxide dismutase; CAT, catalase; THD, NADH transhydrogenase; TR, thioredoxin reductase; TPx, thioredoxin peroxidase; TRxred, reduced thioredoxin; TRox, oxidized thioredoxin; GSH, glutathione; GSSG, glutathione disulfide; IMAC, inner membrane ion channel; VDAC, voltage dependent anion channel; ΔΨm, membrane potential. [Feisssner et al., 2009]

Several sites of mitochondrial ROS production have been identified using isolated mitochondria respiring with different substrates [Murphy, 2009].
Complex I and III are the main sources of ROS production. Besides these, several other sites have also been identified [Murphy, 2009].

The mitochondria possess various antioxidant defenses designed to eliminate both O$_2^-$ and H$_2$O$_2$ [Cadenas & Divies, 2000; Murphy, 2009]. The elaborate antioxidant defence system present in mitochondria comprises of antioxidant enzymes such as Mn-SOD, glutathione peroxidase, glutathione reductase, phospholipid hydroperoxide, glutathione peroxidase and peroxiredoxins as also non-enzyme molecules like reduced glutathione, thioredoxins etc. which can effectively scavenge the generated ROS [Andreyev et al., 2005; Lenaz, 2001; Murphy, 2009]. The inter-membrane space of mitochondria also contains copper- and zinc-containing superoxide dismutase (Cu/ZnSOD) which helps in scavenging superoxide radicals released in to this space.

Mitochondria are not only the major sites of ROS production but they are also highly vulnerable to oxyradical induced damage [Murphy, 2009]. This is because of its close proximity with the site of ROS generation and limited ability of mitochondrial DNA repair system [Karahalil et al., 2002; Stuart et al., 2005]. In many pathological conditions, various oxidative damage markers of lipid, protein and DNA accumulate within mitochondria [Beckman & Ames, 1998; Halliwell & Gutteridge, 1999; Mariani et al., 2005]. The radicals of oxygen set off a chain reaction by abstracting a H-atom form the unsaturated fatty acid [Fig. 10] and produce toxic intermediates like peroxyl radicals, lipid hydroperoxides, lipid peroxides and finally form smaller compounds like aldehyde, ketones, carboxylic acids and
hydrocarbons like ethane, pentane etc. are formed. Some of the products of lipid peroxidation like manoldialdehyde (MDA), acrolein, 4-hydroxynonenal (4-HNE) etc are more toxic than free radical and can form adducts rapidly with protein and DNA.

Fig. 10: Outline of lipid peroxidation.

The protein are also highly vulnerable to radical attack and several classes of damage have been documented including oxidation of sulphydryl groups, reduction of di sulfides, oxidative adduction of amino acid residues close to metal-binding sites via metal-catalyzed oxidation, reactions with aldehydes, protein-protein cross-linking, and peptide fragmentation [Stadman & Oliver, 1991; Stark Reed & Oliver, 1989].
Fig. 11: Generation of protein carbonyls by glycation and glycoxidation and by reactions with lipid peroxidation products of polyunsaturated fatty acids. (A) Reactions of protein amino groups (P–NH₂) with the lipid peroxidation product, malondialdehyde. (B) Michael addition of 4-hydroxy-2-nonenal to protein lysine (P–NH₂), histidine (P–His), or cysteine (P–SH) residues. (C) Reactions of sugars with protein lysyl amino groups (P–NH₂). “Me” represents “metal ions.”

3.8. Mitochondrial biogenesis

Mitochondrial biogenesis is a complex process which involves multiplication of mtDNA, increase in mitochondrial mass and proliferation of mitochondria [Patti & Corvera, 2010]. Only 13 subunits of respiratory chain in mitochondria are coded by mtDNA, while a large number of nuclear genes codes for different proteins of the mitochondria especially those required for mtDNA replication, transcription and mitochondrial protein synthesis [Kelly & Scarpulla, 2004; Scarpulla, 2008].
Several factors like NRF 1 (nuclear respiratory factor 1), NRF 2 (nuclear respiratory factor 2), PPARα, ERRα (estrogen receptor-related receptor α), Sp1 (specificity protein 1) and Tfam take part in this process. They together bring about the co-ordination between the mitochondrial and nuclear genome necessary for biogenesis [Kelly & Scarpulla, 2004; Lee, 2005; Scarpulla, 2008]. The target genes for these transcription factors especially of NRF 1, NRF 2, Sp 1 and Tfam have been identified and they work in concert with each other to bring about mtDNA replication, transcription, synthesis and assembly of respiratory chain complexes and protein import within mitochondria. The integration of the functions of such diverse
transcription factors requires a high level of transcriptional co-ordination which is brought about by PGC 1 (PPAR-γ coactivator 1) family of co-activators (PGC 1α, PGC 1β, PGC-1-related coactivator or PRC etc.) [Kelly & Scarpulla, 2004; Lee, 2005; Scarpulla, 2008]. PGC 1α, is considered as the master regulator of mitochondrial biogenesis [Kelly & Scarpulla, 2004; Lee & Wei, 2005; Scarpulla, 2008]. Cellular energy requiring conditions like exercise, cold exposure, hypoxia and fasting can cause activation of PGC 1α and it is highly expressed in muscle, liver, and brown fat [Lin et al., 2005; Patti & Corvera, 2010]. NRF 1 and NRF 2 are considered as the main target transcription factors for PGC 1α [Lin et al., 2005]. NRF1 and NRF2 bind to the promoter region of a broad range of mitochondrial genes encoded in the cell nucleus, including β-ATP synthase, cytochrome c, cytochrome c oxidase subunit IV, and Tfam [Puigserver & Spiegelman, 2003]. NRFs switches on Tfam which then translocates to the mitochondria and activates mitochondrial DNA replication and transcription [Puigserver & Spiegelman, 2003] [Fig. 12]. Besides NRF1 and NRF2, many other targets of PGC 1α have now been identified including some nuclear and cytoplasmic receptors [Kelly & Scarpulla, 2004; Scarpulla, 2008]. PGC 1α has an inducible nature and is regulated by cyclic AMP (cAMP) and cyclic AMP response element-binding protein (CREB), AMP activated protein kinase, calcium/calmodulin-dependent protein kinase type IV (CAMK IV), endothelial nitric oxide synthase (eNOS) etc [Cheng et al., 2011; Kelly & Scarpulla, 2004; Zong et al., 2002]. Mitochondrial fission is carried out with the help of dynamin related protein 1 (DRP1) for the outer membrane and OPA1 for the inner
membrane of mitochondria while mitofusins (Mfn) control mitochondrial fusion [Patti & Corvera, 2010].

3.9. Relationship between mitochondrial biogenesis and ROS

Several studies indicate how ROS can induce Tfam or NRF 1 to initiate mitochondrial biogenesis. In rat hepatoma cells, the induction of Tfam by redox activation of NRF 1 through Akt dependent phosphorylation has been shown [Piantadosi & Suliman, 2006]. Likewise mitochondrial binding of CO leads to increased formation of H$_2$O$_2$ which is often linked to Akt activation and activation of NRF 1, NRF 2 and Tfam [Suliman et al., 2007]. In human endothelial cells, homocysteine is found to induce ROS production which activates mitochondrial biogenesis through involvement of NRF 1 and Tfam [Zinellu et al., 2009]. In HeLa cells with depleted mtDNA, the increase in endogenous ROS has been shown to activate mitochondrial biogenesis through the involvement of NRF 1 and Tfam [Lee et al., 2005]. Another study has elucidated the molecular pathway of ROS-dependent activation and nuclear translocation of Nrf 2 (Nuclear factor erythroid-2 related factor 2). The Nrf 2 in turn causes promoter activation of NRF 1 to start mitochondrial biogenesis programme [Piantadosi et al., 2008]. Other studies have also reported down-regulation of mitochondrial biogenesis by increased ROS [Chevtzoff et al., 2010]. It appears when mitochondrial functional impairment is mild to moderate, ROS may try to compensate it by stimulating mitochondrial biogenesis, but which in turn may lead to an
overload of ROS production which can further damage the cellular components including the mitochondria [Chakrabarti et al., 2011].

3.10. Roles of mitochondria in adipose tissue

Although the important role of mitochondria in brown adipose tissue and muscles has been highlighted often in the literature, several recent studies indicate that mitochondrial density increases remarkably during differentiation in white adipose tissue and the mitochondria in the latter group have diverse type of proteins than mitochondria from heart, skeletal muscle or brain [Kim et al., 2004; Luo et al., 2008; Wilson-Fritch et al., 2003]. The most important functions performed by mitochondria in white adipose tissue are the anaplerotic generation of metabolic intermediates for fatty acid synthesis and esterification [Owen et al., 2002], the maintenance of adiponectin synthesis, folding and secretion [Koh et al., 2007], and the modulation of interactions of the insulin signaling pathway [Shi et al., 2008].

The relevance of white adipocyte mitochondria to whole-body metabolism and metabolic disease may depend on the extent to which mitochondrial respiratory capacity and/or the total mass of white adipose tissue will be sufficient to impact circulating free fatty acid levels. Higher mitochondrial density and even uncoiler protein 1 (UCP1) can be induced in response to pharmacological or genetic alterations of white adipocytes [Bogaka et al., 2005; Hims-Hagen et al., 2000; Loncar, 1991; Orci et al., 2004; Strom et al., 2008; Tiraby et al., 2003; Toh et al., 2008; Wilson-Fritch et al., 2004]. These
suggest that white adipose tissue can potentially be induced to acquire more oxidative metabolic phenotypes, promoting increased fuel consumption and energy expenditure. Further respiratory chain uncoupling mediated through the induction of UCP1 in white adipocytes alone can be important in regulating free fatty acid synthesis and release. More interestingly, in cultured adipocytes, impairment of respiratory chain function through depletion of Tfam during adipocyte differentiation results in impaired insulin stimulated glucose transport [Shi et al., 2008].

3.11. Evidence for reduced adipose tissue mitochondrial capacity in diabetes and obesity

White adipocyte mitochondrial content is decreased in both rodent and human obesity [Choo et al., 2006; Okamoto et al., 2007; Rong et al., 2007; Sutherland et al., 2008; Wilson-Fritch et al., 2004] which correlates with insulin resistance that accompanies obesity. In humans, white adipocyte mtDNA copy number is found to be inversely correlated with age and BMI and directly correlated with basal and insulin-induced lipogenesis [Kaaman et al., 2007]. Down regulation of genes coding for subunits of respiratory complexes, decreased number of mitochondria with diminished mt DNA content, reduction of mitochondrial size, diminished levels of transcription factors involed in mitochondrial biogenesis and decreased mitochondrial respiration have been reported in human and animal models of obesity and type 2 diabetes [Abdul-Ghani & DeFronzo, 2008; Bonard et al., 2008; Choo et al., 2006; Dahlman et al., 2006; Heilbronn et al., 2007; Hojlund et al., 2008; Kraunsoe et al., 2010; Laye et al., 2009; Wang CH et al., 2010 ].
MtDNA content can reduce adipocyte capacity for lipid storage, causing ectopic lipid accumulation in peripheral tissues such as muscle and liver [Dahlman et al., 2006]. Administration of thiazolidinediones, adrenergic stimulation, β-3 agonists, and CB1 blockade induces changes in mitochondrial content and remodeling in white adipocytes and causes an improvement in insulin sensitivity [Laplante et al., 2006; Tedesco et al., 2008; Wilson-Fritch et al., 2004]. Some evidence suggests that lack of insulin signaling does not reduce mitochondrial capacity in adipose tissue. For example, mice with adipose tissue-specific removal of the insulin receptor (FIRKO mice) display high levels of mitochondrial genes involved in fatty acid oxidation and OXPHOS over the lifespan of the animals [Katic et al., 2007].

3.12. Evidence for reduced muscle mitochondrial capacity in diabetes and obesity

Obesity is associated with reductions in citrate synthase, malate dehydrogenase, carnitine palmitoyltransferase 1 (CPT1), and cytochrome oxidase (COX) activity in the fasting state [Simoneau & Bouchard, 1995; Simoneau et al., 1995] and with parallel increases in activity of the glycolytic enzymes hexokinase and phosphofructokinase [Simoneau & Kelly, 1997]. Another study has shown that the enzymatic activity of OXPHOS complex I, as assessed by the activity of rotenone-sensitive NADH:O2 oxidoreductase, is reduced by about 40% in skeletal muscle biopsy samples from individuals with type 2 diabetes mellitus and by 20% in obese individuals [Ritov et al., 2005] and modest reductions in ADP and succinate-stimulated oxygen
consumption in permeabilized muscle fibers from obese individuals with type 2 diabetes mellitus has also been found [Boushel et al., 2007]. Electron microscopy has also demonstrated diminished mitochondrial size in obesity and diabetes [Kelly et al., 2002] particularly in subsarcolemmal fractions [Ritov et al., 2005]. Nuclear magnetic resonance (NMR) spectroscopy has also been used to assess mitochondrial function in vivo and similar reductions in oxidative function were found in both insulin resistance and type 2 diabetes mellitus [Peterson et al., 2004]. In offsprings of type 2 diabetes subjects decreased rates of OXPHOS system have been observed by NMR studies and electron microscopic examinations of the same have indicated reduced mitochondrial mass [Befroy et al., 2007]. Decreases in maximal ADP-stimulated respiration (state 3, malate and pyruvate as substrates) in mitochondria isolated from obese subjects with diabetes mellitus as compared with obesity alone has been observed [Mogensen et al., 2007]. Further, reduction in mRNA expression levels for multiple nuclear-encoded genes of the OXPHOS pathway as well as transcription factors of mitochondrial biogenesis is found in humans with type 2 diabetes mellitus [Mootha et al., 2003; Patti et al., 2003; Sreekumar et al., 2002].

3.13. Evidence for reduced liver mitochondrial capacity in diabetes and obesity

Human obese and type 2 diabetes subjects are associated with reduced expression of seven of 25 genes encoding OXPHOS genes and the expression of these genes is inversely correlated with hepatic lipid accumulation and paralleled by reduction in expression of PGC-1α and genes known to be
regulated by thyroid hormone [Pihlajamaki et al., 2009]. Reduced expression of OXPHOS genes (e.g., COX7C, ATP5C1) is also observed in mice fed a high-fat diet and normalized by acute therapy with thyroid hormone T3 [Pihlajakami et al., 2009]. Whereas in contrast, studies in Japanese individuals with established diabetes mellitus and modest obesity (BMI 27 kg/m2) have shown a modestly increased expression of multiple genes within all complexes of OXPHOS complexes, in parallel with BMI and insulin resistance (measured by homeostasis model assessment of insulin resistance, HOMA-IR) [Takamura et al., 2008] and is also positively correlated with expression of several genes linked to mitochondrial biogenesis (e.g., PGC-1α, ERR α, NRF, thyroid hormone receptor) and both ROS generation (e.g., NADPH oxidase) and attenuation (e.g., glutathione peroxidase). Enzymatic activity of complexes I-V is reduced in liver extracts from patients with NASH and is inversely correlated with BMI and HOMA-IR [Greenfield et al., 2008; Perez-Carreras et al., 2003]. Moreover, NASH is characterized by prominent abnormalities in mitochondrial ultrastructure such as increased size, loss of cristae, and paracrystalline inclusion bodies similar to those observed in some mitochondrial myopathies [Sanyal et al., 2001]. Reduced OXPHOS activity is also accompanied by increased tissue long-chain acylcarnitines and reduced short-chain acylcarnitines, despite normal CPT1 activity and increased expression of β-oxidation genes [Kohjima et al., 2007; Misu et al., 2007]. Similarly, circulating β-hydroxybutyrate levels are increased in NASH [Sanyal et al., 2001].
In insulin resistance, excessive ROS/ RNS generation occurs as part of several, partially interrelated, pathophysiological mechanisms, which include metabolic overload—the overabundance of glucose and FFAs, inflammation, endoplasmic reticulum (ER) stress and the unfolded protein response (UPR), and dysregulated hormonal and growth factors regulation (Fig. 3). These in turn rely on the main cellular sources for ROS or RNS (reactive nitrogen species) generation in physiological conditions, such as the NOX family of NADPH oxidases, mitochondrial respiration, the ER, and nitric oxide synthases [Bashan et al., 2009].

Fig. 13: Major sources of ROS and RNS in pathophysiology related to insulin resistance and obesity. The processes responsible include the following: i) High metabolic load ii) Inflammation iii) ER stress iv) Endocrine dysregulation.[Bashan et al., 2009]
3.14.1. **NADPH oxidase.**

NADPH oxidases are membrane-bound enzymatic complexes consisting of an electron transport chain that transfers electrons from the donor, cytosolic NADPH, to the acceptor, O$_2$ and consequently, O$_2^-$ (and H$_2$O$_2$) are generated. ROS generation through NADPH oxidase is required for diverse physiological functions, including the response to various hormones and growth factors. Insulin-stimulated ROS production relies on NOX4 in adipocytes [Mahadev et al., 2004] and also on NOX2 expressed in skeletal and cardiac muscle [Heymes et al., 2003; Javesghani et al., 2002]. In adipose tissue of obese mice, NOX4 is found to be increased, as are p22 Phox, NOX2, as well as other components of its multiprotein complex (p67 Phox, p47 Phox, and p40 Phox) [Furukawa et al., 2004]. In adipose tissue of obese mice, NOX2 may arise either from obesity-associated upregulation of NOX2 in the adipose cells, or from phagocytic cells infiltrating this tissue in obesity [Weisberg et al., 2003; Xu et al., 2003]. Increased ROS production in 3T3-L1 adipocytes incubated with FFA or in hyperglycemic conditions is inhibited by chemical inhibitors thought to inhibit mainly NADPH oxidases [Furukawa et al., 2004; Wu et al., 2005]. In high fat fed mice, increased ROS production by adipocytes is inhibited by inhibitors of NADPH oxidases [Talior et al., 2005]. In humans, the expression of p47 Phox in endothelial cells has been shown to correlate with body mass index and is significantly elevated in obese and overweight compared with lean controls [Silver et al., 2007]. It has been demonstrated that hypoxia [Suliman et al., 2004; Vallet et al., 2005], inflammatory cytokines like TNF [Moe et al., 2006] and angiotensin II
[Higashi et al., 2003; Wingler et al., 2006], and ER stress [Pedruzzi et al., 2004] all induce upregulation of NOX4 mRNA and protein levels. All of these conditions or factors have also been implicated as causative factors in insulin resistance of adipose tissue and/or muscle [Gregor & Hotamisligil, 2007; Hosagai et al., 2007; Hotamisligil et al., 1999; Hwei et al., 2006]. PPARγ ligands used as insulin sensitizing agents have been shown to downregulate protein and mRNA expression of NOX4 (and NOX2) in endothelial cells [Hwang et al., 2005].

3.14.2. Mitochondrial ROS generation

The mitochondrial electron transport chain is a major source of ROS production as discussed earlier. Increased mitochondrial ROS generation has been strongly implicated as a mediator between hyperglycemia and its pathological consequences in various tissues such as the vasculature [Nishikawa et al., 2000], kidney [Kiritoshi et al., 2003], neurons [Vincent et al., 2002; Vincent et al., 2004], retina [Lin et al., 2006], and pancreatic beta cells [Sakai et al., 2003]. In 3T3-L1 adipocytes, high glucose has been shown to induce mitochondrial ROS, which mediated insulin resistance and activation of inflammatory pathways [Lin et al., 2005]. Increased reactive oxygen species production with lower abundance of complex I subunits and carnitine palmitoyltransferase 1b protein despite normal mitochondrial respiration have been shown in mitochondria isolated from insulin-resistant human skeletal muscle [Lefort et al., 2010]. In another study, it has shown that the mitochondrial mass, membrane potential, and superoxide-production are not changed in diabetic myotubes compared to lean controls.
but $H_2O_2$ production and ATP production are significantly reduced [Minet & Gaster, 2011]. A recent study has proposed that in muscle of diet-induced insulin resistant mice, mitochondrial dysfunction is in fact a consequence, not the cause, of increased ROS production [Bonnard et al., 2008].

### 3.14.3 Endoplasmic reticulum ROS production

The ER recently has attracted attention as a source of ROS generation [Bashan et al., 2009]. It is estimated that oxidative protein folding may account for as much as 25% of total cellular ROS generation [Cullinan & Diehl, 2006; Malhotra & Kaufman, 2007]. When the requirement for ER-supported protein folding increases, whether as a consequence of increased demand for newly synthesized proteins or secondary to accumulation of misfolded proteins that initiate the unfolded protein response (UPR), ER-derived ROS generation may also increase [Malhotra & Kaufman, 2007] [Fig. 14]. Inter organellar communication between mitochondria and ER may cause increased mitochondrial ROS generation originating from ER-derived signals. Excessive activation of the UPR, or “ER stress,” has been implicated in the pathogenesis of insulin resistance associated with obesity and with pancreatic beta cell failure related to type 2 diabetes [Bashan et al., 2009]. Oxidative protein folding in the ER requires an electron transfer chain, which is less characterized than that in mitochondrial respiration, but share some common features. Electrons freed from the two cysteines that form a disulfide bond are donated to ER oxidoreductases like protein disulfide isomerase (PDI), then to ER oxidoreductin 1 (Ero1), a flavin adenine dinucleotide (FAD)-containing protein, and finally, to molecular oxygen [Tu
ER stress has been proposed as a putative mechanism for insulin resistance, particularly in adipose tissue and the liver [Ozcan et al., 2004; Ozcan et al., 2006]. In nutritional and genetic models of obesity, adipose tissue exhibit markers of increased ER stress including phosphorylation of eIF2α, PERK, and elevated expression of the ER chaperone GRP78 [Ozcan et al., 2004]. IRE-mediated phosphorylation of the stress-activated MAP kinase JNK is thought to be the molecular mechanism connecting ER stress and impaired insulin signaling [Ozcan et al., 2004].

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**Fig. 14 ROS generation by the ER and its interrelations to mitochondrial ROS generation.** ROS can be generated in the ER as part of oxidative protein folding. To generate correctly folded protein with disulfide bonds formed between the correct cysteines (in green), electrons are donated to oxidoreductases (PDI, ErO1) and ultimately to molecular oxygen, yielding H₂O or ROS. When incorrect disulfide bonds form (in orange), they need to be reduced by GSH, resulting in a further decrease of GSH/GSSG ratio, altering the redox state within the ER. Alternatively, misfolded proteins can be directed to degradation through ER-associated degradation machinery (ERAD). Accumulation of misfolded proteins in the ER initiates the unfolded protein response (UPR), part of which is programmed to enhance antioxidant defense. Calcium ions released from the ER can augment mitochondrial ROS generation. This occurs through the elevation in electron donors to the electron transport (respiratory) chain through the stimulation of the tricarboxylic acid cycle. In addition, calcium ions increase cytochrome c release, impairing electron transfer and increasing ROS generation. This in turn can impair respiratory complexes, further augmenting ROS generation. ATF6, activating transcription factor 6; ATF4, activating transcription factor 4; eIF2α, eukaryotic translational initiation factor 2α; ERAD, ER-associated degradation; IRE, inositol-requiring enzyme-1; Nrf2, nuclear respiratory factor 2; ox/red-Ero1, ER oxidoreductin 1; ox/red-PDI, oxidized/reduced protein disulfide isomerase; PERK, PKR-like eukaryotic initiation factor 2α kinase; XBP1, X-box protein 1. [Bashan et al., 2009]
3.14.4. RNS generation: NO synthases

NO synthases (NOS) are the major source of NO production. nNOS is expressed in all major insulin target tissues (except for adipose tissue), as well as in liver [Esteban et al., 1997] and skeletal muscle [Kapur et al., 1997; Nakane et al., 1993]. eNOS is mainly expressed in the endothelial cell component of various insulin target tissues such as liver [Shah et al., 1997], skeletal [Kapur et al., 1997], and cardiac muscles [Balligand et al., 1995], and in adipose tissue [Kapur et al., 1997]. Its major physiological function is the generation of NO to promote vasodilatation. Increased $\text{NO}_2^-$ and $\text{NO}_3^-$ have been reported, mainly in persons with late diabetes complications [Chiarelli et al, 2000; Izumi et al., 2006].

Genetic and nutritional animal models of obesity have shown increased iNOS expression in skeletal and cardiac muscle, in adipose tissue [Perreault & Marette, 2001; Zhou et al., 2000], and in $\text{ob}/\text{ob}$ mice also in liver [Fujimoto et al., 2005]. In Zucker rats, total NOS activity and eNOS immunoreactivity in skeletal muscle decreases compared with lean controls [St-Pierre et al., 2006]. In obese humans, eNOS and iNOS, but not nNOS, are shown to be more highly expressed at mRNA levels in adipose tissue and isolated adipocytes [Elizalde et al., 2000; Engeli et al., 2004]. iNOS knockout mice are protected against insulin resistance, attributed to improved skeletal muscle, but not adipose tissue, insulin action, and glucose disposal [Perreault & Marette, 2001]. iNOS also plays a role in hepatic insulin resistance in $\text{ob}/\text{ob}$ mice [Fujimoto et al., 2005].
3.15. Role of oxidative stress in type 2 diabetes and obesity

Oxidative stress plays a pivotal role in the development and progression of diabetes and its complications due to increased production of free radicals and impaired antioxidant defences [Bashan et al., 2009]. It has also been implicated in the progression of long-term diabetes complications, including microvascular and macrovascular dysfunction. In diabetic condition, glucose uptake in muscle and fat is impaired by oxidative stress [Bashan et al., 2009]. Many reports have suggested increased oxidative stress in obesity [Atabek et al., 2004; Bakker et al., 2000]. Decreased antioxidant capacity, increased production of ROS, and elevated oxidation products of lipids, DNA, and proteins have been reported in various tissues [Bashan et al., 2009].

Evidence for increased oxidative stress is found in adipose tissue in obesity and diabetes which includes elevated oxidation products of both lipids and proteins [Bashan et al., 2009]. Obese mice before developing diabetes have shown increased H$_2$O$_2$ generation by adipose tissue, along with decreased mRNA levels of SOD, catalase, and glutathione peroxidase [Furukawa et al 2004]. Elevated levels of thiobarbituric acid reactive substances (TBARS)[Furukawa et al 2004] or malondialdehyde (MDA) [Garcia-Diaz et al., 2007], measures of lipid peroxidation are also found in adipose tissue of obesity. Total protein carbonylation is reported to be two- to threefold higher in adipose tissue of high-fat fed (HFF) mice [Grimsrud et al., 2007]. Concomitantly, mRNA levels of glutathione-S-transferase 4, a key enzyme responsible for reversing lipid peroxidation adducts (4-hydroxynonenal, 4-
HNE) on proteins, is decreased three- to fourfold in obesity [Bashan et al., 2009]. In cultured adipocytes, elevated levels of fatty acids increased oxidative stress via NADPH oxidase activation, and oxidative stress is found to cause dysregulated production of adipocytokines such as adiponectin, plasminogen activator inhibitor-1, IL6, and monocyte chemotactic protein-1. In obese mice, treatment with NADPH oxidase inhibitor reduced ROS production in adipose tissue, attenuated the dysregulation of adipocytokines, and improved diabetes, hyperlipidemia, and hepatic steatosis [Furukawa et al., 2004].

Increased markers of lipid peroxidation has been reported in the livers of animal models of diabetes and obesity [Desco et al., 2002; Feillet-Coudray et al., 1999; Svegliati-Baroni et al., 2006]. However, these are not matched by a decrease in the levels of dietary antioxidants like vitamin E. Furthermore, no protective effect is shown by antioxidant supplementation [Feillet-Coudray et al., 1999; Svegliati-Baroni et al., 2006]. Obesity and the related insulin resistance are frequently associated with increased accumulation of lipids (triglycerides) in the liver and it remains unclear if the elevation in lipid peroxidation in liver really reflects increased oxidative stress, or simply the increase in available substrates for lipid peroxidation [Svegliati-Baroni et al., 2006]. A decrease in the ratio of reduced to oxidized GSH (GSH/GSSG) in the liver is reported in a model of type 1 diabetes, but not in type 2 diabetes or obesity model [Galinier et al., 2006].

A recent study exhibited that there is no increase in 4-HNE in muscle of obese diabetics compared with obese nondiabetic persons [Mogensen et al.,...
2007]. In experimental models of type 1 diabetes, decreased high- or low-molecular-weight thiols have been reported in skeletal or cardiac muscles, as well as decrease in antioxidant enzymes [Desco et al., 2002; Khamaisi et al., 1999]. Furthermore, increased S-nitrosylation of total muscle proteins as well as of key proteins in the insulin signaling cascade has been seen in nutritional and genetic obesity in rodents [Carvalho-Filho et al., 2005; Yasukawa et al., 2005]. Despite these, in the KKAy mouse model of obesity and diabetes skeletal muscle, \( \text{H}_2\text{O}_2 \) production, TBARS and the expression of antioxidant enzymes are found to be unaltered, although they are affected in adipose tissue [Furukawa et al., 2004].