As type 2 diabetes mellitus (T2DM) is multi-factorial disease, use of several oral hypoglycemic agents (OHAs) is the main stay of pharmacological treatment. Though many new OHAs with heterogeneous mode of action have become available for the treatment of T2DM in recent years, these OHAs are associated with several unwanted side effects. Complementary approaches such as use of medicinal plants and dietary polyunsaturated fatty acids (PUFA) with hypoglycemc and hypolipidemic activities are therefore imperative. This chapter reviews various studies in relation to effects of OHAs and dietary PUFA on lipid metabolism in T2DM.

3.1 Antidiabetic Drugs:

Treatment of type 2 diabetes is based on interplay of patient characteristics, severity of hyperglycemia and available therapeutic options. Biguanides, thiazolidinediones (TZDs) and sulfonylureas are the most widely used oral medications.

3.1.1 Biguanides:

Metformin (Dimethyl-biguanide) (Fig. 5) is approved by the U.S. Food and Drug Administration (USFDA) to treat T2DM and its safety profile is probably superior to other insulin sensitizing drugs (Kittappa and Mitra, 2012). United Kingdom Prospective Diabetes Study (UKPDS) study reported reduction in the diabetic complications and mortality by 32% and 42% respectively, which further strengthened the use of metformin as a first line treatment in T2DM (Rojas and Gomes, 2013). Hepatic organic cation transporter-1 (OCT-1) facilitates selective uptake of metformin into hepatocytes (Jin et al. 2009).

Biguanides are known to reduce hepatic glucose production and increase the peripheral glucose uptake in skeletal muscles. Reports suggest favorable effect of metformin on body weight, hyperinsulinaemia, lipid profile, arterial vascular disease, removal of small blood clots and endothelial dysfunction (Kittappa and Mitra, 2012). Metformin is also found to be effective in controlling blood glucose levels in obese patients with T2DM (Rojas and Gomes, 2013; Pala et al. 2014).
Mechanism of Action:

The improvement in insulin sensitivity by metformin could be ascribed to its positive effects on insulin receptor expression, tyrosine kinase activity and inhibition of glucose production by disrupting gluconeogenesis (Viollet et al. 2012). Metformin is reported to increase plasma levels of glucagon-like peptide 1 (GLP-1) and it also induces expression of incretin receptor gene in islet cells by activating peroxisome proliferator activated receptor alpha (PPARα) (Maida et al. 2011). Metformin is also known to activate adenosine monophosphate-activated protein kinase (AMPK) and decrease the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity, leading to reduction of reactive oxygen species in cultured podocytes (Piwkowska et al. 2010). It has also been shown to reduce lipid accumulation in macrophages by repressing forkhead box protein and hence may serve as a therapeutic agent for treating atherosclerosis (Kittappa and Mitra, 2012). The mechanism of action of metformin is shown in Fig. 6.
**Fig. 6: Mechanism of Action of Metformin**


Figure modified from Viollet et al., 2012, Clinical Science 122(6): 253-270.

**Side Effects:**

Some reports suggest that metformin reduces vitamin B_{12} absorption due to calcium-dependent ileal membrane antagonism where vitamin B_{12} deficiency is related to dose and duration of the treatment (Mazokopakis and Starakis, 2012). A study detected 19% decrease in vitamin B_{12} and 5% decrease in folate concentration in serum with metformin treatment (de Jager et al. 2010). Rare side effects of metformin include cholangiohepatitis with marked elevations in serum liver transaminases, intrahepatic cholestasis (Biyyani et al. 2009), diarrhoea, nausea, vomiting, bloating and flatulence and lactic acidosis in T2DM patients (Bouchoucha et al. 2011).
3.1.2 Thiazolidinediones:

Currently available thiazolidinediones (TZDs) are rosiglitazone and pioglitazone and were introduced in the USA in 1999 and in the UK in 2000. They act as insulin sensitizers providing a novel means to improve glycemic control by reducing insulin resistance (Nicholson and Hall, 2011). The structures of different TZDs are shown in Fig. 7.

TZDs lower blood glucose concentrations mainly through increased peripheral glucose utilization, improving insulin sensitivity and to a lesser extent through decreased hepatic glucose output (Pandey et al. 2011; Koppaka et al. 2013). TZDs are reported to reduce plasma cholesterol, triglycerides, VLDL, atherogenic small dense LDL particles and increase HDL levels in T2DM patients (Miller et al. 2011). Pioglitazone is reported to decrease the incidence of cardiac events in people with T2DM who have already had a heart attack (Nitta et al. 2013).

**Fig. 7: Structure of Thiazolidinediones**

![Structure of Thiazolidinediones](image)

*Figure source: Panchapakesan et al., 2005, Nature Clinical Practice Nephrology 1(1): 33-43.*

**Mechanism of Action:**

TZDs mediate their function through binding to the peroxisome proliferator activated receptor gamma (PPAR\(\gamma\)) that is expressed predominantly in adipocytes. Glitazones act as agonists of PPAR\(\gamma\) to enhance the actions of insulin, leading to improvement in insulin dependent glucose disposal, reductions in hepatic glucose output and lower fatty acid synthesis (Scheen, 2004; Phielix et al. 2011). The TZDs also suppress the expression of tumor necrosis factor alpha (TNF-\(\alpha\)) in adipocytes (Cawthorn et al. 2008). Effects of TZDs on AMPK activity is mediated indirectly via
inhibition of the respiratory chain complex I and modulation in cellular adenine nucleotide levels (Brunmair et al. 2004) (Fig. 8).

**Fig. 8: Mechanism of Action of Thiazolidinediones**

*AMPK*: Adenosine monophosphate-activated protein kinase; *PPARγ*: Peroxisome proliferator activated receptor γ; *SREBP*: Sterol regulatory element binding protein; *ACC*: Acetyl-coenzyme A carboxylase; *TNF-α*: Tumor necrosis factor-α; *VLDL*: Very low density lipoprotein

Figure modified from Phielix et al., 2011, Trends in Pharmacological Sciences 32(10): 607-616.

**Side Effects:**

Troglitazone was withdrawn in 2000 following reports of fatal hepatotoxicity (Della-Morte et al. 2014). Rosiglitazone has shown to be associated with a significant increase in the risk of myocardial infarction that led to drastic decline in the use of drug, and it being banned in several countries including India (Nissen and Wolski, 2007). Side effects of TZDs include weight gain, fluid retention, atypical fractures and bladder cancer (Defronzo et al. 2013).
3.1.3 Sulfonylureas:

The sulfonylureas were initially developed in the 1920s and have become important in the management of T2DM as they improve both first and second phases of insulin secretion (Basit et al. 2012) (Fig. 9).

**Fig. 9: Structure of Sulfonylurea**

![Structure of Sulfonylurea](image)

*Figure source: Derosa and Salvadeo, 2009b, Clinical Medicine Insights: Therapeutics 1: 835-845.*

**Mechanism of Action:**

Sulfonylureas stimulate insulin secretion from pancreatic β-cells. The mechanism of action involves a direct secretory effect on the pancreatic β-cells through its binding with adenosine triphosphate sensitive potassium channels (K$^+$ATP) which serve as sulfonylurea receptors. K$^+$ATP channels of the β-cells play an important role in releasing insulin (Seino et al. 2011). It also has a hypoglycemic effect through inhibiting opening of K$^+$ATP channel which increases K$^+$ concentration inside the cell leading to depolarization of β-cell membrane (Panten et al. 1996). Depolarization of membrane opens voltage-dependent Ca$^{2+}$ channels leading to insulin release (Brady and Terzic, 1998; Whirl-Carrillo et al. 2012) (Fig. 10).
**Fig. 10: Mechanism of Action of Sulfonylureas**

ATP: Adenosine triphosphate; ADP: Adenosine diphosphate

*Figure modified from Brady and Terzic, 1998, Journal of the American College of Cardiology 31(5): 950-956.*

**Side Effects:**

Sulfonylureas are reported to cause hypoglycemia, weight gain, skin reactions, acute porphyria, rarely hyponatraemia and acute cholestatic hepatitis (Sarkar et al. 2011). Sulfonylureas are also reported to increase cardiovascular mortality among diabetic patients (Simpson et al. 2015).

### 3.1.4 Alpha-glucosidase Inhibitors:

The first alpha-glucosidase inhibitor, voglibose, was discovered in Japan in 1981, after its isolation from *Streptomyces hygroscopicus* var. *limonons* (Dabhi et al. 2013). It delays the digestion and absorption of maltose and sucrose. Voglibose and acarbose are commonly prescribed alpha-glucosidase inhibitors (Derosa and Maffioli, 2012b). The structure of voglibose is shown in Fig. 11.
**Fig. 11: Structure of α-glucosidase Inhibitor (Voglibose)**

![Structure of α-glucosidase Inhibitor (Voglibose)](image)

*Figure source: Deruiter, 2003, Endocrine Pharmacotherapy Module, Spring 2003.*

**Mechanism of Action:**

Voglibose is an alpha-glucosidase inhibitor, known for its ability to increase and prolong glucagon like peptide-1 (GLP-1) secretion in T2DM subjects which shows inhibitory action on glycogen breakdown and lowers fasting glucose levels (Dabhi et al. 2013; Gupta, 2013). Increased release of GLP-1, which is an insulinotropic hormone, enhances insulin secretion and insulin sensitivity (Tahrani et al. 2011). It also delays the absorption as well as digestion of dietary polysaccharides by reversibly inhibiting carbohydrate digestive enzymes (Derosa and Maffioli, 2012a). Voglibose improves glucose tolerance by inhibiting digestion and absorption of glucose from intestine and decreases postprandial glucose without inducing over secretion of insulin (Naik, 2014). The mechanism of action of α-glucosidase inhibitor is shown in Fig. 12.
**Fig. 12: Mechanism of Action of α-glucosidase Inhibitor**

GLP-1: Glucagon like peptide-1

*Figure modified from Arungarinathan et al., 2011, British Journal of Cardiology 18(2): 78-81.*

**Side Effects:**

The common side effects include abdominal flatulence and gastrointestinal side effects (Lee et al. 2014a) and it has also been shown to raise liver enzymes. Voglibose hypersensitivity in diabetic patients is known to increase the risk of hepatitis and severe cholestasis (Dabhi et al. 2013).

### 3.1.5 Other Therapeutic Agents with Anti-Hyperglycemic Effects

#### 3.1.5.1 Angiotensin II Type 1 Receptor (AT1) Blocker (Telmisartan):

Telmisartan is a well-established angiotensin II type 1 receptor (AT1) blocker having the longest half-life of about 24 hours (Michel et al. 2013). Telmisartan is reported to control blood pressure and improve insulin resistance (Li et al. 2013). The structure of telmisartan is shown in Fig. 13.
Fig. 13: Structure of AT1 Blocker (Telmisartan)

Figure source: Amano et al., 2012, Hypertension Research 35(7): 715-719.

Mechanism of Action:

Telmisartan acts as a partial agonist of PPARγ, a transcription factor that plays a role in regulation of carbohydrate and lipid metabolism and insulin resistance (Mori et al. 2012). Telmisartan is highly specific for AT1 which induces decrease in blood pressure and also blocks harmful effects like vasoconstriction (Fig. 14). It also activates protein kinase, NADPH oxidase, Janus kinase transcription cascade and cell proliferation (Billecke and Marcovitz, 2013).

Fig. 14: Mechanism of Action of AT1 Blocker (Telmisartan)

AngI: Angiopoietin I; AngII: Angiopoietin II; ACE: Angiotensin I converting enzyme; AT1: Angiotensin II type 1 receptor blocker

Figure modified from Saavedra, 2012, Clinical Science 123(10): 567-590.

Side Effects:

Telmisartan has rare side effects like headache, paresthesias, weakness, anorexia, nausea, vomiting and diarrhea (Garg et al. 2012).
3.1.5.2 Hydroxymethylglutaryl-CoA (HMG-CoA) Reductase Inhibitor (Statin):

Mevastatin is the first 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) (Fig. 15) reductase inhibitor isolated from *Penicillium citrinum*. Pravastatin, lovastatin and simvastatin are derivatives while atorvastatin, cerivastatin, fluvastatin, pitavastatin and rosuvastatin are chemically synthesized compounds (Rao et al. 2011). Several studies reported favorable effects of atorvastatin, fluvastatin, lovastatin, simvastatin and rosuvastatin on lipid levels in T2DM (Hu et al. 2012; Agouridis et al. 2013). Atorvastatin has shown to increase total protein level and decrease the level of serum glutamic oxaloacetic transaminase, serum glutamate pyruvate transaminase and alkaline phosphatase (Pichandi et al. 2011). It also exhibits antioxidant properties by reducing lipid peroxidation and increasing catalase activity (Hadi et al. 2010).

*Fig. 15: Structure of HMG-CoA Reductase Inhibitor (Statin)*

![Fig. 15: Structure of HMG-CoA Reductase Inhibitor (Statin)](image)

*Figure source: Istvan and Deisenhofer, 2001, Science 292(5519): 1160-1164.*

**Mechanism of Action:**

Statins are known to block the HMG-CoA reductase, which catalyzes the key step in cholesterol synthesis. HMG-CoA reductase prevents the conversion of HMG-CoA to L-mevalonate resulting in the inhibition of downstream cholesterol biosynthesis (Kotyla, 2010) (Fig. 16).
**Fig. 16: Mechanism of Action of HMG-CoA Reductase Inhibitor (Statin)**

*HMG-CoA: 3-hydroxy-3-methylglutaryl-coenzyme A*

*Figure modified from Bonetti et al., 2003, European Heart Journal 24(3): 225-248.*

**Side Effects:**

Statins are reported to reduce the production of inflammatory cytokines like TNF-α and interleukin (IL) 1β, IL8 and IL6, which are associated with natural immunity (Tandon et al. 2010). Statins have been shown to disrupt oxidative stress and inflammation cycle by decreasing PPARα and PPARγ, which are responsible for the release of inflammatory mediators and peroxidation of lipids (Rondi et al. 2014). It increases the production of liver enzymes which may cause hepatotoxicity (Maji et al. 2013). Daily statin treatment has become controversial and is associated with adverse effects like increased blood glucose, muscle pain, fatigue, weakness, rhabdomyolysis and disturbed mitochondrial function (Golomb and Evans, 2008).

Overall mechanism of oral hypoglycemic agents and their targets in T2DM is summarized in Fig. 17.
In T2DM effective treatment of hyperglycemia is a first priority to achieve specific glycemic goals which can considerably reduce morbidity (Nathan et al. 2009; American Diabetes Association, 2008). While the management of hyperglycemia has historically taken center stage in the treatment of diabetes, therapies directed at other coincident features, such as dyslipidemia, hypertension, obesity and insulin resistance, have also been a major focus of research and therapy. However, availability of modern treatments for management of diabetes failed to demonstrate a beneficial effect of intensive diabetes therapy on CVD caused by dyslipidemia or altered lipid metabolism in type 2 diabetes (Gerstein et al. 2008; Abraira et al. 2009; Nathan et al. 2009; Nyenwe et al. 2011).

3.2 Lipid Metabolism:

Liver is the main lipid metabolizing site in the body. Lipids are essential for energy homeostasis, organ physiology and numerous aspects of cellular biology (Lee

FFA: Free fatty acid

Figure modified from Cheng and Farese, 2005, Canadian Medical Association Journal 172(2): 213-226.
et al. 2003). Lipid metabolism consists of catabolic processes in which primary metabolites of fatty acids and energy are generated and anabolic processes in which biologically important molecules are synthesized from fatty acids and other dietary sources. Hepatic fatty acid pool can be derived from diet and/or through de novo synthesis (inside the body) in case of excess carbohydrates. These fatty acids are further oxidized or esterified to glycerol for the synthesis of triglycerides (Bechmann et al. 2012). The hepatic lipid metabolism involves a balance between expressions of lipogenic genes and genes involved in fatty acid oxidation (Jump, 2008, 2011).

3.2.1 Fatty Acid β-Oxidation:

Fatty acid β-oxidation is a mitochondrial process in which the conserved energy of the fatty acids is released in a stepwise manner. At the time of β-oxidation, two carbon molecules of fatty acids are removed producing activated 2-carbon and acetyl-CoA during each step (Dashty, 2014). Fatty acid oxidation occurs in two stages in the cytoplasm and mitochondria (Fig. 18).

Fig. 18: Fatty Acid β-Oxidation

CoA-SH: Coenzyme A; ATP: Adenosine triphosphate; AMP: Adenosine monophosphate; PPi: Inorganic pyrophosphate; CPT-I: Carnitine palmitoyltransferase I; CPT-II: Carnitine palmitoyltransferase II; TCA: Tricarboxylic acid

Dietary PUFA entering into the cell cannot directly enter into mitochondria because of their long chains. Therefore they must be converted to acyl-CoA by fatty acyl CoA synthetase (ACS) in order for it to enter the mitochondria for β-oxidation. These acyl-CoA are shuttled into the mitochondria through carnitine palmitoyltransferase 1 (CPT1) for β-oxidation (Tisdale, 2009). Mitochondrial β-oxidation oxidizes short to long chain fatty acids, while very long chain fatty acids are β-oxidized in peroxisomes (Nguyen et al. 2008; Bender, 2014).

The acyl-CoA is shuttled into the mitochondria through CPT1 for β-oxidation to generate acetyl-CoA. Acetyl-CoA is oxidized through the citric acid cycle to yield energy, H₂O and CO₂ or it is converted to different components or metabolites such as 1) citrate, which exits to the cytosol and generates acetyl-CoA through ATP citrate lyase (ACLY), or to 2) ketone bodies, through the HMG-CoA system, or 3) take part in cholesterol synthesis, a reaction catalyzed by HMG-CoA reductase. Low fatty acid oxidation has been observed in T2DM patients which could be a result of lower expression of genes like PPAR and CPT1. This further leads to increased fatty acid synthesis and lipid accumulation resulting into insulin resistance (Muioio and Newgard, 2008a; Wakil and Abu-Elheiga, 2009).

### 3.2.2 Fatty Acid Synthesis:

Fatty acid synthesis is a central event in lipid metabolism. Fatty acids are utilized for the formation of triglycerides (where fatty acids are esterified with glycerol to form fats) (Harwood, 2005). Carbons from glucose (or acetate) are incorporated into fatty acids through a series of enzymatic reactions starting with the formation of malonyl-CoA from acetyl-CoA by acetyl CoA carboxylase (ACC). The synthesis occurs in the cytoplasm. The ACC generated malonyl-CoA is utilized by fatty acid synthase (FAS) for the synthesis of fatty acids in the cytosol. Fatty acid synthesis takes place via the enzymatic activity of a single, homodimeric, multifunctional protein, acyl carrier protein (ACP) and FAS complex (Wakil, 1961).

The synthesis of fatty acids by FAS requires acetyl-CoA, malonyl-CoA and NADPH. The first committed step in fatty acid synthesis is catalyzed by FAS; a multifunctional cytosolic protein that primarily synthesizes palmitate, a 16 carbon saturated fatty acid (Nguyen et al. 2008; Lodhi et al. 2011). Thus in the cytosol,
acetyl-CoA is carboxylated to malonyl-CoA by ACC1 and utilized through FAS to generate saturated fatty acids. Elongation phase of fatty acid synthesis starts with the formation of acetyl ACP and malonyl ACP. This reaction is catalyzed by acetyl transacylase and malonyl transacylase which separate CoA from acetyl-CoA and malonyl-CoA (Wakil, 1961). Afterward, saturated fatty acids undergo elongation and desaturation to form monounsaturated fatty acids with the help of stearoyl-CoA desaturase 1 (SCD-1) (Fig. 19).

**Fig. 19: Fatty Acid Synthesis**

![Fatty Acid Synthesis Diagram](image)

**ACLY**: ATP citrate lyase; **ACC1**: Acetyl CoA carboxylase 1; **ACC2**: Acetyl CoA carboxylase 2; **FAS**: Fatty acid synthase

*Figure modified from Wakil, 1961, Proceedings of the National Academy of Sciences USA 52(1): 106-114.*

Increased concentration of acetyl-CoA in the cytoplasm triggers fatty acid synthesis. Excess of acetyl-CoA, through stimulation of the Krebs cycle and citrate synthesis, is converted to malonyl-CoA and stored as fatty acids (Foster, 2012). Phosphorylation of ACC by AMPK decreases the level of malonyl-CoA and inhibits fatty acid synthesis (Foster, 2012). AMPK is activated when the intracellular energy is low. AMPK also stimulates fatty acid oxidation by augmenting the inhibitory effect of malonyl-CoA for transport of fatty acids to mitochondria (Xue and Kahn, 2006).
When glucose level is lower, cAMP or protein kinase A (PKA) pathway gets stimulated by glucagon and epinephrine (Jiang and Zhang, 2003) which inhibits AMPK pathway. This leads to acetyl-CoA being consumed in ketogenesis pathway instead of fatty acid synthesis (Liljenquist et al. 1974). When glucose levels are lower in the body, ketone bodies are used as alternative source for energy. When glucose levels are higher, insulin stimulates the pentose phosphate pathway (Ceddia et al. 2003), ACC and fatty acid synthesis (Witters et al. 1988). This process is regulated by transcription factors like upstream regulatory factors (URFs) and sterol regulatory element binding factor 1 (SREBP1) (Kersten, 2001). Several reports suggest that higher expression of SREBP activates lipogenic genes like ACC and FAS resulting in increased fatty acid synthesis in T2DM (Teran-Garcia et al. 2007; Strable and Ntambi, 2010; Devarshi et al. 2013).

**Triglyceride Synthesis:**

Esterification of three fatty acid molecules with glycerol produces triglycerides. The first step of triglyceride synthesis is consumption of glycerol in the form of glycerol-3-phosphate (G3P) (Wendel et al. 2009). Liver and adipose tissue synthesize G3P by two different ways. In the liver, glycerol undergoes phosphorylation to produce G3P with the help of glycerol kinase and ATP (Watford, 2000). In adipose tissue, G3P is synthesized by reduction of dihydroxy acetone phosphate with the help of glycerol-3-phosphate dehydrogenase. Thereafter, two molecules of acyl-CoA (fatty acids) join to G3P using phosphatidic synthetase or fatty acyl-CoA transferase to from phosphatidic acid (Dashty, 2014). Phosphatidic acid, in presence of phosphatase, loses one phosphate group and produces diacylglyceride. Diacylglycerol combines with one extra acyl-CoA and produces triglycerides with the help of triglyceride synthase (Chen and Farese, 2005) (Fig. 20).

Triglycerides are then transported in the form of VLDL (Chao et al. 1986). Glycerol can also follow gluconeogenesis to produce glucose and glycogen (Dashty 2013). Higher triglyceride and VLDL levels have been reported in T2DM patients (Mooradian 2009; Laakso, 2010), which could due to attributed to the higher expression of genes like SREBP, SCD, ACSL (Yan et al. 2015; Phillips et al. 2010).
**Cholesterol Synthesis:**

Acyl CoA combines with acetyl-CoA to form acetoacetyl-CoA. The acetyl-CoA acts as precursor in the cytosol and hydroxymethylglutaryl (HMG)-CoA synthase is the first enzyme for the cholesterol synthesis (Dashty, 2013). HMG-CoA is formed by condensation of acetyl-CoA and acetoacetyl-CoA. This reaction is catalyzed by HMG-CoA synthase. Further, HMG-CoA gets converted into mevalonate with the help of second enzyme HMG-CoA reductase which is a rate limiting step in cholesterol synthesis (DeBose-Boyd, 2008) (Fig. 21). In diabetes, high levels of cholesterol and LDL are reported (Steiner, 2005) partly as a result of higher expression of SREBP, HMG-CoA synthase and HMG-CoA reductase (Maron et al. 2000; Eberle et al. 2004).
3.3 Transcription Factors and Genes Regulating Lipid Metabolism and Inflammation:

The hepatic lipid metabolism involves synthesis as well as oxidation of fatty acids. These biochemical reactions are regulated by transcription factors like peroxisome proliferator activated receptors (PPARs), sterol regulatory element binding proteins (SREBPs), and genes like acetyl CoA carboxylase (ACC), fatty acid synthase (FAS), carnitine palmitoyltransferase (CPT), stearoyl-CoA desaturase (SCD), long-chain acyl-CoA synthetases (ACSL), malonyl-CoA-acyl carrier protein transacylase (MCAT) etc. (Jump, 2011).

3.3.1 Peroxisome Proliferator Activated Receptors (PPARs):

PPAR is a ligand activated transcription factor that belongs to steroid hormone receptor super family (Yoon, 2009). PPARs form heterodimers with retinoid X receptors (RXRs) and these heterodimers regulate transcription of various genes (Pawlak et al. 2012). PPARs regulate the expression of genes critical for lipid
metabolism, fatty acid oxidation, fat cell development and lipoprotein metabolism (Yoon, 2009). Three subtypes of PPARs are known: PPARα, PPARδ and PPARγ. PPARγ is a regulator of adipocyte differentiation (Evans et al. 2004; Siersbaek et al. 2010). Additionally, PPARγ has been implicated in the pathology of various diseases including obesity, diabetes, atherosclerosis and cancer (Tyagi et al. 2011). PPARγ is expressed in adipose tissue, where it regulates adipocyte differentiation, secretion of adipocytokines and insulin sensitivity of adipocytes (Evans et al. 2004; Siersbaek et al. 2010). PPARγ, besides being a key component of adipose tissue development and a target of insulin-sensitizing drugs, also has a role in immune cell differentiation and function (Szatmari et al. 2007). PPARγ is expressed in inflammatory cells and regulates the production of inflammatory mediators, having anti-inflammatory action (Szanto et al. 2008).

PPAR ligands include several naturally occurring long chain PUFAs, such as docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), arachidonic acid (AA) and linoleic acid (LA) (Grygiel-Gorniak, 2014). Ligand activated PPAR enhances the expression of genes related to fatty acid oxidation (Jump, 2008). DHA is considered as a more preferred ligand since it fits into the long and large hydrophobic ligand binding pocket of PPAR (Itoh and Yamamoto, 2008). After binding to its ligand, PPAR heterodimerizes with RXR. This heterodimer binds to peroxisome proliferator response elements of the target genes and allows the transcription of genes involved in oxidation of fatty acids (Pawlak et al. 2012).

### 3.3.2 Sterol Regulatory Element Binding Proteins (SREBPs):

Sterol regulatory element binding proteins (SREBPs) are transcription factors that regulate lipid homeostasis by controlling the expression of various enzymes required for cholesterol, fatty acid, triglyceride and phospholipid synthesis (Horton et al. 2002; Ye and Debose-Boyd, 2011). SREBPs are known to regulate fatty acid and cholesterol metabolism in liver (Horton et al. 2002). The three SREBP isoforms, SREBP1a, SREBP1c and SREBP2, have different roles in lipid synthesis. SREBP1c is known to enhance the transcription of genes involved in fatty acid, triglyceride and phospholipid synthesis while SREBP1a and SREBP2 activate genes involved in cholesterol synthesis (Pegorier et al. 2004). SREBP1c expression is induced by cholesterol and repressed by PUFAs. PUFAs promote PPAR and suppress SREBP,
thus inhibiting lipogenesis (Jump, 2008). SREBP1c is also activated by insulin at the post-translational level. Homodimer of activated SREBP1c binds to sterol regulatory element (SRE) sequences on the promoters of its target genes and induces genes like ATP-citrate lyase (ACLY), acetyl-CoA synthetase (ACS), ACC, FAS, SCD1 and glycerol-3-phosphate acyltransferase (GPAT), involved in fatty acid and triglyceride synthesis. Studies demonstrate that a decrease in SREBP transcription leads to hepatic inhibition of FAS and ACC and results in lower levels of fatty acids, triglyceride and VLDL (Teran-Garcia et al. 2007). Additionally, in humans, hepatic steatosis is associated with increased levels of SREBP1c (Higuchi et al. 2008). Thus, down-regulation of SREBP1 in the liver has a therapeutic value in treating diabetic hepatic steatosis and carbohydrate induced hypertriglyceridemia (Moon et al. 2012).

3.3.3 Fatty Acid Synthase (FAS):

Fatty acid synthase is a complex multifunctional enzyme comprising of two identical monomers. Each FAS monomer of about 270 kDa contains six catalytic activities (Chirala and Wakil, 2004). FAS plays important role in de novo lipogenesis by converting acetyl-CoA and malonyl-CoA into the final end product palmitate, which is further converted into triacylglycerols and stored in adipose tissues (Griffin and Sul, 2004). SREBP 1 and upstream stimulatory factors play important role in regulating FAS transcription. Omega-3 fatty acids suppress the transcription of lipogenic genes like FAS (Teran-Garcia et al. 2007).

3.3.4 Long-Chain Acyl-CoA Synthetases (ACSLs):

Long chain acyl CoA synthetases (ACSLs) include five different ACSL isoforms, each encoded by a separate gene (Mashek et al. 2006). ACSLs located on outer membrane of endoplasmic reticulum and mitochondria activate fatty acids, with chain lengths from 12 to 20 carbon atoms, to form acyl-CoAs, which act as intermediates for lipid metabolism (Soupene and Kuypers, 2008). ACSL isoforms are involved in fatty acid metabolism, membrane modifications and different physiological processes (Yan et al. 2015). ACSLs plays a crucial role in regulating fatty acid metabolism by converting free fatty acids (FFA) into fatty acyl CoA derivatives (fatty acid activation) (Saraswathi and Hasty, 2009). ACSL catalyzes the first step in fatty acid activation for intracellular metabolism by converting long-chain fatty acids into acyl-CoA thioesters (Hall et al. 2003). Activated fatty acids can take
part into several metabolic pathways like β-oxidation, synthesis of triglycerides and cholesterol esters (Saraswathi and Hasty, 2009). ACSL1 is highly expressed in energy-metabolizing tissues like liver, adipose tissue and muscles (Coleman et al. 2000; Mashek et al. 2006). In the liver, ACSL1 accounts for about 50% of total hepatic ACSL activity (Li et al. 2009). ACSL plays an important role in triglyceride synthesis by partitioning fatty acids towards triglyceride synthesis (Yan et al. 2015). ACSL1 over-expression increases the proportion of oleic acid in diacylglycerol and phospholipids. Over-expression of ACSL is reported to increase the synthesis of triglycerides (Li et al. 2006; Ellis et al. 2010). Deficiency of ACSL1 causes reduced triglyceride synthesis and increased β-oxidation of lipids in liver cells (Li et al. 2009). Increased expression of ACSL1 leads to higher synthesis of triglyceride and its accumulation in hepatoma cells (Parkes et al. 2006). ACSL is regulated by PPAR (Schoonjans et al. 1996; Martin et al. 1997), and it is also implicated in the pathogenesis of diabetes (Phillips et al. 2010). Fatty acyl derivatives have been implicated in the pathogenesis of T2DM, including insulin resistance and pancreatic dysfunction (McGarry, 2002), and triglyceride biosynthetic enzymes such as ACSL are increased in obesity (Coleman et al. 2000). Metformin has been shown to lower ACSL expression and triglyceride levels in diabetic rats (Forcheron et al. 2009).

3.3.5 Malonyl-CoA-Acyl Carrier Protein Transacylase (MCAT):

*De novo* biosynthesis of fatty acids may take place through two different pathways (Hiltunen et al. 2009). In the cytosol, palmitate is synthesized with the help of megasynthase complex. In the mitochondria, octanoyl moieties are synthesized by using several enzymes (Bunkoczi et al. 2009). Initiation of fatty acid biosynthesis in two different compartments is dependent upon the substrates. In cytosol, acetyl CoA and malonyl-CoA is required for fatty acid synthesis (White et al. 2005) while in mitochondria only malonyl-CoA is translocated with the help of MCAT system (Zhang et al. 2003). The protein encoded by MCAT gene is found exclusively in the mitochondrion. Besides triglyceride synthesis by ACSL, MCAT catalyzes transfer of malonyl-CoA moiety to free thiol group on the acyl carrier protein in mitochondria, indicating its role in mitochondrial fatty acid synthesis (Zhang et al. 2003). Lower expression of both MCAT and ACSL has beneficial effects in lowering the fatty acid synthesis and triglyceride levels (Zhang et al. 2003; Li et al. 2009).
3.3.6 **Nuclear Factor Kappa β (NFκβ):**

NFκβ is a key transcription factor and acts as a master switch for regulation of gene expressions encoding proinflammatory cytokines like TNF-α, several interleukins like IL1, IL6, IL8, adhesion molecules, and nitrous oxide synthase (Kumar et al. 2004; Sigal, 2006). In the cytosol, inactive NFκβ is present in the form of a trimer. Extracellular signals, such as inflammatory and oxidative stress, activate NFκβ by phosphorylation of its inhibitory subunit, Iκβ. This leads to dissociation of Iκβ from the NFκβ leading to formation of active NFκβ as a dimer. After activation, NFκβ (dimer) translocates to nucleus and activates expression of genes involved in synthesis of proinflammatory cytokines such as TNF-α (Hayden et al. 2006; Perkins, 2007; Oeckinghaus and Ghosh, 2009).

3.3.7 **Tumor Necrosis Factor Alpha (TNF-α):**

TNF-α is an important pro-inflammatory cytokine with several biological actions in different cells, tissues, organs and also regulates and/or interferes in lipid metabolism (Zhang et al. 2002; Chen et al. 2009). TNF-α is produced predominantly by activated macro-phages and T lymphocytes. The actions of TNF-α are facilitated by two distinct receptors, such as tumor necrosis factor receptor I and II (Bradley, 2008). These two receptors have different binding affinities and activate different signaling pathways (Tartaglia et al. 1993; Aggarwal, 2003). Effects of TNF-α on lipid metabolism through different mechanisms like reduction in uptake of free fatty acids, increase in lipogenesis, inhibition of enzymes involved in lipid metabolism etc. in turn regulates cholesterol metabolism and adipokines secretion (Fon Tacer et al. 2007; Chen et al. 2009; Srivastava et al. 2012). TNF-α activates SREBP1, up-regulates FAS and suppresses PPARγ gene expression thereby leading to increase in fatty acid synthesis and lowered fatty oxidation (Cawthorn and Sethi, 2008; Choi et al. 2012). Diverse physiological roles of TNF-α are thus important in the development of atherosclerosis, insulin resistance and T2DM (Popa et al. 2007; McArdle et al. 2013). Overall mechanism of transcription factors and genes regulating lipid metabolism and inflammation is summarized in Fig. 22.
3.4 Effects of OHAs on Lipid Metabolism and Inflammatory Markers:

Metformin is an antidiabetic drug which has ability to lower blood glucose by decreasing gluconeogenesis in the liver, stimulating glucose uptake in the skeletal muscles and increasing fatty acid oxidation in adipose tissues (Stumvoll et al. 2005; Shu et al. 2007). The antidiabetic effect of metformin is mainly attributed to hepatic activation of AMPK which is known to regulate lipid metabolism (Shu et al. 2007). It is well known that after metformin induced phosphorylation, AMPK deactivates ACC and HMG-CoA reductase, lowers FAS expression and activates malonyl-CoA carboxylase which decreases fatty acid and cholesterol synthesis (Kohjima et al. 2008). Metformin also inhibits adipogenesis and modulates adipokine secretion which consequently lowers the adipose tissue expansion (Huypens et al. 2005). Metformin has been shown to increase adiponectin which activates AMPK and inhibit hepatic
lipid accumulation through increased β-oxidation and/or lowering fatty acid synthesis (Coughlan et al. 2014).

TZDs like rosiglitazone and pioglitazone also stimulate AMPK activity. TZDs activate AMPK by a mechanism independent of PPARγ regulated gene transcription, which appears to be associated with change in cellular energy state (Brunmair et al. 2004; Saha et al. 2004). TZDs act as ligand for PPARγ (Spiegelman, 1998) which activate PPARγ and thus increase β-oxidation and lower fatty acid synthesis (Srivastava 2009, 2011). It has been suggested that TZDs increase adiponectin secretion (Bays et al. 2004) which is known to stimulate glucose uptake and fatty acid oxidation in muscles and inhibit gluconeogenesis in the liver (Yamauchi et al. 2002; Kadowaki et al. 2007). In the skeletal muscles of diabetic patients treated with TZDs, an increase in peroxisome proliferator-activated receptor gamma coactivator-1-alpha (PPARGC1α) was found, leading to increased glucose uptake and fatty acid oxidation (Mootha et al. 2003).

TZDs are reported to lower lipolysis through the reduction of TNF-α and accelerated uptake of free fatty acids by adipocytes which lower fatty acids in circulation (Schimmack et al. 2006; Srivastava et al. 2012; Kaur, 2014). Rosiglitazone shows anti-inflammatory effects by inhibiting the binding of NFκβ to DNA in the nucleus of PBMCs in obese and obese diabetic patients (Mohanty et al. 2004). TZDs also inhibit the secretion of several inflammatory adipocytokines like IL-6 and resistin which induce insulin resistance in muscle. It is well known that monocytes respond to PPARγ ligands by reducing the expression of inflammatory cytokines ex vivo, such as TNF-α, IL6 and IL1β (Jiang et al. 1998).

Increased glucose levels lead to altered lipid profile in T2DM patients (Mooradian, 2009) which can be managed by lipid lowering drugs like statins (Maron et al. 2000; Barakat et al. 2013). Statins inhibit HMG-CoA reductase which is involved in the cholesterol synthesis (Istvan, 2002). Such altered lipid profile is a consequence of modulation of several genes in different tissues. Human tissues are not easily accessible because of which peripheral blood mononuclear cells (PBMCs) are preferred for the study of gene expression. Several reports suggest that altered lipid metabolism in different tissues is reflected in PBMCs (Marx et al. 2001, 2002; Bouwens et al. 2007, 2008, 2010).
3.5 Peripheral Blood Mononuclear Cells (PBMCs) as Surrogate Cells to Monitor Gene Expression:

Genomic approaches are used to determine differential gene expression profiles by expression analysis of genes (Kannel and McGee, 1979; Cras-Meneur et al. 2004). Gene expression analysis is now providing global views towards the possible genes and pathways that are associated with diabetes (Rao et al. 2004). In humans, approximately 800 genes have been implicated in diabetes based on differential gene expressions from the tissues like pancreas, muscle and fat (Rome et al. 2003). Gene expression analysis is an interesting way to check the effects of pharmacological and nutritional supplementations on the target tissues. However, it is difficult in humans to get the target tissues like pancreas, liver, muscles, adipose tissues etc. due to inaccessibility as well as several ethical issues including the need for surgical procedures to obtain these tissues. Use of blood to monitor gene expression profiles has several advantages. Blood is easily accessible in contrast to the tissues listed above (Bragt-van Wijngaarden, 2011; Koncarevic et al. 2014). In addition, blood cells that circulate through the body are suggested to serve as sentinels, which pick up physiological and pathological changes and capture these changes by altering their transcriptome (Liew et al. 2006). PBMCs are cells that contain a round nucleus and consist of different lymphocytes like T-cells, B-cells and NK-cells; and monocytes which play an important role in the immune system (Mallone et al. 2011). PBMCs are easy to isolate from a whole blood. They can also be used for mRNA extraction and gene expression analysis. Changes in PBMC gene expression due to nutritional (van Erk et al. 2006), pharmacological (Wibaut-Berlaimont et al. 2005), and toxicological stimuli (van Leeuwen et al. 2005) and exercise (Connolly et al. 2004) are well known.

It has been suggested that PBMCs express approximately 80% of the genes encoded by the human genome and these genes respond to the macro and micro environmental changes occurring in body (Liew et al. 2006). PBMCs have the same transport proteins present in liver, muscles, myocardial tissue and other target cells affected in diabetes mellitus. In humans, liver plays an important role in coordinating and regulating lipid metabolism (Jump, 2011). PBMCs reflect important metabolic changes in the liver (Telle-Hansen et al. 2013; Konieczna et al. 2014). The expression pattern and regulation of genes involved in lipid metabolism is also reflected in
human PBMCs (Marx et al. 2001, 2002; Bouwens et al. 2007, 2008, 2010; Telle-Hansen et al. 2013; Konieczna et al. 2014). Thus, PBMCs have been described as a model system to study the pathophysiology of diabetes and its complications (Balasubramanyam et al. 2002; Bouwens et al. 2007).

3.5.1 Gene Expression Analysis in PBMCs for Disease Diagnosis and Prediction of its Progression:

PBMCs act as early biomarkers of metabolic health and provide valid biological information for the study of metabolic processes in humans (Konieczna et al. 2014). In peripheral arterial disease patients, expression of 47 genes is reported to be higher while expression of 39 genes was lower in PBMCs (Masud et al. 2012). Individuals at the risk of obesity were reported to show higher free fatty acid levels while reduced PBMC gene expression level of uncoupling protein 2 (mitochondrial protein carrier), hormone-sensitive lipase and PPARδ as compared to obese subjects (Telle-Hansen et al. 2013). Genes involved in lipid metabolism such as CPT1, FAS, ACC1/2, acylcarnitine/carnitine translocase, very long chain acyl-CoA dehydrogenase and acyl-CoA thioesterase-2 were reported to be altered in human PBMCs (Bouwens et al. 2007; Konieczna et al. 2014). Altered expression of transcription factors like PPARγ and NFκβ in PBMCs has been reported in metabolic syndrome patients (Macias-Gonzalez et al. 2008; Garcia-Fuentes et al. 2010). Several reports suggest that inflammatory genes like NKκβ, TNF-α, IL8 etc. are altered in the PBMCs (Crujeiras et al. 2008; Navarro-Gonzalez et al. 2010; Elliott et al. 2014; Gjevestad et al. 2015).

3.6 Omega-3 Fatty Acids:

Omega-3 fatty acids were first discovered in 1929 by George Burr and Mildred at the University of Minnesota Medical School (Burr, 1981; Spector and Kim, 2015). Omega-3 and omega-6 fatty acids are of primary importance for their role in normal growth and development (Coletta et al. 2010). LCPUFA are constituents of cell membrane phospholipids and maintain fluidity, permeability and conformation of cell membranes. They also perform membrane-associated functions and act as intracellular mediators of gene expression (Dutta-Roy, 2000). AA and DHA are important structural fatty acids in neural tissues. They provide energy and
act as the precursors of the metabolically active compounds such as the prostacyclins, prostaglandins, thromboxanes, leukotrienes, resolvins and perform structural and functional roles within the body (Patterson et al. 2012).

### 3.6.1 Sources of Omega-3 Fatty Acids:

EPA and DHA are present in fish oils, egg oil, squid oils, krill oil, while some plants or plant based products like walnut, chia seeds, clary sage seed, algal, flaxseed, sacha inchi, Echium, and hemp oil contain ALA (Kris-Etherton et al. 2003; Tur et al. 2012). Fish oil (EPA and DHA) and flaxseed oil (ALA) are the richest sources of omega-3 fatty acids (Bradberry and Hilleman, 2013).

### 3.6.2 Health Benefits of Omega-3 Fatty Acids in Metabolic Syndrome:

Omega-3 fatty acids important in human physiology are ALA, EPA and DHA (Jump et al. 2012). Fatty acids are mainly located in membrane phospholipids (Haber et al. 2006) and changes in fatty acid composition can alter membrane structure and function of islet cells. Such alteration leads to changes in the activities of β-cell channels and receptors (Stutzer et al. 2012). Further, phospholipids, diacylglycerol, phosphatidic acid and triacylglycerol are implicated in different cellular mechanisms influencing a range of glucose and lipid signaling pathways (Haber et al. 2006; Zechner et al. 2012).

Although ALA, EPA and DHA are grouped together as omega-3 fatty acids, there is significant evidence suggesting that the individual fatty acids may have selective and potentially independent effects on cardiovascular health (Lee et al. 2009a; Saremi and Arora, 2009; Yashodhara et al. 2009). The ratio of omega-6:omega-3 fatty acids in early human diets was probably almost equal (1:1). However, due to changes in dietary habits (especially with the increased use of vegetable oils such as soybean, corn, sunflower, safflower oil, and cotton seed oils rich in LA, there is increase in the ratio of omega-6:omega-3 leading to increased risk of metabolic syndrome (Connor, 2000; Russo, 2009).

It has been suggested that reduction of omega-6:omega-3 ratio may reduce the risk of metabolic syndrome (Stanley et al. 2007; Griffin, 2008). Irrespective of the amount of omega-6 fatty acids in the diet, there is a growing consensus that incorporation of high levels of ALA and the metabolically more active EPA and DHA.
in the diet is important for reducing the risks of CVDs (Stanley et al. 2007; Griffin, 2008).

3.6.3 Effects of Omega-3 Fatty Acids on Glucose Homeostasis and Insulin Resistance:

Omega-3 fatty acid supplementation has been shown to reduce insulin resistance and improve glucose tolerance in metabolic syndrome and type 2 diabetes rat models (Sener et al. 2009). ALA deprivation in rats is reported to increase adipose tissue mass, plasma glucose and insulin concentrations, and insulin resistance index (Sener et al. 2009). Rats deprived of omega-3 fatty acids when fed with flaxseed oil enriched diet showed decreased plasma glucose concentrations and insulin resistance index (Sener et al. 2009). The replacement of LA (from safflower oil) with omega-3 fatty acid (from fish oil) has been shown to prevent the development of insulin resistance in liver and skeletal muscle in rats fed on high fat diet (Storlien et al. 1987). These effects were accompanied by decreased intra muscular triglyceride content in skeletal muscles (Jucker et al. 1999). Treatment of high fat diet fed C57BL/6 mice, with DHA/EPA and rosiglitazone lead to increase in adiponectin secretion, reduction in hepatic lipogenesis and improvement in glucose tolerance thereby preventing obesity, adipose tissue hypertrophy and inflammation, dyslipidemia and insulin resistance in mice (Kuda et al. 2009).

The beneficial effects of omega-3 fatty acids are also evident from several intervention trials in obese children and young obese individuals (Lopez-Alarcon et al. 2011; Dangardt et al. 2012; Juarez-Lopez et al. 2013). IGT and insulin resistance was found to be ameliorated in obese individuals after treatment with omega-3 fatty acids (Ramel et al. 2008; Dangardt et al. 2012). Individuals with IGT, when treated with fish oil, showed improvement in insulin sensitivity and their CVD risk was also lowered owing to decrease in the levels of total cholesterol, triglycerides and LDL (Fasching et al. 1991). In spite of several clinical trials, there is still a lack of consensus about the most effective dosages and formulae for determining the ratio of fatty acids for different pathologies (Lorente-Cebrian et al. 2013).
3.6.4 Effects of Omega-3 Fatty Acids on Lipid Profile:

Omega-3 fatty acids reduce the risk for cardiovascular disease partly by improving serum lipid profile. However, both responses and mechanism of actions of the medium chain and long chain omega-3 fatty acids appear to be independent. ALA exerts most of its effects by modulating lipoproteins, while EPA and DHA may reduce triglyceride synthesis and adiposity. Daily supplementation with 20-50g ALA rich flaxseed has been shown to reduce total cholesterol and LDL cholesterol concentrations in normolipidemic (Cunnane et al. 1993, 1995) as well as hypercholesterolemic patients (Jenkins et al. 1999; Mandasescu et al. 2005). Supplementation of EPA+DHA (4g/day) along with fluvastatin (80mg/day) in hyperlipidemic type 2 diabetics lead to reduction of serum triglycerides, VLDL, and triglyceride/HDL ratio and increase in HDL content (Valdivielso et al. 2009).

In healthy males, fish oil and DHA oil supplementation (4g/day) for 15 weeks has been shown to lower fasting plasma triglyceride concentrations and postprandial total chylomicron concentration (Agren et al. 1996). The Look AHEAD (Action for Health in Diabetes) study examining the effects of omega-3 fatty acid intake in diabetic individuals found that omega-3 fatty acid intake is inversely associated with blood triglyceride levels (Belalcazar et al. 2010). In obese individuals, body mass index, waist circumference and hip circumference are reported to be inversely correlated with EPA and DHA intakes (Micallef et al. 2009). In patients with dyslipidemia, consumption of EPA+DHA (3g/day) increased HDL-cholesterol and decreased triglyceride in blood at 3 and 6 months of treatment (Derosa et al. 2009a). Fish oil supplementation (180mg EPA+120mg DHA capsule/day) in patients with metabolic syndrome, has been shown to reduce body weight and serum concentrations of total cholesterol, LDL cholesterol and triglycerides (Ebrahimi et al. 2009). It has been suggested that the triglyceride lowering effects of DHA and EPA are due to decreased hepatic triglyceride secretion and enhanced clearance of triglyceride from the plasma (Miller et al. 2011; Bradberry and Hilleman, 2013).

3.6.5 Mechanistic Effects of Omega-3 Fatty Acids on Lipid Metabolism and Inflammatory Genes:

Omega-3 fatty acids affect the expression of several key enzymes/proteins involved in lipid metabolism and inflammation (Deckelbaum et al. 2006; Calder,
It is well known that omega-3 fatty acids promote lipid metabolism and reduce inflammation (Scorletti and Byrne, 2013). However, omega-3 fatty acids can up-regulate or down-regulate the expression of different genes in different tissues (Calder, 2012).

PPARs are activated by non-covalent binding of ligands that include omega-3 fatty acid and various eicosanoid mediators (Forman et al. 1995, 1997; Kliewer et al. 1997). In dendritic cells, DHA has been reported to induce PPARγ (Kong et al. 2010) as well as several PPARγ target genes (Zapata-Gonzalez et al. 2008). Omega-3 fatty acids and their metabolites act as natural ligands for PPAR, promoting fatty acid oxidation and suppressing the transcription of lipogenic genes like FAS (Teran-Garcia et al. 2007). These effects are consequently linked to decreased production of inflammatory cytokines TNF-α and IL6 (Kong et al. 2010). Thus, through activation of PPAR, omega-3 fatty acids are able to regulate lipid metabolism, inflammation and several other cell and tissue responses (Madsen et al. 2005; Wang and Huang, 2015). It has been previously demonstrated that, omega-3 fatty acids bring about their hypotriglyceridemic effects by lowering SREBP1c (Davidson, 2006; McKenney and Sica, 2007; Skulas-Ray et al. 2008; Devarshi et al. 2013).

It is well known that EPA and DHA exhibit triglyceride lowering effect by down-regulating SREBP1 and reducing the expression levels of lipogenic genes like HMG-CoA reductase, ACC1 and FAS (Kaur et al. 2011). Dietary omega-6 and omega-3 fatty acids, but not mono-unsaturated or saturated fatty acids, coordinately suppress the transcription of several hepatic genes encoding lipogenic enzymes like FAS, ACC, SCD1 and glycolytic pyruvate kinase (Katsurada et al. 1990; Wilson et al. 1990; Jump et al. 1994; Xu et al. 1999). This suppressive effect of omega-3 fatty acids is associated with a significant reduction in the rate of hepatic malonyl-CoA production and fatty acid biosynthesis (Wilson et al. 1990).

The down-regulation of expression and nuclear content of hepatic SREBP1 by dietary omega-3 fatty acid is correlated with an inhibition of FAS gene expression, suggesting that omega-3 fatty acids inhibit hepatic FAS gene transcription by reducing nuclear SREBP1 (Worgall et al. 1998). However, Teran-Garcia et al. (2007) have reported that omega-3 fatty acids inhibit FAS transcription at two different response regions at FAS promoter. Several evidences indicate that SREBP1 is a weak
activator of transcription and it functions efficiently only when activated by co-activating transcription factors such as NF-Y and specificity protein 1 (Dooley et al. 1998; Ericsson and Edwards, 1998; Bennett and Osborne, 2000). It has also been reported that the omega-3 fatty acids also down regulate ACSL gene involved in triglyceride synthesis (Sampath and Ntambi, 2006).

DHA supplementation shows increases in peroxisomal fatty acid oxidation and activity of fatty acyl-CoA oxidase in the rat liver (Willumsen et al. 1993). Fiamoncini et al. (2013) reported that fish oil enriched diets increase peroxisomal β-oxidation and prevents obesity and glucose intolerance. EPA and DHA phospholipids decrease hepatic as well as serum total cholesterol and triglyceride levels in mice through improvement of lipid metabolism (Tang et al. 2012). EPA also leads to increased CPT activity, peroxisomal β-oxidation and CPT1α mRNA level (Liu et al. 2013). A combination of omega-3 fatty acids, polyphenols and L-carnitine reduces the plasma lipid levels and increases expression of genes like CPT1α/b, OCT and carnitine acetyl transferase in human PBMCs and HepG2 cells (Radler et al. 2011).

Pure EPA and DHA can inhibit the production of a range of inflammatory proteins like TNF-α, IL1, IL6, IL8 and IL12, including COX-2 and inducible NO synthase in cultured endothelial cells (De Caterina and Libby, 1996; Calder, 2012), monocytes (Lo et al. 1999; Babcock et al. 2002), macrophages and dendritic cells (Kong et al. 2010; Draper et al. 2011). These inhibitory effects of omega-3 fatty acids seem to involve decreased activation of NFκβ (Lee et al. 2001; Novak et al. 2003). In agreement with the in vitro studies, feeding fish oil to animals also decreases NFκβ activation (Xi et al. 2001), and production of inflammatory cytokines (Billiar et al. 1988; Renier et al. 1993; Yaqoob and Calder, 1995). Various studies suggest that fish oil supplementation decreases production of TNF-α, IL1β, IL6 and various growth factors by monocytes or mononuclear cells which are stimulated by bacterial endotoxin in healthy humans (Endres et al. 1989; Meydani et al. 1991; Trebble et al. 2003). Diet enriched with flaxseed oil suppresses the synthesis of TNF-α and IL1β (Caughey et al. 1996). Similarly in other study, both flaxseed oil or fish oil diets showed significant down-regulation of transcripts of TNF-α and IL6 in diabetic rat liver (Jangale et al. 2013).
3.7 **Need for Animal Study:**

Epidemiological studies in humans have several limitations due to the invasive nature of certain analyses and the size and randomness of the study populations (Ghezzi et al. 2012). Also, it is not possible to control levels of nutrient restriction in human experiments (Nathanielsz, 2006). Experimental animal studies are important tool which can overcome these issues allowing uniform and standardized interventional studies. The studies in animal models are helpful in answering the key questions relating to exposure of intervention, its mechanism of action and its effect on disease status (Nathanielsz, 2006; Kaplan and Wagner, 2006). Animal studies offer several advantages over human epidemiological studies. Animal studies are carried out in a relatively homogeneous group of animals instead of a heterogeneous group of patients. It is possible to control different environmental and confounding factors and to examine the pathology and mechanisms of disease as well as interventions (Hooijmans and Ritskes-Hoitinga, 2013). Invasive techniques like biopsies can be performed in animals (Baran et al. 2011).

Animal studies are carried out either in large (e.g. pig and sheep) or small (e.g. mouse, rat and guinea-pig) animal models. Large animal models have major advantage of genetic make-up being similar to humans (Rabada-Diehl and Nathanielsz, 2013; Pinnapureddy et al. 2015). However, their use in research is difficult due to their high maintenance cost and requirement of large space (Wang et al. 2010). Small animals have the advantage of short lifespan, requirement of less space and low maintenance cost (Doke and Dhawale, 2013). Rodents are considered as useful and valid species for the study of metabolic syndrome, dyslipidemia and atherosclerosis (Russell and Proctor, 2006). Wistar rats exhibit changes similar to those seen in human metabolic syndrome, such as hyperglycemia, dyslipidemia, abdominal obesity and hypertension (Hill et al. 1992; Aleixandre and Miguel, 2009; Ghezzi et al. 2012). Blood can be withdrawn from retro orbital plexus on a regular interval in rats (Conybeare et al. 1988; Ayers et al. 2012). Further, the rodent diets are well defined and hence it is easy to examine the effects of various nutritional interventions (McMullen and Mostyn, 2009). Several challenges exist in translating animal research to humans such as biological differences between species and strains, type of methodology used in animal experiments, variation in study design and insufficient reporting of details of animals (Hooijmans and Ritskes-Hoitinga, 2013).
Nevertheless, rats have often been used to evaluate the effects of nutritional, herbal and pharmaceutical interventions on disease biology.

Both human and animal studies are equally important and they have their own advantages and limitations. The present work involves biochemical and gene expression analysis of blood samples from type 2 diabetic individuals and nicotinamide-streptozotocin induced diabetic rats.