The spiraling of diabetes epidemic in India is partly due to sheer lack of awareness of this metabolic disorder. The studies about awareness of this disorder till date have consistently reported such lack of awareness in healthy as well as diabetic population with relation to their predisposition to the disorder, the risk factors, importance of regular medical examination etc. (Mohan et al. 2010; Limaye et al. 2015). The improvement in lifestyle with good dietary habits and regular exercise is known to have beneficial effects in delaying metabolic disorders and in the management of hyperglycemia in clinically diagnosed diabetic individuals (Colberg et al. 2010). Several new therapeutic agents are now available in the market for management of hyperglycemia and associated secondary complications of type 2 diabetes mellitus (T2DM) (Nyenwe et al. 2011). The availability of such drugs with complementary mechanism of action has recently lead to multidrug therapy being more common in T2DM (Halimi et al. 2008). Despite all such new treatment regimens, long-term management of hyperglycemia in diabetics is still a challenge. Type 2 diabetic individuals in the present study were also found to be on multidrug therapy with drugs like metformin, pioglitazone, statins, glimepiride, voglibose, amlodipine, telmisartan etc.

The present study investigated the long-term effects of anti-diabetic drugs and chronicity of the disorder in male and female diabetic patients. The influence of insulin resistance and glycemic status on biochemical markers and metabolic measures was also analyzed. Animal experiment was undertaken to study the effects of dietary interventions in the form of omega-3 fatty acids on abnormal lipid profile in diabetic animals.

I. HUMAN STUDY:

6.1 Metabolic Variables in Healthy and Diabetic Individuals:

6.1.1 BMI and WHR:

It is well known that BMI and WHR are important predictors of diabetes (Vazquez et al. 2007; Kodama et al. 2012). In the present study, BMI was found to be comparable in diabetic and healthy males while diabetic females had higher BMI than healthy females. An increase in BMI is generally associated with a high risk of
developing metabolic diseases such as T2DM (Garber, 2012). In the present study, WHR was comparable in both diabetic males and females than healthy individuals of corresponding genders.

BMI is strongly and independently associated with the risk of T2DM. Ganz et al. (2014) have reported that the incremental association of BMI on the risk of T2DM is stronger for people with a higher BMI than people with a lower BMI in the United States. In Asian individuals, the risk association with diabetes and cardiovascular diseases occurs at lower levels of BMI when compared with the white population (McKeigue et al. 1991; Enas et al. 1992; Banerji et al. 1999). This is attributed to body fat distribution in Asian Indians with a tendency towards abdominal deposition of adipose tissue. Such site specific deposition of adipose tissue in Asian Indians is known to cause higher insulin resistance, despite having normal BMI (Banerji et al. 1999; Chandalia et al. 1999). Similar results were also observed in Australian aboriginals, who had an epidemic proportion of glucose intolerance and had BMI values much lower than the obesity limits suggested by Western standards (Daniel et al. 2002). It has been consistently observed that in urban Asian Indian individuals, even minor changes in BMI could tilt the metabolic balance toward hyperglycemia (Ramachandran et al. 1992). It is also observed that Indians have higher upper-body adiposity, measured as the waist-to-hip ratio (WHR) or waist circumference (WC), although they have lean body mass (UKPDS, 1994; Ramachandran et al. 1997). These reports indicate that central obesity is one of the risk factors for development of metabolic disorders. Significant weight loss is also observed in many individuals after development of T2DM (Kaur, 2014). Such weight loss during long standing diabetes may also lead to decrease in WHR which consequently may be comparable to healthy individuals (Blaak et al. 2012). Findings in the present study also report that the WHR is comparable in both healthy and diabetic individuals which may be an outcome of weight loss after development of frank hyperglycemia.

6.1.2 Blood Pressure:

Accumulating evidence suggests that hypertension is associated with type 2 diabetes (DeFronzo and Tripathy, 2009). Several risk factors are responsible for the development of hypertension and diabetes such as altered lipid profile, inflammation and oxidative stress, insulin resistance, obesity, physical inactivity etc. (Cheung and
The risk for cardio-vascular diseases (CVD) is approximately twice for diabetics than non-diabetics (Chung and Won, 2011).

In the present study, systolic and diastolic BP was found to be significantly higher in diabetic males while BP was comparable in diabetic females as compared to healthy individuals of corresponding genders. It is well known that the high BP increases the risk of macrovascular and microvascular complications in T2DM (Ganesh and Viswanathan, 2011; Mohan et al. 2013), suggesting the need to evaluate the risk factors associated with BP. Several studies report that, lowering BP in T2DM can reduce deaths from strokes, overall mortality, and CVD events and also progression of renal disease in T2DM patients (Curb et al. 1996; Martin-Timon et al. 2014).

6.2 Biochemical Parameters in Healthy and Diabetic Individuals:

6.2.1 Glucose, Insulin and HOMA-IR:

In the present study, glucose levels were higher in both diabetic male and female patients as compared to healthy individuals. Elevated levels of glucose in blood are found in T2DM and such high levels of glucose are associated with abnormal carbohydrate metabolism (American Diabetes Association, 2014). Diabetic individuals have decreased insulin sensitivity in target tissues for glucose utilization, which is responsible for increased blood glucose levels (DeFronzo and Tripathy, 2009).

Many longitudinal studies in adults have demonstrated that insulin resistance is a risk factor for development of T2DM (Bunt et al. 2007; DeFronzo and Tripathy, 2009). Type 2 diabetic patients usually have lower insulin levels due to decreased number of pancreatic beta cells (Donath et al. 2005). HOMA-IR index is a tool used for assessment of insulin resistance from fasting glucose and insulin concentrations (DeFronzo and Ferrannini, 1991) and is a method for assessing insulin resistance. In the present study, though lower levels of insulin were found in diabetic males and females as compared to healthy individuals of corresponding gender, the differences were not statistically significant. On the other hand, HOMA-IR was significantly higher in diabetic females as compared to healthy females. Several studies report
higher insulin resistance in type 2 diabetic patients (Mohan et al. 2010) which is attributed to defective insulin utilization (American Diabetes Association, 2014). In line with the results in the present study, a study from Korea also showed that diabetic females had higher BMI and HOMA-IR than men with diabetes (Kwon, 2014).

It has been suggested that sustained hyperglycemia induces insulin resistance. Insulin resistance and β-cell function is estimated by the HOMA-IR model using fasting insulin and glucose levels. The relationship between glucose and insulin reflects the balance between liver glucose output and insulin synthesis, which is maintained by a feedback mechanism between the liver and pancreatic β-cells (DeFronzo and Ferrannini, 1991; Donath et al. 2005). Therefore association of HOMA-IR with different biochemical markers was analyzed in diabetic population receiving oral hypoglycemic agents or antidiabetic drugs. A positive association of HOMA-IR with glucose and insulin levels was observed in diabetic males while diabetic females showed a positive association of HOMA-IR with insulin. In the group of diabetic male and female individuals receiving only metformin, a positive association between HOMA-IR and insulin was seen. Further, a positive association between HOMA-IR, glucose and insulin was seen in the group of diabetic male and female individuals consuming metformin in combination with other drugs. Metformin is considered as insulin sensitizer and has glucose lowering effect (Viollet et al. 2012). Several reports suggest favorable effect of metformin treatment on insulin resistance and endothelial function and the treatment also improves association between glucose and insulin (Mather et al. 2001; Kadhim et al. 2012). Bermudez-Pirela et al. (2007) have reported that the T2DM patients receiving metformin with other drugs showed positive association of HOMA-IR with glucose and insulin. Addition of rosiglitazone to sulfonylurea has been shown to improve glycemic control in patients with T2DM previously treated with sulfonylurea monotherapy and produced a positive effect on insulin resistance and β-cell function (Pfutzner et al. 2006).

6.2.2 Lipid Profile:

Several studies report alterations in lipid profile in T2DM patients (Mooradian, 2009; Laakso, 2010). Diabetic individuals often show elevated triglyceride and LDL levels and low HDL levels (Mooradian, 2009). This altered lipid
profile is secondary to insulin resistance which develops due to increased free fatty acid flux (Mooradian, 2009). Dyslipidemia in diabetes is uniquely displayed in the form of increased triglycerides and LDL and decreased levels of good cholesterol, HDL (Steiner, 2005). In the present study, serum triglyceride levels were lower in diabetic males while serum HDL and LDL levels were lower in diabetic females as compared to healthy individuals of corresponding genders. All diabetic patients in the present study were either on oral hypoglycemic agents with or without insulin treatment while individuals with dyslipidemia were prescribed lipid lowering drugs. Hence, the variation in the levels of lipids in the present study may be due to these drugs. Oral hypoglycemic drugs with or without lipid lowering agents are known to affect the lipid profile in diabetic individuals (Buse et al. 2004). Metformin is a preferred first-line drug for the treatment of T2DM which has lipid lowering activity through the activation of AMP-activated protein kinase (AMPK) (Thevenod, 2008; Gong et al. 2012). AMPK activation by metformin is of therapeutic potential. AMPK activation is the inhibition of energy-consuming biosynthetic pathways (such as fatty acid synthesis in liver and adipocytes, cholesterol synthesis in liver and insulin secretion from β-cell) and the activation of ATP-producing catabolic pathways (such as fatty acid uptake and β-oxidation in multiple tissues, glycolysis in heart and mitochondrial biogenesis in muscles) (Viollet et al. 2009). Moreover, TZD is class of OHA used as insulin-sensitizer in the treatment of diabetic patients. TZDs activate the transcription factor peroxisome proliferator activated receptor gamma (PPARγ) and stimulate AMPK. Activation of PPARγ and AMPK modulates the expression of genes involved in glucose and lipid metabolism leading to decreased insulin resistance, improved β-cell function and lipid profile (Goldberg et al. 2005; Flockhart and Desta, 2009). Several antidiabetic drugs also act on adipose tissues and directly inhibit adipogenesis and thereby modulate adipokine secretion and activate lipid metabolism (Goldberg et al. 2005; Guilherme et al. 2008). American Diabetes Association (2003) has also reported that the oral hypoglycemic agents and lipid lowering drugs have effects on lipid profile levels of diabetic individuals. Most widely prescribed lipid lowering drug, statin, has its effect through inhibition of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase enzyme which is involved in cholesterol synthesis (Istvan, 2002). Among several types of statins, pravastatin has protective role against the development of hyperlipidemia and related metabolic disorders (Anderson, 2005). Pravastatin treatment has also been shown to
ameliorate the insulin resistance in metabolic syndrome because of which it is considered as a treatment option for hypercholesterolemic patients with metabolic syndrome and vascular diseases (Anderson, 2005). However, Golomb and Evans (2008) have reported that statin causes adverse effects, such as muscle problems, elevated liver function test markers and increased risk of cancer. With this background, there is now a consensus for the need to search alternative therapies for normalization of lipid metabolism in diabetes (Golomb and Evans, 2008).

A positive association of HOMA-IR with cholesterol, triglyceride, and VLDL was observed in diabetic males in the present study. However, a positive association between HOMA-IR and cholesterol, HDL, LDL was seen in the diabetic females. Several reports indicate that increased insulin resistance is positively associated with cholesterol, triglyceride and LDL levels in type 2 diabetic individuals (Bonora et al. 2002; Mooradian, 2009; Jung and Choi, 2014). Insulin resistance is known to enhance hepatic free fatty acid synthesis which increases lipid synthesis and thus directly contribute to increase in triglyceride and LDL cholesterol levels (Stalder et al. 1981). Insulin resistance also affects the activity of lipoprotein lipase in peripheral tissues contributing to elevated triglyceride and LDL cholesterol levels (Pykalisto et al. 1975; Sadur et al. 1984). It has been found that insulin resistance may be responsible for reduced levels of HDL in T2DM patients. HDL cholesterol concentration was significantly reduced in type 2 diabetes than control individuals; this decrease in plasma HDL cholesterol was entirely accounted by an increase in the rate of apolipoprotein A1/HDL cholesterol degradation, which exceeded the rate of its synthesis (Golay et al. 1987).

6.2.3 Liver Function Test Markers:

Hyperglycemia in T2DM has been reported to affect liver functions adversely (Harris, 2005). Elevated levels of liver enzymes, non-alcoholic fatty liver disease and acute liver failure are some of the common manifestations of hepatic complications in diabetes (Tolman et al. 2007). Higher levels of SGPT, SGOT and ALP serve as markers of liver damage (Harris, 2005). Higher serum SGPT and bilirubin levels were observed in diabetic men while diabetic women showed increase in SGPT levels as compared to healthy individuals of corresponding genders. Insulin resistance in diabetic men from the present study was also found to be positively associated with
levels of SGPT. Some reviews report that metformin (Cone et al. 2010) and troglitazone affect LFT markers in T2DM patients (Della-Morte et al. 2014). Metformin decreases SGPT, SGOT and ALP levels in N-nitrosodimethylamine initiated hepatocellular carcinoma in rats (Afzal et al. 2012). Metformin and glibenclamide have been shown to reduce SGOT and SGPT in diabetic rats (Bugudare et al. 2011).

Studies on diabetic individuals have shown that atorvastatin increases the total protein level and lower SGOT, SGPT and ALP levels (Colhoun et al. 2004) while telmisartan improves insulin resistance and liver injury (Enjoji et al. 2008). Moreover, long-term treatment with statins is known to induce oxidative stress and inflammation (Fenster et al. 2003) apart from its adverse effects on liver enzymes (Argo et al. 2008; Maji et al. 2013). However, some studies claim that high bilirubin levels delay the progression of diabetic complications due to negative association of bilirubin with cardiovascular diseases, diabetic nephropathy and their risk factors (Vitek, 2012; Mashitani et al. 2014).

6.2.4 Antioxidant Enzymes:

Hyperglycemia generates reactive oxygen species (ROS), which cause damage to the cells in different ways. Antioxidant enzymes provide protection against such type of oxidative damage (Tiwari et al. 2013). In the current study, SOD and catalase activity from erythrocytes was studied. As against healthy individuals, significantly higher SOD activity was found in diabetic individuals irrespective of their genders. Increase in SOD activity provides protection against oxidative damage (Kowluru et al. 2006). Erythrocyte catalase activity was found to be lower in diabetic participants in the present study which is in accordance with earlier reports mentioning decrease in erythrocyte catalase activity after oral hypoglycemic therapy (Aydin et al. 2001). Similar findings have been reported in Hungarian diabetic patients (Goth, 2008). Streptozotocin (STZ) induced diabetic rats also exhibit increased SOD activity and lower catalase activity (Erejuwa et al. 2010). In contrast, lower SOD activity and higher erythrocyte catalase activity is reported in T2DM patients with microvascular complications (Kesavulu et al. 2000).

Insulin resistance and SOD activity in diabetic males was found to be positively associated in the present study. Earlier studies indicate that treatment with oral hypoglycemic agents has effects on SOD and catalase activity (Pavlovic et al.
These studies indicate that there is increase in erythrocyte SOD and decrease in catalase activity. Results in the present study are in accordance with these reports. Several animal studies on diabetic rats have demonstrated that treatment with metformin and glibenclamide has no detectable effect on the SOD activity but erythrocytes show reduced catalase activity (Erejuwa et al. 2010). It has been demonstrated that the efficacy of metformin on scavenging reactive oxygen species (ROS) depends on the nature of ROS generator (Bonnefont-Rousselot et al. 2003). If O$_2$ and H$_2$O$_2$ are the most produced oxidant species during oxidative stress, metformin is not able to scavenge ROS (Bonnefont-Rousselot et al. 2003; Elia et al. 2006; Erejuwa et al. 2010). Therefore metformin may not be able to modulate the activity of catalase enzyme as observed in the present study. Reports suggest that SOD and catalase activities remain unaltered by statins (Passi et al. 2003) while atorvastatin causes increase in the catalase activity (Wassmann et al. 2002). Metformin is reported to reduce ROS by inhibiting mitochondrial respiration chain complex 1 (Viollet et al. 2012). Metformin and glimepiride have been reported to decrease oxidative stress mediated nuclear damage in diabetic rats (Rabbani et al. 2009). Treatment with metformin is supposed to have its antioxidant and insulin sensitizing effects based on its ability to confer better membrane protection by improving the levels of reduced glutathione which is one of the important antioxidants (Faure et al. 1999).

6.2.5 Interleukin 8:

Chronic low-grade inflammation is one of the hallmarks of T2DM (Fuentes et al. 2013). Inflammation in diabetes is closely linked to oxidative stress and failure of antioxidant defense system to scavenge the free radicals completely (Bajaj and Khan, 2012; Galassetti, 2012; Tiwari et al. 2013). Higher oxidative stress leads to increase in interleukin-8 (IL8) secretion, which in turn causes recruitment of inflammatory cells, further inducing the oxidative stress mediators, making it a key parameter in localized inflammation (Reuter et al. 2010; Kolluru et al. 2012). In the present study, serum IL8 levels were lower in diabetic females but were similar in the males as compared to healthy individuals of corresponding gender. In contrast, elevated circulating IL8 levels have been reported in type 1 and type 2 diabetic patients (Zozulinska et al. 1999; Esposito et al. 2003) and in obese individuals with impaired glucose tolerance.
suggesting higher inflammation (Straczkowski et al. 2003; Herder et al. 2005; Jung and Choi, 2014). It has been shown that antidiabetic drug consumption reduces inflammation (Isoda et al. 2006; Zhang et al. 2008; Kewcharoenwong et al. 2013). Several reports suggest that oral hypoglycemic agents such as thiazolidinedione, ciglitazone and biguanide like metformin decreases IL8 gene expression in different tissues (Bruun et al. 2001; Isoda et al. 2006). A recent report Yasser et al. (2013), found that metformin monotherapy significantly lowered serum IL8 levels in T2DM patients, which could be the possible reason for the reduced IL8 levels seen in the present study.

6.2.6 Adipocytokines:

In the current study, adiponectin levels were significantly higher while leptin levels were lower in both male and female diabetic individuals as compared to their corresponding controls. A negative correlation between HOMA-IR and adiponectin was seen only in the diabetic females. Ahsan et al. (2014) also found higher adiponectin levels in diabetic females and showed negative association with insulin resistance. However, reports indicate markedly lower plasma adiponectin and higher leptin levels in insulin resistant individuals (Lee et al. 2009b; Mente et al. 2010; Kim et al. 2013). On the other hand, the treatment of insulin resistant diabetic patients with PPARγ agonist drugs, such as the thiazolidinediones, pioglitazone and rosiglitazone, has reported to increase plasma adiponectin levels 2-3 fold along with an improvement in insulin sensitivity (Rasouli et al. 2005). A study by Rasouli et al. (2005) suggests that plasma adiponectin is very sensitive to PPARγ agonist drugs and pioglitazone treatment increases plasma adiponectin through post-transcriptional mechanisms. It has been shown that administration of rosiglitazone for 24 h increases the otherwise steady state mRNA of adiponectin in differentiated 3T3-L1 adipocytes (Lin et al. 1999). Rosiglitazone is a synthetic PPAR-agonist enhancing insulin sensitivity and is now widely used to treat patients with T2DM (Whitcomb and Saltiel, 1995; Yang et al. 2002). The high molecular weight (HMW) adiponectin complex is negatively correlated with insulin resistance (Aso et al. 2006; Eglit et al. 2013; Lee and Kwak, 2014b). The amount of HMW adiponectin complex, but not the total amount of adiponectin is reported to be correlated with thiazolidinediones mediated improvement in insulin sensitivity (Pajvani et al. 2004).
6.3  Effect of Fasting Blood Glucose Levels and Duration of Disease on the Metabolic Measures and Biochemical Parameters in Diabetic Individuals:

6.3.1  Metabolic Measures:

6.3.1.1 BMI, WHR and Blood Pressure:

In the present study, WHR was higher in the male patients with high FBG levels than the male patients with normal FBG levels and females with high FBG. Moreover, BMI was lower in females while systolic blood pressure was higher in males with longer duration of disease. A study by Nemesure et al. (2008) reported that WHR is more strongly associated with hypertension, whereas BMI has a stronger association with diabetes. Several studies report that appropriate management of body mass help in lowering hyperglycemia in diabetic subjects (Boule et al. 2001; Hamman et al. 2006). Asian-Indian phenotype is characterized by increased abdominal obesity and visceral fat despite low BMI. Hyperglycemia has been reported to be strongly associated with BMI, WHR and hypertension (Huang et al. 2002; Feng et al. 2012). Studies have also indicated increase in blood pressure with the duration of diabetes in T2DM patients (Knowler et al. 1980; Leske et al. 2005; Nemesure et al. 2008). Koivisto et al. (1996) found that as duration of diseases increases, WHR, and hypertension was more common in diabetic men with CVD while in diabetic women, a greater BMI was associated with CVD.

6.3.2  Biochemical Parameters:

6.3.2.1 Glucose, Insulin and HOMA-IR:

In the present study, glucose levels and HOMA-IR were significantly higher in both male and female patients with higher FBG levels as compared to their corresponding gender with normal FBG levels. Insulin levels were comparable in both FBG groups of male and female patients. In the present study, as expected, glucose levels were higher in both male and female individuals as disease progresses. A study by Verma et al. (2006) showed progressive increase in glucose levels with increase in diabetes duration. In contrast, a study by Kabadi, (1988) showed no association between duration of disease and glucose levels. Insulin sensitivity and fasting glucose levels are known to be negatively correlated in diabetic men (Toyoda et al. 2008). Olefsky et al. (1973) have reported an inverse relationship between fasting insulin
levels with insulin sensitivity and increase in the insulin levels with chronicity of diabetes. Chronic hyperglycemia with increasing levels of fasting glucose leads to significant increase in insulin resistance (Rhee et al. 2006). Though increase in the levels of fasting glucose were observed in chronic diabetes, significant correlation between glucose, fasting insulin levels and insulin resistance was not observed in the present study.

6.3.2.2 Lipid Profile:

In the present study, male patients with high FBG levels showed elevated triglyceride and VLDL levels while FBG did not affect lipid profile in females. Further, males with high FBG levels showed a positive association between cholesterol, triglycerides and VLDL with glucose while diabetic females did not show such association. Several studies report elevated triglyceride and VLDL in T2DM patients (Laakso, 2010; Miller et al. 2011). A study by Simonen et al. (2002) showed a positive association between blood glucose and cholesterol synthesis in obese diabetic population without any mention about gender of the participants. Diabetic male individuals are more susceptible to develop dyslipidemia and consequent complications like CVD (Mohammed et al. 2014). Poor glycemic control in diabetic individuals is believed to result in high serum triglyceride, total cholesterol and LDL cholesterol (Mullugeta et al. 2012). Serum triglyceride levels vary with glucose levels and a better management of hyperglycemia is known to reduce serum triglycerides in T2DM patients (Abdel-Gayoum, 2004).

In the present study, HDL and LDL levels were higher in males having diabetes for more than 7 years. No significant change was observed in lipid profile of female individuals. A recent report suggests that triglyceride, cholesterol, HDL, LDL and VLDL levels significantly increase with duration of diabetes (Naheed et al. 2003; Sultana, 2010; Parmar et al. 2013). Secondary diabetic complications not only depend upon duration of diabetes but also on management of hyperglycemia (Fowler, 2008; Weber and Schnell, 2009).

6.3.2.3 Liver Function Test Markers:

High glucose output and free fatty acid synthesis in diabetic individuals is known to affect hepatic cells resulting in elevated liver enzymes (Harris, 2005).
Poorly controlled glycemia, insulin resistance and high glycogen synthesis elevates liver function test markers (LFT) (Chatila and West, 1996; Hanley et al. 2005). In the present study, SGPT and SGOT levels were significantly higher in the males with high FBG levels. Recent reports suggest that hyperglycemia is associated with increase in SGPT and SGOT levels in diabetic males (Al-Jameil et al. 2014; Oka et al. 2014). Chronic hyperglycemia elevates LFT markers also in experimentally induced diabetes in animals, in the absence of any interventions (Kumar et al. 2013a; Rao et al. 2013; Ali et al. 2015).

In the present study, chronicity of diabetes did not have significant effect on LFT markers in diabetic individuals. A study by Ni et al. (2012) reported lower SGPT levels in diabetic patients with longer duration of diabetes. In contrast, Meybodi et al. (2008) and Judi et al. (2010) observed increase in SGPT levels with disease duration in diabetic individuals. Recent studies have also reported absence of any significant correlation between SGOT, ALP and bilirubin with duration of diabetes (Belay et al. 2014; Hind and AbdElkarim, 2014). Hence, LFT markers seem to be sensitive to high FBG levels rather than to duration of diabetes.

6.3.2.4 Antioxidant Enzymes:

Lower levels of antioxidant enzymes indicate elevated reactive oxygen species (ROS) leading to increased oxidative stress (Tiwari et al. 2013). Hyperglycemia and increased fatty acid synthesis induce oxidative stress in the form of ROS which leads to development and progression of diabetic complications (Giacco and Brownlee, 2010; Matough et al. 2012; Fiorentino et al. 2013). In the present study, SOD and catalase activity were comparable in high and normal FBG groups of diabetic individuals. Several studies report lower SOD and catalase activities in diabetic individuals (Fujita et al. 2009, 2011; Goth and Nagy, 2013) and absence of association between disease duration and SOD and glutathione peroxidase levels (Walter et al. 1991; Akkus et al. 1996; Hartnett et al. 2000). In present study, no significant variation was observed in SOD activity in diabetic individuals with disease progression but reduced catalase activity was observed in male diabetic patients with chronic hyperglycemia.
6.3.2.5 Interleukin 8:

Excessive oxidative stress is also known to lead to inflammation in diabetes (Giacco and Brownlee, 2010; Matough et al. 2012). Increased oxidative stress and chronic inflammation are important contributors to the pathology of diabetes and associated secondary complications (Laakso, 2010; Pitocco et al. 2013; Tiwari et al. 2013). IL8 levels were comparable and also did not show any association with glucose in both high and normal FBG groups of male and female individuals in the present study. In contrast, a study by Zozulinska et al. (1999) reports increase in IL8 levels in T2DM patients. Glucose levels influence IL8 production (Urakaze et al. 1996; Srinivasan et al. 2003) while IL8 mRNA expression and protein synthesis is inhibited by antidiabetic drugs (Bruun et al. 2000; Yasser et al. 2013). There are also some contrasting reports which show increase in IL8 levels with increase in disease duration (Doganay et al. 2002; Lee et al. 2008; Huseynova and Efendiyev, 2009). Treatment with antidiabetic drugs such as metformin, pioglitazone, glibenclamide etc. reduces the levels of IL8 in T2DM individuals (Zhang et al. 2008; Kewcharoenwong et al. 2013). In the present study also, IL8 levels were found to decrease in males as disease progresses.

6.3.2.6 Adipocytokines:

The present study found comparable levels of adiponectin and leptin in males. Females with high FBG levels had higher adiponectin levels and lower leptin levels than females with normal FBG group. However, there was no association between FBG and adipocytokines in both male and female individuals. Diabetic patients are known to have lower adiponectin and higher leptin levels (Ley et al. 2008), which are associated with high risk of diabetic complications (Al-Hamodi et al. 2014). However, Ryan et al. (2003) have reported higher adiponectin levels in diabetic patients with fasting blood glucose more than 144 mg/dl. The above studies did not report variation in the levels of adipocytokines in males and females. A recent report by Al-Hamodi et al. (2014) shows that diabetic females had higher adiponectin levels than males, suggesting gender-wise variation. In present study also, higher adiponectin was found in females with high FBG as compared to normal FBG group.
In the present study, higher adiponectin levels were found only in females but not in males, with increase in the duration of the disease. Adiponectin levels are higher in patients with diabetes for more than 10 years (Looker et al. 2004). Additionally, females are reported to have higher adiponectin levels than males which could be attributed to the sexual dimorphism and differences in sex hormones between males and females (Nishizawa et al. 2002; Combs et al. 2003; Xu et al. 2005).

6.4 Gene Expression:

6.4.1 Expression of Genes Involved in Lipid Metabolism:

PPARγ and SREBP1 are transcription factors that play important roles in the regulation of lipid metabolism (Keller and Wahli, 1993; Vidal-Puig et al. 1997; Sewter et al. 2002). The activation of PPAR has been shown to stimulate beta-oxidation of fatty acids by enhancing the expression of genes like CPT1a, acyl-CoA oxidase 1 and thereby reduce lipid synthesis and lower triglyceride levels (Schoonjans et al. 1996). SREBP activates the genes critical for lipogenesis (Teran-Garcia et al. 2007), such as fatty acid synthase (FAS) and acetyl-CoA carboxylase alpha (ACACA) (Ronnebaum et al. 2008) while a down regulation of SREBP leads to inhibition of hepatic genes essential for lipid synthesis. In the present study, no significant alteration was found in the expression of PPARγ while SREBP1 expression was significantly higher in diabetic patients as compared to healthy individuals. Several studies report lower PPARγ and higher SREBP1 expression in patients with type 2 diabetes (Sewter et al. 2002; Chiarelli and Di Marzio, 2008), indicating lower oxidation of fatty acids and increased lipid synthesis. In accordance with these reports and consequent to higher expression of SREBP1 in diabetic individuals, an increase in expression of FAS was reported in the present study.

ACSL is a target of PPAR and it is also implicated in the pathogenesis of diabetes (Phillips et al. 2010). ACSL plays an important role in triglyceride synthesis by partitioning fatty acids towards triglyceride synthesis. Over-expression of ACSL increases the synthesis of triglycerides (Li et al. 2006; Ellis et al. 2010). MCAT, a mitochondrial protein catalyzes transfer of acyl CoA through the acyl-carrier protein in mitochondria which takes part in mitochondrial fatty acid synthesis (Bunkoczi et al.
2009). This is the first report of gene expression of MCAT from PBMCs in diabetic patients. In the present study, ACSL and MCAT gene expression was higher in diabetic males while it was comparable in diabetic females than the respective healthy individuals. Over-expression of ACSL is known to decrease the turnover of intracellular triglycerides and phospholipids, possibly through altering re-acylation of lysophospholipids (Li et al. 2006). Increase in ACSL gene expression has also been observed in PBMCs of type 1 diabetic patients (Kanter et al. 2012).

6.4.2 Expression of Genes Involved in Inflammation:

T2DM is a chronic inflammatory disease wherein over-expression of pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), interleukin 6 (IL6) and monocyte chemo-attractant protein-1 (MCP-1) is suggested to be involved in the pathogenesis of T2DM (McArdle et al. 2013). A transcription factor; nuclear factor kappa beta (NFκβ) activates the transcription of inflammatory cytokines like TNF-α, thereby increasing the risk of secondary complications owing to their pro-atherogenic nature (Jagannathan-Bogdan et al. 2011). In the present study, NFκβ gene expression was higher in diabetic males while no significant change was observed in female diabetic patients. There are some studies which indicate that there is no significant change in NFκβ gene expression in PBMCs in diabetic patients (Bragt et al. 2009; Horvath et al. 2015) while some studies show higher expression of NFκβ and TNF-α in PBMCs of T2DM patients (Navarro-Gonzalez et al. 2010; Prattichizzo et al. 2015).

Expression of NFκβ in turn controls TNF-α gene expression through inflammatory signaling pathway. In present study, TNF-α gene expression was found to be higher in diabetic patients as compared to healthy individuals which is consistent with the higher expression of NFκβ in diabetic patients. Inflammatory signals can cause phosphorylation of inhibitor of κβ, thereby releasing it and activating NFκβ, followed by translocation of NFκβ to the nucleus and activation of genes involved in the pro-inflammatory response, such as TNF-α (Oeckinghaus and Ghosh, 2009). Several antidiabetic drugs are known to down regulate lipolysis through reduction of TNF-α and accelerating uptake of free fatty acids by adipocytes, reducing fatty acids in circulation (Srivastava et al. 2012).
II. ANIMAL STUDY:

Apart from overt hyperglycemia due to impaired glucose metabolism, type 2 diabetes frequently leads to qualitative and quantitative abnormalities in serum lipids (Ozder, 2014). The abnormal lipid metabolism is believed to be due to modulation of expression of transcription factors and genes involved in lipid synthesis, transport and metabolism (Erejuwa et al. 2012). In line with this, altered lipid profile and expression of genes involved in lipid metabolism and inflammation was observed in diabetic individuals as compared to healthy individuals. Poorly controlled serum glucose and duration of disease was found to be associated with altered lipid profile and liver function test markers which varied with the type of treatment in diabetic individuals. Statins are the most commonly prescribed drug for management of altered lipid profile in diabetic patients (Maron et al. 2000; Barakat et al. 2013). However, there are several reports suggesting increased serum glucose levels after statin treatment in diabetic individuals while healthy, obese individuals had increased risk and/or incidence of diabetes (Rajpathak et al. 2009; Sattar et al. 2010; Preiss et al. 2011; Marx, 2012; Carter et al. 2013). Recent literature suggests the use of nutraceuticals and functional foods as supplementary treatment to lipid abnormalities (Alissa and Ferns, 2012; Calder, 2012; Mozaffarian and Wu, 2012). Omea-3 fatty acids are one of such recommended candidates due to their anti-inflammatory, anti-atherogenic, vasodilatory and lipid lowering properties which have been established in several chronic diseases including diabetes (Connor, 2000; Ander et al. 2003; Calder, 2012; Mozaffarian and Wu, 2012; Wu et al. 2012; Jangale et al. 2013). With this background, the effects of omega-3 fatty acids on lipid profile and underlying molecular mechanisms were analyzed in nicotinamide (NIC)-streptozotocin (STZ) induced diabetic rats.

6.5 Glucose Levels:

The general characteristics of STZ treated diabetic rats include low body weights and elevated blood glucose (Howarth et al. 2005). Significant decrease in body weights and sustained hyperglycemia was observed in animals treated with NIC-STZ indicating successful induction of diabetes. In the present study, both metformin and omega-3 fatty acids could not normalize the glucose levels at day 15 and they
remain higher till day 30 of treatment. However, fish oil treatment lowered the glucose levels at day 30 as compared to day 15. It has been reported that metformin alone, may not adequately control hyperglycemia (Salama et al. 2013) and omega-3 fatty acids do not directly affect glucose homeostasis (Woodman et al. 2002). However, several studies have reported hypoglycemic and insulin sensitizing effects of metformin (Erejuwa et al. 2011; Mohammadi et al. 2012; Maheshwari et al. 2014) and omega-3 fatty acids (Jangale et al. 2013) in NIC-STZ induced diabetic rats.

Metformin and omega-3 fatty acids are known to improve insulin sensitivity and lower glucose in hyperglycemic rodents or humans. The anti-hyperglycemic and insulin sensitizing effects of metformin have been demonstrated in NIC–STZ induced diabetic rats where metformin is administered at a dose of 500 mg/kg for 28 to 42 days (Mohammadi et al. 2012; Maheshwari et al. 2014). In contrast, metformin treatment at a dose 500 mg/kg for 8 weeks could not lower blood glucose (Alhaider et al. 2011). Flax oil or fish oil, at a dose of 10% w/w for 35 days were found to lower glucose levels in NIC-STZ induced diabetic rats (Jangale et al. 2013). In the present study, metformin was administered at a lower dose of 200 mg/kg and 500 mg/kg omega-3 fatty acids for 30 days, neither of them could improve glucose levels. These differences might be due to variations in the treatment time and/or dose of metformin / omega-3 fatty acids which needs further investigation.

In addition, the aim of the present study was to investigate the anti-inflammatory and lipid normalizing effects of omega-3 fatty acids and not their glucose lowering effects.

6.6 Lipid Profile:

Significant lipid abnormalities, like elevated cholesterol, triglycerides, LDL and VLDL have been reported in NIC-STZ induced diabetic rats (Ohno et al. 2000; Nasrolahi et al. 2012). In the present study, increase in serum cholesterol, triglycerides and VLDL levels was observed in STZ group. However, metformin treatment for 30 days showed significant reduction in the levels of lipids. The beneficial lipid lowering effects of metformin have been reported in diabetic rats (Nasrolahi et al. 2012). Long-term use of OHAs is also known to significantly reduce
Flax oil and fish oil interventions normalized all lipid profile parameters at day 30 as compared to day 15. There are many reports indicating triglyceride lowering effects of dietary fish oil (De Caterina et al. 2007; Hartweg et al. 2008; Devarshi et al. 2013; Lorente-Cebrian et al. 2013; Bremer et al. 2014) which are primarily attributed to eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in fish oil (Egert et al. 2009; Skulas-Ray et al. 2011). The present study indicated activation of PPAR and modulation of SREBP1 in the animals treated with flax oil and fish oil which are the principle mechanisms for lipid normalizing action of omega-3 fatty acids (Davidson, 2006; Moon et al. 2012; Devarshi et al. 2013).

6.7 Liver Function Test Markers:

SGPT, ALP levels were higher in the STZ group at day 15 while ALP remained higher at day 30 as compared to control group. The biochemical alterations in these hepatic markers were also reflected in liver with destructive changes in hepatocytes and accumulation of lipid droplets. Hepatic dysfunction and higher levels of liver enzymes such as SGPT, SGOT and ALP have been reported in STZ induced diabetic rats (Chandran et al. 2012). A result from the present human study has also showed higher serum SGPT and bilirubin levels in diabetic individuals.

Metformin treatment in the present study did not affect other markers. Liver section analysis of these animals displayed destruction of some hepatocytes and congestion of central vein. In contrast, a study in diabetic rats treated with metformin is reported to have elevated bilirubin levels (Nasrolahi et al. 2012). A recent study reported mild granular degeneration, mild swelling (narrow sinusoidal capillaries) and normal hepatic architecture in the metformin treated diabetic rats (Motshakeri et al. 2014). Some case reports have even demonstrated that metformin induces hepatotoxicity in diabetic patients raising concern about its efficacy and safety profile (Cone et al. 2010; Miralles-Linares et al. 2012).

Omega-3 fatty acid supplementation is known to improve liver function markers in chronic diseases (Heller et al. 2004; Lee et al. 2007; Wu et al. 2012b).
Chavan et al. (2013) have reported hepatoprotective activity of flax and fish oil in chemically induced hepatotoxicity in rats. Flax and fish oil intervention, in the present study, showed progressive effects in lowering ALP levels at day 30 as compared to day 15. However, Jangale et al. (2013) have reported that dietary supplementation of flax and fish oil lowered the inflammatory genes expression and oxidative stress in liver which is the possible mechanism for the improvement of hepatic damage in diabetic rats. In the present study, flax and fish oil groups exhibited near normal hepatic architecture and displayed significant recovery of destructive changes.

6.8 Gene Expression:

6.8.1 Expression of Genes Involved in Lipid Metabolism:

STZ induction markedly reduced the expression of PPARγ and increased SREBP expression which was restored by metformin as well as by flax and fish oil. Metformin treatment is known to regulate incretin receptor axis via PPAR dependent pathway in mice (Maida et al. 2011) and reduce SREBP expression by regulating AMP-activated protein kinase activity in rats (Zhou et al. 2001). Metformin has been shown to reduce fat content by decreasing SREBP1 and FAS expression in rat kidneys (Wang et al. 2006). A recent study reports reduction in PPAR expression and increase in SREBP expression in diabetic rats which was restored by dietary fish and flax oil supplementation (Devarshi et al. 2013). 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).

ACSL, catalyzing the thioesterification of fatty acids, is a target of PPAR and is implicated in the pathogenesis of diabetes (Phillips et al. 2010) and also plays an important role in triglyceride synthesis (Yan et al. 2015). In the present study, STZ treated animals showed significantly higher ACSL expression with high triglyceride levels. However, metformin, flax and fish oil interventions lowered the expression and consequently had significant reduction in serum triglyceride levels. Metformin has been shown to lower ACSL expression and triglyceride levels in diabetic rats (Forcheron et al. 2009). Besides triglyceride synthesis by ACSL, MCAT, a mitochondrial protein catalyzes transfer of 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 MCAT along with ACSL has beneficial effects in normalizing the levels of free fatty acids and triglycerides (Li et al. 2009; Zhang et al. 2013). In the present study also, rats treated with metformin, flax and fish oil exhibited reduced MCAT expression indicating lower fatty acid synthesis which may contribute to normalize the lipid profile (Fig. 32).

6.8.2 Expression of Genes Involved in Inflammation:

In the present study, NFκβ expression was higher in the STZ group which was significantly lowered by flax and fish oil treatment (Fig. 32). However, TNF-α expression was not significantly altered in the STZ group and treatment groups. Hyperglycemia induced NFκβ activation in ex-vivo, isolated PBMCs has been reported in type 1 diabetic patients (Hofmann et al. 1998). Metformin administration has also been shown to down regulate the expression of NFκβ and TNF-α and ameliorate β-cell dysfunction in diabetes (Hyun et al. 2013; Liu et al. 2014). Recent studies also suggest the role of omega-3 fatty acids in reducing TNF-α expression (Jangale et al. 2013; Ellulu et al. 2015).
Fig. 32: Diagrammatic Representation of the Possible Mechanism of Action of Metformin, Flax and Fish Oil on the Lipid Metabolism and Inflammation in Rats

PPARγ: Peroxisome proliferator activated receptor γ; SREBP1: Sterol regulatory element binding protein 1; NFκβ: Nuclear factor kappa β; FAS: Fatty acid synthase; ASCL: Long chain acyl CoA synthetase; MCAT: Malonyl-CoA-acyl carrier protein transacylase; TNFα: Tumor necrosis factor α

↑: Up-regulation; ↓: Down-regulation