INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online atwww.ijrpc.com

Review Article

MOLECULAR PATHWAYS IMPAIRED IN ALCHOLIC AND NON ALCOHOLIC FATTY LIVER DISEASE

Mandeep Kaur, Gagandeep Kaur and Saurabh Sharma*

Department of Pharmacology, I.S.F College of Pharmacy, Moga, Punjab, India.

ABSTRACT

Fatty liver disease is gaining increasing recognition as a major health issue worldwide.If left untreated, liver may become so seriously scarred that it can no longer heal itself, progresses to cirrhosis which is life threatening. Fatty liver disease (FLD), whether it is alcoholic FLD (AFLD) or nonalcoholic FLD (NAFLD), encompasses a morphological spectrum consisting of hepatic steatosis (fatty liver) and steatohepatitis. FLD may be considered a single disease with multiple etiologies. This review summarizes recent cellular and molecular advances in the understanding of the mechanisms leading to end-stage FLD. Key risk factors including overweight, insulin resistance, a sedentary life-style and an altered dietary pattern have been identified in recent years. Pathogenesis of Fatty liver disease involves the role of nuclear receptors, proinflammatory cytokines, apoptosis, mitochondrial dysfunction, oxidative stress, sphingolipids, C-Jun-N-terminal kinase (JNK) pathway, phosphatidylinositol 3-kinase (PI3K/AKT) pathway, AMP Activated Protein Kinase **(**AMPK) pathway, DiacylglycerolAcyltransferase (DGAT), Visceral Adipocyte Tissue (VAT), Peroxisome Proliferators-Activated Receptors (PPAR's), ceramides, Tumor suppressor gene, Sterol regulatory element binding proteins (SREBPs) and NF-E2-related factor-2 (Nrf_2-ARE) pathway. Prevention and therapy opposing the development of steatosis can be achieved by a multifactorial approach: control of alcohol consumption, avoidance of obesity and of excess dietary long-chain fatty acids, or their replacement with medium-chain fatty acids. Progress in the understanding of the pathogenesis of Fatty liver disease and its progression to inflammation and fibrosis will results in prospects for their better prevention and treatment.

Keywords: Fatty liver disease, hepatic steatosis, steatohepatitis, alcohol, fibrosis, apoptosis.

INTRODUCTION

Fatty liver is the accumulation oflipid within hepatocytes. Clinically, FLD is divided into two broad categories, alcoholic FLD (AFLD) and nonalcoholic FLD (NAFLD), to bring the emerging nonalcoholic causes into existence and to distinguish them from alcoholic liver disease. Fatty Liver Disease (FLD), whether it is alcoholic FLD (AFLD) or nonalcoholic FLD (NAFLD), possesses a morphological spectrum consisting of hepatic steatosis (fatty liver) and steatohepatitis. FLD, if not treated can progress toward the development of cirrhosis and hepatocellular carcinoma[1]. FLD is a single pathological condition with multiple etiologies that include alcohol consumption, obesity associated-insulin resistance, and many metabolic disorders. When there is increased consumption of energy and decreased combustion of calories, the unburnt energy is conserved in the form of fat in adipose tissue, leading to obesity[2]. These pathogenic eventsare responsible for the metabolic syndrome consisting of Type 2 diabetes mellitus, dyslipidemia, atherosclerosis, hypertension, and hepatic steatosis which leads to FLD[3, 4].

CAUSES OF FATTY LIVER

It is worldwide known that behavioral factors are main factors in the pathophysiology of fatty liver and in this aspect, an increased energy intake and diet composition is considered to be a major player.Furthermore, studies showed that a sedentary lifestyle with decreased physical activity represents another factor for fatty liver. Although these risk factors may successfully beabolished by adopting moderate lifestyle, the existence of other risk factors may need intense treatment. Among them, a disproportionate fat distribution, particularly with high visceral adiposity releasing humoral factors regulating liver fat are relevant. Finally, abnormal hepatic lipid oxidation as well as dysregulatedlipogenesis, affected by genetics, may be of pathophysiological relevance[5].Mitochondria play significant role in metabolism of hepatocyte and it representsthe primary site for the oxidation of fatty acids and oxidative phosphorylation. Mitochondrion occupiesabout 18% of the liver cell volume[6]. Thus, the above mentioned findings readily suggest that mitochondrial function is a major regulator of liver fat. In addition, when there is abnormal mitochondrial function or even when an excess of free fatty acid is available, which is a case of fatty liver, reactive oxygen species (ROS) can occur leading to oxidative stress, which is thought to be important for the progression of disease and leading to NASH and fibrosis[7].

METABOLIC CONSEQUENCES OF FATTY LIVER

a) **Dyslipidemia:** An increased rate of synthesis of hepatic triglycerideand production of VLDL particle, which results in low HDL cholesterol and increased density of LDL particle, is considered to be main factor of dyslipidemia. Furthermore, a decrease in lipoprotein lipase activityis also an underlying mechanism for dyslipidemia.

b) **Dissociation of fatty liver and insulin resistance**: Although hepatic fat accumulation, both in animals and in humans, is mainly associated with a insulin resistance, and other parameters regulating insulin sensitivity such as overall obesity, body fat distribution, or circulating adipokines. In other words, for the identification of hepatic steatosis, subjects can be divided who have very high and very low insulin resistance suggesting that a dissociation of fatty liver and lowerinsulin senstivity exists.

c) Fat accumulation in the liver induces **hyperglycemia**, inflammationand the secretion of parameters that can be referred to as “hepatokines” for examplefetuin-A, thereby inducing**atherosclerosis**, and possibly hepatocyte dysfunction and cell death. However, the same amount of hepatic fat accumulation may be strongly associated with hepatic lipotoxicity, resulting in aggravation of high glucose level, subclinical inflammation, and an imbalance in hepatokine production as well as in their metabolic consequences. This condition may be referred to as malign fatty liver[5].

ALCOHOLIC FATTY LIVER DISEASE

Alcohol from a long time known to cause fatty liver by altering $NADH/NAD^+$redox potential inliver, which, in turn, inhibits fatty acid oxidation and the activity of tricarboxylic acid cycle reactions. More recent studies indicate that metabolism of ethanol impair fatty acid oxidation and stimulate lipogenesis. Ethanol interferes with DNA binding and transcriptionactivating properties of peroxisome proliferator-activated receptor-α(PPAR-α). Ethanol-fed mice when treated with a PPARagonist can reverse fatty liver even in case of continued ethanol consumption. Ethanol also activated sterol regulatory element binding protein 1, and stimulate lipogenic enzymes. These effects are due to inhibition of AMP-dependent protein kinase, reduction in plasma adiponectin, or increased levels of TNF-α in the liver. The understanding of these ethanol effects provides new therapeutic targets to reverse alcoholic fatty liver[8].

NONALCOHOLIC FATTY LIVER DISEASE

Nonalcoholic fatty liver disease (NAFLD) is a condition in which large amount of fat deposits in the liver of a patient without a history of alcohol abuse[9].NAFLD is categorized into simple steatosis and nonalcoholic steatohepatitis (NASH). In NASH, there is not only steatosis but also intralobular inflammation and hepatocellular ballooning is present, which often lead to progressive fibrosis[10]. NAFLD is associated with obesity and insulinresistance; the current Western diet, high in saturated fats and fructose, plays an important role in pathogenesis of NAFLD. There are several mechanisms which

can accumulate triglycerides in hepatocytes. In NAFLD, there is formation of steatotic droplets in liver. Visceral adipose tissue dysfunction in obesity and insulin resistance results in overexpression of many cytokines which have a role in liver injury in NAFLD[11].

MOLECULAR SIGNALING PATHWAYS INVOLVED IN FATTY LIVER DISEASE

COMMON PATHWAYS IN AFLD AND NAFLD

NAFLD and AFLD both are leading causes of non-viral chronic liver diseases, and the prevalence of these two clinical disorders is gradually growing worldwide. The pathogenic difference between both the disorders is not easy to differentiate, so expertise from new evidence of clinical classification of FLD agree that similar major molecular mechanisms are shared between NAFLD and AFLD, including inflammatory pathways and fibrogenesis. Apoptosis is acommon cell death process shared by NAFLD and AFLD, but AFLD is mainly associated with extrinsic pathway related to tumor necrosis family, which is derived by proapoptotic BCl_2 family members. In contrast, NAFLD is associated with FAS-induced apoptosis, which is related intricate network of metabolic stressors, activation of caspases, and that stimulate apoptosis, necrosis, and inflammation. It is worth mentioning that opening of mitochondrial membrane permeability transition pore and increased endoplasmic reticulum stress are central features in both AFLD and NAFLD. Hence, these data suggest thatsimilar disease mechanisms lead to the clinical outcome of NAFLDand AFLD[12].

1. Tumor suppressor gene

The p53 gene was identified as the first tumor-suppressor gene. The p53 protein prevents multiplication of stressed cells or causes them to undergo programmed cell death (apoptosis). The stresses that activate p53 are vast, ranging from DNA damage to oxidative stress, hypoxia, and heat shock[13]. In ob/ob mice p53 is activated in adipocytes and it is also involved in the mechanisms of dysregulated gene expression in adipose tissue of obese animals. These mice have excessive fat deposition in adipocytes and liver cells thereby activating the p53 pathway, the same mechanism could be followed in the steatotic hepatocytes of obese animals, possibly leading to hepatocellular injury[14]. Steatotic hepatocytes are under various stresses like oxidative stress and DNA damage that induce p53, which leads to liver cell damage and plays an important role in the development of fatty liver disease[15]. Excess fat accumulation in the hepatocytes leads to hepatocellular injury, and that this is possibly caused by the cellular toxicity of excess free fatty acids, oxidative stress, and lipid peroxidation[16]. P53 has been shown to maintain balance between oxidative and glycolytic metabolism in cancer cells. Hence it is well known to be a key regulator of cellular metabolism[17]]. Recently, p53 has also been involved in NAFLD because of its role in regulating fatty acid oxidation. Similar to its role in alcoholic fatty liver disease, p53 has been shown to be upregulated in the livers of mice with NAFLD[14]and has been linked to hepatocytes apoptosis in NAFLD[18].To test the preventive effect of p53 as a therapeutic target in NAFLD, Derdak et al. looked at the effect of a p53 inhibitor, pifithrin-αp-nitro (PFT) on various parameters of NAFLD in a murine model[19]. In HFD fed C57BL/6 mice, PFT treatment was found to decrease hepatic steatosis, apoptosis, and oxidative stress. These effects are believed to be secondary as reduction in the upregulation of miRNA34a was also found. Another recent study confirmed miR-34a upregulation and involvement of SIRT1 and p53 in liver steatosis and observed that treatment with ursodeoxycholicacid reversed p53 upregulation and restored SIRT1 expression[20].

2. Nuclear receptors (NRs)

NRs are ligand-activated transcription factors. They play various metabolic and regulatory functions. Levels of many natural and synthetic ligands including hormones, biomolecules (lipids), vitamins, bile acids, metabolites, drugs, and xenobiotic toxins have great influence on activity of NRs. In liver, NRs control hepatic inflammation, regeneration, fibrosis, and tumor formation[21]. Body's metabolic needs, includingthe synthesis and control of the pathways involved in themetabolism of cholesterol, fatty acids, carbohydrates, aminoacids, serum proteins, and bile acids, and the detoxificationof drugs and xenobiotics are dependent on hepatocytes. Some members of the NR superfamily provide hepatic mechanisms for self-regulation in hepatocytes[22].

2.1 Liver X Receptor

The transcriptional factor liver X receptor (LXR) is involved in metabolism of cholestrol. The LXR gene encodes two different units, LXRα and LXRβ,both have diverse patterns of expression but have similar target DNA binding elements

and ligands[23]. On activation, LXR induces the expression of a bunch of genes which helps in lipid metabolism; LXR helps in cholesterol absorption, efflux, transport, and excretion[24].Besides its metabolic role, LXRs is also involved in immune and inflammatory responses in macrophages[25].LXR forms heterodimers with the retinoid X receptor (RXR) within the nucleus. Binding of the RXR to LXR results in complex formation along with corepressors.Corepressors involved in this complex are silencing mediator ofretinoic acid, thyroid hormone receptor, and nuclear corepressor[26].LXR positively regulates several enzymes involved in lipoprotein metabolism including lipoprotein lipase (LPL), human cholesteryl ester transport protein, and the phospholipid transfer protein[27], Proinflammatory molecules are involved in NAFLD whose expression are repressed by LXR. These include inducible nitric oxide synthase (iNOS), cyclooxygenase 2, interleukin-6 (IL-6), IL-1β, chemokine monocyte chemoattractant protein-1, and chemokine monocyte chemoattractant protein-3[28].

2.2 Farnesoid X Receptor

The farnesoid X receptor (FXR) is also a member of the NR superfamily. It has a typical NR structure and contains a hydrophobic pocket that possesses lipophilic molecules such as bile acids[29]. Bile acids bind to FXR and activate this NR. Activation of the FXR decreases both hepatic lipogenesis and plasma triglyceride and cholesterol levels, induces the genes involved in lipoprotein metabolism/clearance, and represses hepatic genes involved in the synthesis of triglycerides[30].FXR-mediated suppression of hepatic lipase expression increases the hepatic uptake of HDL and also promotes reverse transport of cholesterol[31]. Hepatic lipase is an enzyme which decreases HDL particle size by hydrolyzing its triglycerides and phospholipids in hepatic sinusoids, which facilitates hepatic uptake of HDL cholesterol. Activation of the hepatic FXR regulates carbohydrate metabolism by regulating gluconeogenesis, glycogen synthesis, and insulin sensitivity[32].

2.3 The Pregnane X Receptor (PXR) and Constitutive Androstane Receptor (CAR)

PXR induces lipogenesis in a SREBP-independent manner. In PXR-transgenic mice, Lipid accumulation and marked hepatic steatosisare associated with increased expression of the fatty acid translocase CD36. CD36 is a multiligand scavenger receptor which is present on the surface of a number of cell types. It may contribute to hepatic steatosis by facilitating the highaffinity uptake of fatty acids from the circulation. PXR-mediated activation of PPARγ may also promote hepatic steatosis by increasing the expression of CD36 directly or indirectly[33].PXR activation is also suppresses several genes which are involved in fatty acid β-oxidation, such as PPARα and thiolase[34]. Activation of the CAR might suppress lipid metabolism and lower serum triglyceride levels by reducing the level of SREBP-1. SREBP-1 is a master regulator of lipid metabolism. The inhibitory effects of the CAR on lipid metabolism might also be attributed to induction of Insig-1. Insig-1 is a protein with antilipogenic properties[35]. During fasting, CAR interacts with PPARαand interferes with fatty acid metabolism by binding to DNA elements overlapping with the PPARα-binding site in the promoter region of 3-hydroxyacyl CoA dehydrogenase. 3-hydroxyacyl CoA dehydrogenase is an important enzyme in peroxisomal fatty acidβ-oxidation[36].

3. PI3K/AKT/PTEN Pathway

Inflammation is found to be the major reason behind the diseasesandmaylead to fibrosisandsubsequent cirrhosis. In metabolic dysfunctions including obesity, metabolic syndrome, and the FLD, deregulation of the phosphatidylinositol 3-kinase (PI3K)/AKT pathway in hepatocytes is a common molecular event. PTEN is a tumor suppressor which negatively regulates the PI3K/AKT pathways through its lipid phosphatase activity. PTEN plays an important role in hepatic insulin senstivity and the development of steatosis, steatohepatitis, and fibrosis[37]. Phosphatidylinositol-3 kinase (PI3K) and serine-threonine protein kinase AKT (also known as protein kinase B) activates immune cells by regulating key inflammatory cytokines[38], and modulates PI3K/AKT signaling pathway which may contribute to specific therapeutic effects for the NAFLD. The second messengers generated by the activation of PI3K is dephosphorylated by PTEN which is its physiological function, thereby suppressing insulin signaling downstream of PI3K[39]. In PTEN-deficient mice, synthesis and storage of triglyceride in hepatocytes is found to be increased because of theupregulation of PI3K/AKT activity. As a result of the lack of PTEN activity, there may be increased hepatocyte fatty acid uptake and increased fatty acid synthesis[40].

4. JNK Pathway

C-Jun-N-terminal kinase (JNK) is a mitogen-activated protein kinase (MAPK) family member. There are 3 isoforms of JNK in mammals: JNK1, JNK2, and JNK3. JNK1 and JNK2 are expressed in almost all cells, including liver parenchymal cells, whereas JNK3 is mainly expressed in brain, heart, and testis. In hepatocytes, JNK signaling is associated with cell death, survival, differentiation, proliferation, and tumorigenesis. In nonparenchymal liver cells, such as hepatic macrophages (Kupffer cells) and hepatic stellate cells (HSCs), JNK is involved in inflammation and fibrosis. TNF activates NF-B via the IKK complex and induces expression of antiapoptotic genes that are regulated by NF-B and block caspase-8 – dependent cell death and prolonged activation of JNK[41]. Long term JNK activation increases ROS accumulation, which progressively leads to oxidative inhibition of JNK dual-specificity phosphatases[42]. Movement of activated JNK towards the outer membrane of mitochondria is another important step in induction of JNK-mediated hepatocyte death. Mitochondrial Bcl-XL, Mcl-1, and Sab are substrates for JNK. TNF helps in movement of phosphorylated JNK and MKK4 and theBcl-2 family memberBax to the mitochondrial outer membrane where they promotes generation of mitochondrial ROS and sustained activation of JNK, inducing hepatocyte death[43].

5. Role of Visceral Adipose Tissue (VAT)

VAT has greater lipolytic potential than subcutaneous adipose tissue, and the release of FFA from visceral fat directly into the portal circulation creates a first-pass effect. Serum FFA derived from VAT by lipolysis is the main source of hepatic TG in NAFLD[44]. VAT supplies fat to the liver via portal vein hence plays an important role in the pathogenesis of hepatic steatosis.Visceraladiposity has also been shown, together with insulin resistance, to be an independent predictor of the presence of a steatosis-associated increase in portal pressure[45].The mechanisms linking VAT and the liver are currently poorly understood. The drainage of the venous blood of the gastrointestinal system, including the VAT, via the portal system tothe liver represents a unique anatomical link between the two. VAT can directly influence the liver via portal vein. NAFLD-induced changes in liverhaemodynamics mightinversely influence VAT[46].

6. Nrf2-ARE Pathway

A series of antioxidant genes are induced by elevated ROS and electrophiles through which there is activation of antioxidant response element (ARE) which ultimately protect cells against oxidative stress[47]. NF-E2-related factor-2 (Nrf2) is a member of the cap'n'collar family of bZIP transcription factors which primarily regulates ARE containing gene expression[48] . Oxidative stress and electrophiles leads to activation of Nrf2 in a variety of tissues and cells and plays a role as a multiorgan protector through target gene induction[49].In chronic alcohol-fed mice increased levels of Nrf2 protein and mRNA levels were observed in liver tissues or hepatocytes. HepG2 cells overexpressing CYP2E1 (E47 cells) showed increased Nrf2 mRNA and protein expression compared with control HepG2 (C34 cells). Nrf2 is activated in E47 cells as shown by an increase in nuclear translocation of Nrf2 and Nrf2-ARE binding activity and upregulation of Nrf2-regulated genes such as GCLC and HO-1[50].Nrf2- knockout mice have reduced ability to detoxify acetaldehyde, leading to accumulation of the toxic metabolite. Due to loss of Nrf2 a marked steatosis and inflammatory response was observed in Kupffer cells in ethanol-fed mice. Furthermore, chronic ethanol consumption led to a progressive depletion of total and mitochondrial reduced GSH, which was associated with more pronounced structural and functional changes to mitochondria of Nrf2-knockout mice[51].Yates et al. carried out a global analysis of mouse hepatic gene expression and revealed that both genetic and pharmacologic activation of Nrf2 induce a larger cluster of genes associated with lipid metabolism[52].

7. Diacylglycerolacyltransferase (DGAT)

Hepatic TG production is result of uptake of free fatty acids (FAs) to the liver and de novo synthesis both. Hepatic TG removal is result of FA -oxidation and formation of very low density lipoprotein (VLDL) particles. The final step and rate-limiting reaction in TG synthesis is catalyzed by acyl CoA:diacylglycerolacyltransferase (DGAT), which covalently joins a fatty acyl-CoA and a diacylglycerol (DG) molecule to form TG. In mammals, DGAT occurs in two isoforms, DGAT1 and DGAT2, from distinct gene families[53, 54]. Both isoforms of DGAT are widely expressed in white adipose tissue and are present in high levels. DGAT1 is most highly expressed in the small intestine, whereas

DGAT2 is primarily expressed in the liver[53, 55]. DGAT1 is associated with VLDL synthesis and DGAT2 is associated with steatosis thus both the isoforms play different roles in TG metabolism. Overexpression of liver-specific DGAT2 in mice results in hepatic steatosis[56]. Chronic alcohol exposure contributes to upregulation of hepatic DGAT2 which ultimately leads to the development of ALD. Abnormal hepatic metabolism of methionine, specifically the suppression of transmethylation reactions, plays a mechanistic role in alcohol-induced DGAT2 upregulation by suppressing MEK/ERK1/2 activation. Therefore, inhibition of ERK1/2 activation represents a critical link between methionine metabolism and fatty liver development via upregulation of hepatic DGAT2 expression[57].

8. AMP Activated Protein Kinase (AMPK)

AMP Activated Protein Kinase (AMPK) possesses to inhibit lipid synthesis by the acute inhibition of glycerol-3-phosphate acyltransferase (GPAT) activity and transcriptional regulation via sterol regulatory element binding protein-1c (SREBP-1c). AMPK helps to regulate energy metabolism, fatty acid metabolism, protein synthesis, glucose uptake, increase lipid oxidation and inhibit lipid synthesis. AMPK helps in acute inhibition of glycerol-3-phosphate acyltransferase (GPAT) which is an integral enzyme in triglyceride accumulation. GPAT is the rate-limiting enzyme catalyzing the first committed step in triglyceride synthesis[58, 59]. An AMP-analog aminoimidazolecarboxamideribonucleotide (AICAR) chemically activates AMPK and results in reduced fat accumulation in the hepatocyte by decreasing GPAT1 activity by 30 to 40 percent. AMPK inhibits acetyl-CoA carboxylase (ACC), an enzyme that catalyzes the formation of malonyl-CoA. Malonyl-CoA inhibits carnitinepalmitoyltransferase I (CPT1) resulting in decreased betaoxidation and increased fat synthesis. Decreasing malonylCoA production results in an increase in CPT1 activity. Therefore, through AMPK's acute role of inhibiting GPAT1 and increasing CPT1, there is an overall increase in oxidation relative to triglyceride synthesis[60].

9. PPARs.

The ligand-activated transcription factors belonging to the peroxisome proliferators-activated receptors (PPARs) are a subfamily of the steroid/thyroid/retinoid receptors superfamily. PPARs act as fatty acid sensors to control many metabolic programs including adipocyte differentiation, inflammation and energy homeostasis, lipoprotein metabolism, and FA oxidation that are essential for systematic energy homeostasis and represent an important target for NAFLD[61, 62, 63]. In steatosis development, PPAR-γ involves activation of lipogenic genes and de novo lipogenesis and attributes as a causal role of steatosis. Hence, increased PPAR-γexpression is a feature of the steatotic liver[64]. The specific PPAR subtype PPAR-γ is mainly expressed in the white and brown adipose tissue[65], where it controls the expression of genes related to lipogenesis. PPAR helps in promoting cell differentiation, FA uptake, and TAG accumulation, which reduces FA delivery to the liver[66]. In addition, PPAR-αdownregulation may play a significant role in enhancing the DNAbinding capacity of proinflammatory factors NF-κB and AP-1 in the liver of obese patients, thus constituting one of themajor mechanisms for the progression of simple steatosis tosteatohepatitis.

10. Role of Cytokines

Cytokines are classified into two subtypes as T helper 1 (Th1) and T helper 2 (Th2). The Th1 cytokines are main proinflammatoryand it includes tumor necrosis factor-alpha (TNF-a), interleukin (IL)-1, IL-6 and interferon; whereas the Th2 cytokines are main anti-inflammatory and it includes IL-4 and IL-10. In general, Th1 cytokines induce Th1 cytokines and inhibit Th2 cytokine production and vice versa[67].TNF-a helps to activate intracellular signaling molecules which includes stress related kinases such as Jun N-terminal kinase and inhibitor kappa beta kinase beta. TNF-a helps to make cells resistant to the actions of insulin[68].TNF-aactivates harmful proatherogenic pathways partially by reducing HDLcholesterol, elevating expression of cholesterogenic genes and accompanied by raising potentially harmful precholesterol metabolites, andsuppresing cholesterol elimination[69]. TNF-aon activation can induce both hepatocyte cell death and hepatocyte proliferation[70]. Both TNF-aand IL-6 stimulate hepatic lipogenesis[71]. The inhibition of IL-10 promotes the expression of cytokines which are involved in inflammation, deterioration of insulin signal and activation of gluconeogenic and lipidogenic pathways[72].

11. Role of Endoplasmic Reticulum (ER) Stress

The mechanisms by which saturated fatty acids contribute to liver injury aredue todisruption of endoplasmic reticulum (ER) homeostasis, or ER stress, which is a proximal event. ER stress may lead to activation of various intracellular stress pathways that can progress inflammation and lead to hepatocyte cell death and liver damage.ER is responsible for posttranslational modification of proteins destined for intracellular organelles and the cell surface[73]. Highly saturated fat dietsinduce hepatic ER stress and liver damage in male Wistar rats progresses to development of liver dysfunction[74]. In ER, during oxidative protein folding each disulfide bond formed is responsible for production of single ROS and this process is responsible for ~25% of all ROS generated in a cell[75, 76, 77]. As such, loss of ER calcium homeostasis via inhibition of the sarco/endoplasmic reticulum ATPase (SERCA) uptake pump will reduces the folding capacity of the ER and induces ER stress and ultimately this situation will lead toER-associated apoptosis[78, 79]. ER calcium stores disruption may be the reason of saturated fatty acids induces cell death in H4IIE liver cells and primary rat hepatocytes[80]. Chronic ER stress induces numerous intracellular pathways that, if left unabated, can lead to systemic inflammation, hepatic fibrosis and hepatocyte cell death.

12. Sterol regulatory element binding proteins (SREBPs)

The sterol regulatory element binding proteins (SREBPs) is a family of transcription factors which regulates lipid homeostasis in animal cells[81]. SREBPsbelong to the basic helix–loop–helix–leucine zipper family[82]. Till date three SREBPs have been identified: SREBP-1a and SREBP-1c. These are produced from the same gene through the use of different promoters. SREBP-2 is encoded by a separate gene[83, 84].The synthesis and uptake of cholesterol, fatty acids and phospholipids is regulated by SREBPs which activate the expression of genes which are involved in synthesis[85, 86].SREBP-1c preferentially activates genes involved with free fatty acid[87]. LXRαdirectly regulates SREBP-1c which is the master transcriptional regulator of fatty acid synthesis and fatty acid synthase (FAS) which is a key enzyme in the de novo biosynthesis of fatty acids[88, 89]. SREBP-1c remains bound to the ER and the nuclear envelope in the presence of sufficient sterol concentrations. It is synthesized as a precursor protein.This precursor protein undergoes a series of cleavage process upon sterol deprivation and results in release of NH 2-terminal portion[90]. For activation of transcription of genes involved in fatty acid synthesis these mature SREBP-1c then enters the nucleus by binding to a sterol regulatory element (SRE) or to palindromic sequences (E boxes), within promoter regions of target genes, to activate them[91, 92].

13. Role of ceramides/sphingolipids

Ceramides are members of the sphingolipid family of lipids. They are integral to the structure of the lipid bilayer that makes up cell membranes[93]. However, ceramides also have cell signaling properties. They accumulate in the liver especially during periods of increased hepatic influx of free fatty acids (FFA). They also may contribute to insulin resistance[94].Ceramides are produced by three different pathways; de novosynthesis, a sphingomyelinase (SMase) pathway, and a salvage pathway. LDL. An antioxidant, glutathione (GSH) inhibitsdihydroceramidedesaturase and regulates de novoceramide synthesis and cellular redox status[95]. High saturated fat diet can also stimulate ceramide synthesis[96]. Ceramide activatestranscription factor known as nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB). It can also regulate pro-inflammatory gene expression[96]. Ceramidesreduce GLUT4 translocation and glucose uptake. It has been shown to impair insulin sensitivity in skeletal muscle cells[97].Interaction of TNF-α and ceramides causes insulin resistance and also increases mitochondrial generation of ROS. Ceramides promotes apoptosis and further translocation of inflammatory cells to the liver which ultimately leads to a worsening of hepatic inflammation[98].Accumulation of sphingolipids in the cytoplasm, production of de novo ceramide within the mitochondria and Ceramide-derived sphingolipidi.eganglioside GD3 is required by TNF-αinduced mitochondrial damage[99]. Ceramides could acts as a fuel for the cellular damage caused by inflammation andworsening insulin resistance. It promotes mitochondrial dysfunction and oxidative stress,thus facilitates the progression of NASH.

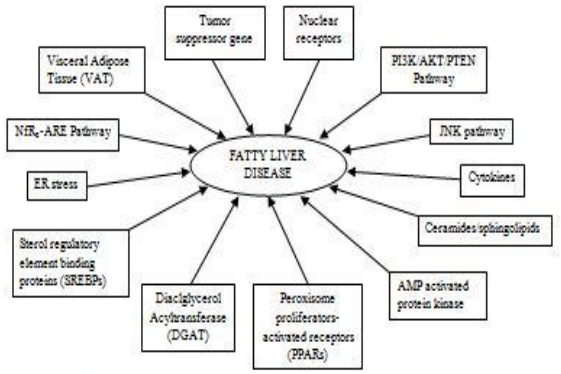

Figure 1: Various mechanisms responsible for causing fatty liver disease.

CONCLUSION

Fatty liver disease is a very complex and multifactorial disorder. Sedentary lifestyle and high intake of dietary fats and alcohol exerts its effects at many levels; individual signaling molecules, cells and finally the entire organ. Integrative approaches providing a comprehensive picture of how alcohol and high fat diet affects intracellular signaling pathways in tissues at different levels is needed. A multidimensional analysis of inflammation and death signaling pathways in immune and non-immune cells of the liver to identify molecular targets in the host leading to systemic and organ inflammation will enhance our understanding of the pathogenesis ofFatty liver disease. Until now various key signaling pathways involved in pathogenesis are nuclear receptors, cytokines, various proinflammatory mediators, AMPK, JNK, VAT, Nrf2/ARE, SREBP, P53, ceramides, stress pathways such as ROS mediated activation of transcription factors, mitochondrial damage and ER stress, have been viewed as separate entities rather than an integrated network of molecular interactions in Fatty liver injury. Future approaches to enable comprehensive analysis of these interactions could offer a powerful tool to understand diagnosis, prognosis, and treatment of FLD.

ACKNOWLEDGEMENT

We are thankful to ShriParveenGarg, chairman, ISF College of Pharmacy, Moga for providing required research facilities.

REFERENCES

1. Reddy JK,Rao MS. Lipid Metabolism and Liver Inflammation: II. Fatty liver disease and fatty acid oxidation.Am J PhysiolGastrointest Liver Physiol2006; 290: G852–G858.
2. Evans RM, Barish GD, Wang YX. PPARs and the complex journey to obesity. Nat Med 2004; 10: 1–7.
3. Browning JD, Szczepaniak LS, Dobbins R et al. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. Hepatology 2004; 40: 1387–1395.
4. Hamaguchi M, Kojima T, Takeda N et al. The metabolic syndrome as a predictor of nonalcoholic fatty liver disease. Ann Intern Med 2005; 143: 722–728.
5. Stefan N, Kantartzis K,Häring H. U. Causes and metabolic consequences of fatty liver. Endocrine Reviews 2008; 29(7): 939-960.
6. Wei Y, Rector RS, Thyfault JP, Ibdah JA. Nonalcoholic fatty liver disease and mitochondrial dysfunction. World J Gastroenterol2008; 14:193–199.
7. Pessayre D, Fromenty B. NASH: a mitochondrial disease.J Hepatol2005; 42:928 –940.
8. You M,Crabb DW. Recent advances in alcoholic liver disease II. Minireview: molecular mechanisms of alcoholic fatty liver. American Journal of Physiology-Gastrointestinal and Liver Physiology 2004; 287(1): G1-G6.
9. Angulo P. Nonalcoholic fatty liver disease. New England Journal of Medicine 2002; 346(16): 1221-1231.
10. Takahashi Y,Fukusato T. Pathology of nonalcoholic steatohepatitis. In: Current Research in Hepatology 2. 2008: 99-112.
11. Tiniakos DG,Vos MB,Brunt EM. Nonalcoholic Fatty Liver Disease: Pathology and Pathogenesis.Annu. Rev. Pathol. Mech. Dis. 2010; 5:145–71.
12. Sookoian S, Pirola CJ. Systems Biology Elucidates Common Pathogenic Mechanisms between Nonalcoholic and Alcoholic-Fatty Liver Disease. PLOS ONE.2013; 8(3). e58895
13. M Oren. Regulation of the p53 tumor suppressor protein. J Biol Chem 1999; 274: 36031–36034.
14. Yahagi N, Shimano H, Matsuzaka T et al. p53 Activation in adipocytes of obese mice.J. Biol. Chem 2003;278: 25395–25400

15. Yahagi N, Shimano H, Matsuzaka T et al. p53 involvement in the pathogenesis of fatty liver disease. Journal of Biological Chemistry 2004; 279(20): 20571-20575.
16. Neuschwander□Tetri BA, Caldwell SH. Nonalcoholic steatohepatitis: summary of an AASLD Single Topic Conference. Hepatology 2003;37(5): 1202-1219
17. Ma W, Sung HJ, Park JY, Matoba S, Hwang PM. A pivotal role for p53: balancing aerobic respiration and glycolysis.J BioenergBiomembr2007; 39(3):243–246.
18. Farrell GC, Larter CZ, Hou JY et al. Apoptosis in experimental NASH is associated with p53 activation and TRAIL receptor expression. Journal of gastroenterology and hepatology 2009; 24(3): 443-452.
19. Derdak Z, Villegas KA, Harb R, Wu AM, Sousa A, Wands JR. Inhibition of p53 attenuates steatosis and liver injury in a mouse model of non-alcoholic fatty liver disease. J Hepatol 2012; 58:785–791.
20. Castro RE, Ferreira D, Afonso MB et al.miR-34a/SIRT1/p53 is suppressed by ursodeoxycholic acid in the rat liver and activated by disease severity in human non-alcoholic fatty liver disease. Journal of Hepatology 2012; 58(1):119–125.
21. Robinson-Rechavi M, Garcia HE,Laudet V. The nuclear receptor superfamily. Journal of cell science 2003; 116(4): 585-586.
22. Karpen SJ. Nuclear receptor regulation of hepatic function. Journal of Hepatology 2002; 36(6): 832–850.
23. Lu TT, Repa JJ,Mangelsdorf DJ. Orphan nuclear receptors as eLiXiRs and FiXeRs of sterol metabolism. Journal of Biological Chemistry 2001; 276(41): 37735–37738.
24. Joseph SB, Laffitte BA, Patel PH et al. Direct and indirect mechanisms for regulation of fatty acid synthase gene expression by liver X receptors. Journal of Biological Chemistry 2002; 277(13): 11019-11025.
25. Castrillo A, Tontonoz P. Nuclear receptors in macrophage biology: at the crossroads of lipid metabolism and inflammation. Annu. Rev. Cell Dev. Biol 2004; 20: 455-480.
26. Chen JD,Evans RM. A transcriptional co-repressor that interacts with nuclear hormone receptors. Nature 1995; 377(6548): 454–457.
27. Zhang Y, Repa JJ, Gauthier K,Mangelsdorf DJ. Regulation of lipoprotein lipase by the oxysterol receptors, LXRα and LXRβ. Journal of Biological Chemistry 2001; 276(46): 43018-43024.
28. Joseph SB, Castrillo A,Laffitte BA, Mangelsdorf DJ,Tontonoz P. Reciprocal regulation of inflammation and lipid metabolism by liver X receptors.Nature Medicine 2003; 9(2): 213–219.
29. Zhang Y, Kast-Woelbern HR,Edwards PA. Natural structural variants of the nuclear receptor farnesoid X receptor affect transcriptional activation. Journal of Biological Chemistry 2003; 278(1): 104–110.
30. Zhang Y, Castellani LW, Sinal CJ, Gonzalez FJ,Edwards PA. Peroxisome proliferator-activated receptor-γ coactivator1α (PGC-1α) regulates triglyceride metabolism by activation of the nuclear receptor FXR. Genes and Development 2004; 18(2): 157–169.
31. Sirvent A, Claudel T, Martin G et al. The farnesoid X receptor induces very low density lipoprotein receptor gene expression. FEBS letters2004; 566(1): 173-177.
32. Cariou B, van Harmelen K, Duran-Sandoval D et al. The farnesoid X receptor modulates adiposity and peripheral insulin sensitivity in mice. Journal of Biological Chemistry 2006; 281(16): 11039-11049.
33. Lee JH, Zhou J,Xie W. PXR and LXR in hepatic steatosis: a new dog and an old dog with new tricks. Molecular pharmaceutics 2007; 5(1): 60-66.
34. Zhou J, Zhai Y, Mu Y et al. A novel pregnane X receptor-mediated and sterol regulatory element-binding protein-independent lipogenic pathway. Journal of Biological Chemistry 2006; 281(21): 15013-15020.
35. Roth A, Looser R, Kaufmann M et al. Regulatory cross-talk between drug metabolism and lipid homeostasis: constitutive androstane receptor and pregnane X receptor increase Insig-1 expression. MolPharmacol 2008; 73: 1282-1289

36. Kassam A, Winrow CJ, Fernandez-Rachubinski F, Capone JP,Rachubinski RA. The peroxisome proliferator response element of the gene encoding the peroxisomal β-oxidation enzyme enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase is a target for constitutive androstane receptor β/9-cis-retinoic acid receptor-mediated transactivation. Journal of Biological Chemistry 2000; 275(6): 4345–4350.
37. Matsuda S, Kobayashi M, Kitagishi Y. Roles for PI3K/AKT/PTEN Pathway in Cell Signaling of Nonalcoholic Fatty Liver Disease. ISRN endocrinology2013; 2013: pages-7. Article ID 472432.
38. Weichhart T, Säemann MD. The PI3K/Akt/mTOR pathway in innate immune cells: emerging therapeutic applications. Annals of the rheumatic diseases 2008; 67(Suppl 3): iii70-iii74.
39. Peyrou M, Bourgoin L, Foti M. PTEN in non-alcoholic fatty liver disease/non-alcoholic steatohepatitis and cancer. Digestive Diseases 2010; 28(1): 236-246.
40. Byrne C. Hypoxia and non-alcoholic fatty liver disease. Clinical science 2010; 118: 397-400.
41. Liu H, Lo CR, Czaja MJ. NF-kappaB inhibition sensitizes hepatocytes to TNF-induced apoptosis through a sustained activation of JNK and c-Jun. Hepatology 2002;35:772–778.
42. Kamata H, Honda S, Maeda S, et al. Reactive oxygen species promote TNF alpha-induced death and sustained JNK activation by inhibiting MAP kinase phosphatases. Cell 2005;120:649 – 661.
43. Win S, Than TA, Han D et al. c-Jun N-terminal kinase (JNK)-dependent acute liver injury from acetaminophen or tumor necrosis factor (TNF) requires mitochondrial Sab protein expression in mice. J BiolChem 2011;286:35071–35078.
44. Vanni E, Bugianesi E, Kotronen A et al. From the metabolic syndrome to NAFLD or vice versa?.Dig Liver Dis. 2010;42:320–330
45. Francque S, Verrijken A, Mertens I et al. Visceral adiposity and insulin resistance are independent predictors of the presence of non-cirrhotic NAFLD-related portal hypertension. Int J Obes (Lond) 2011; 35:270–278.
46. Francque S,WamutuS, Chatterjee S et al. Non-alcoholic steatohepatitis induces non-fibrosis-related portal hypertension associated with splanchnic vasodilation and signs of a hyperdynamic circulation in vitro and in vivo in a rat model. Liver Int 2010;30:365–375.
47. Mathers J, Fraser JA, McMahon M, Saunders RDC, Hayes JD, McLellan LI. Antioxidant and cytoprotective responses to redox stress. Biochemical Society Symposium 2004; 71:157–176.
48. Kobayashi M, Yamamoto M. Molecular mechanisms activating the Nrf2-Keap1 pathway of antioxidant gene regulation. Antioxidants and Redox Signaling 2005;7(3-4):385–394.
49. Lee JM, Li J, Johnson DA et al. Nrf2, a multi-organ protector?. The FASEB Journal 2005;19(9): 1061-1066.
50. Gong P,Cederbaum AI. Nrf2 is increased by CYP2E1 in rodent liver and HepG2 cells and protects against oxidative stress caused by CYP2E1. Hepatology 2006; 43(1): 144–153.
51. Lamlé J, Marhenke S, BorlakJ et al.Nuclear factor-eythroid 2-related factor 2 prevents alcohol - induced fulminant liver Injury. Gastroenterology 2008; 134(4):1159–1168.
52. Yates MS, Tran QT, Dolan PM et al.Genetic versus chemoprotective activation of Nrf2 signaling: overlapping yet distinct gene expression profiles between Keap1 knockout and triterpenoid-treated mice. Carcinogenesis 2009; 30(6): 1024–1031.
53. Cases S, Smith SJ, ZhengYW et al. Identification of a gene encoding an acyl CoA:diacylglycerolacyltransferase, a key enzyme in triacylglycerol synthesis. Proceedings of the National Academy of Sciences 1998; 95:13018 – 13023.
54. Cases S, Stone SJ,Zhou PE et al. Cloning of DGAT2, a second mammalian diacylglycerolacyltransferase, and related family members. J. Biol. Chem 2001; 276:38870 – 38876.
55. Farese RVJr, Cases S, Smith SJ. Triglyceride synthesis: insights from the cloning of diacylglycerolacyltransferase. Curr. Opin. Lipidol 2000;11:229 – 234.

56. Monetti M, Levin MC, Watt MJ et al. Dissociation of hepatic steatosis and insulin resistance in mice overexpressing DGAT in the liver. Cell Metab 2007;6:69 – 78.
57. Wang Z, YaoT,Song Z. Involvement and mechanism of DGAT2 upregulation in the pathogenesis of alcoholic fatty liver disease; Journal of Lipid Research 2010; 51: 3158-3165.
58. Cao J, Li JL, Li D, Tobin JF, Gimeno RE. Molecular identification of microsomal acyl-CoA:glycerol-3-phosphate acyltransferase, a key enzyme in de novo triacylglycerol synthesis. Proceedings of the National Academy of Sciences 2006; 103:19695–19700.
59. Coleman RA, Lee DP. Enzymes of triacylglycerol synthesis and their regulation.Prog Lipid Res 2004; 43:134–176.
60. Henriksen BS, Curtis ME, Fillmore N, Cardon BR, Thomson DM, Hancock CR. The effects of chronic AMPK activation on hepatic triglyceride accumulation and glycerol 3-phosphate acyltransferase activity with high fat feeding. Diabetology& metabolic syndrome 2013; 5(1): 29.
61. Stienstra R, Duval C, Muller M,Kersten S. PPARs,obesity, and inflammation. PPAR Research 2007(2006): 10 pages.
62. Bensinger SJ, Tontonoz P. Integration of metabolism and inflammation by lipid-activated nuclear receptors. Nature 2008; 454(7203): 470–477.
63. Berger JP, Akiyama TE, Meinke PT. PPARs:therapeutic targets for metabolic disease. Trends in Pharmacological Sciences 2005; 26(5): 244–251.
64. Tailleux A, Wouters K,Staels B. Roles of PPARs in NAFLD: potential therapeutic targets. BiochimicaEtBiophysicaActa 2012; 1821(5): 809–818.
65. Neher MD, Weckbach S, Huber-Lang MS,Stahel PF. New insights into the role of peroxisome proliferatoractivated receptors in regulating the inflammatory response after tissue injury. PPAR Research 2012; 2012: 13 pages.
66. Kallwitz ER, McLachlan A, Cotler SJ. Role of peroxisome proliferators-activated receptors in the pathogenesis and treatment of nonalcoholic fatty liver disease. World Journal of Gastroenterology 2008; 14(1): 22–28.
67. Crews FT, Bechara R, Brown LA et al. Cytokines and alcohol. Alcohol ClinExp Res. 2006;30:720–30.
68. Diehl AM, Li ZP, Lin HZ, Yang SQ. Cytokines and the pathogenesis of non-alcoholic steatohepatitis. Gut. 2005;54(2):303–306.
69. FonTacer K, Kuzman D, Seliskar M, Pompon D, Rozman D. TNF-alpha interferes with lipid homeostasis and activates acute and proatherogenic processes. Physiol Genomics. 2007;31(2): 216–27
70. Wullaert A, van Loo G, Heyninck K, Beyaert R. Hepatic tumor necrosis factor signaling and nuclear factor-kappaB: effects on liver homeostasis and beyond. Endocr Rev. 2007;28(4):365–86
71. Brass EP, Vetter WH. Interleukin-6, but not tumour necrosis factor-alpha, increases lipogenesis in rat hepatocyte primary cultures. Biochem J. 1994;301(Pt 1):193–7
72. Cintra DE, Pauli JR, Araujo EP et al. Interleukin-10 is a protective factor against diet-induced insulin resistance in liver. J Hepatol. 2008;48(4): 628–37.
73. Zhang K, Kaufman RJ. Protein folding in the endoplasmic reticulum and the unfolded protein response. HandbExpPharmacol. 2006:69–91.
74. Wang D, Wei Y, Pagliassotti MJ. Saturated fatty acids promote endoplasmic reticulum stress and liver injury in rats with hepatic steatosis. Endocrinology. 2006; 147:943–951.
75. Chakravarthi S, Jessop CE, Bulleid NJ. The role of glutathione in disulphide bond formation andendoplasmic-reticulum-generated oxidative stress. EMBO Rep. 2006; 7:271–275.
76. Malhotra JD, Kaufman RJ. Endoplasmic reticulum stress and oxidative stress: a vicious cycle or a double-edged sword? Antioxid Redox Signal. 2007; 9:2277–2293.
77. Shimizu Y, Hendershot LM. Oxidative folding: cellular strategies for dealing with the resultant equimolar production of reactive oxygen species. Antioxid Redox Signal. 2009; 11:2317–2331.
78. Zhou YP, Teng D, Dralyuk F et al. Apoptosis in insulin-secreting cells.

Evidence for the role of intracellular Ca2+ stores and arachidonic acid metabolism. J Clin Invest. 1998; 101:1623–1632.
79. Luciani DS, Gwiazda KS, Yang TL et al. Roles of IP3R and RyR Ca2+ channels in endoplasmic reticulum stress and beta-cell death. Diabetes. 2009; 58:422–432.
80. Wei Y, Wang D, Gentile CL, Pagliassotti MJ. Reduced endoplasmic reticulum luminalcalcium links saturated fatty acid-mediated endoplasmic reticulum stress and cell death in liver cells. Mol Cell Biochem. 2009; 331:31–40.
81. Kamei Y, Miura S, Suganami T et al. Regulation of SREBP1c gene expression in skeletal muscle: role of retinoid X receptor/liver X receptor and forkhead-O1 transcription factor. Endocrinology 2008; 149:2293 – 2305.
82. Amemiya-Kudo M, Shimano H, Yoshikawa T et al. Promoter analysis of the mouse sterol regulatory element-binding protein-1c gene. J BiolChem 2000; 275:31078 – 31085.
83. Shimano H. Sterol regulatory element-binding proteins (SREBPs): transcriptional regulators of lipid synthetic genes. Prog Lipid Res 2001; 40: 439 – 452.
84. Edwards PA, Tabor D, Kast HR et al. Regulation of gene expression by SREBP and SCAP. BiochimBiophysActa 2000; 1529:103 – 113.
85. Dentin R, Girard J, Postic C. Carbohydrate responsive element binding protein (ChREBP) and sterol regulatory element binding protein-1c (SREBP-1c): two key regulators of glucose metabolism and lipid synthesis in liver. Biochimie 2005; 87:81 – 86
86. Takeuchi Y, Yahagi N, Nakagawa Y et al. In vivo promoter analysis on refeeding response of hepatic sterol regulatory element-binding protein-1c expression. BiochemBiophys Res Commun 2007; 363:329 – 335.
87. Chen G, Liang G, Ou J et al. Central role for liver X receptor in insulin-mediated activation of SREBP-1c transcription and stimulation of fatty acid synthesis in liver. ProcNatlAcadSci USA 2004; 101:11245 – 11250.
88. Zhang H, Shi Z, Liu Y et al. Lipid homeostasis and oxidative stress in the liver of male rats exposed to perfluorododecanoic acid. ToxicolApplPharmacol 2008; 227:16 – 25.
89. Gentile CL, PagliassottiMJ. The role of fatty acids in the development and progression of nonalcoholic fatty liver disease. J NutrBiochem 2008; 19:567 – 576.
90. Horton JD, Goldstein JL, Brown MS. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. J Clin Invest 2002; 109:1125 – 1131.
91. Deng X, Yellaturu C, Cagen L et al. Expression of the rat sterol regulatory element-binding protein-1c gene in response to insulin is mediated by increased transactivating capacity of specificity protein 1 (Sp1). J BiolChem 2007; 282:17517 − 17529.
92. Yoshikawa T, Shimano H, Amemiya-Kudo M et al. Identification of liver X receptor–retinoid X receptor as an activator of the sterol regulatory element-binding protein 1c gene promoter. Mol Cell Biol 2001; 21:2991 − 3000.
93. Hannun YA, Obeid LM. Principles of bioactive lipid signalling: lessons from sphingolipids. Nat Rev Mol Cell Biol. 2008; 9:139–150.
94. Summers SA. Ceramides in insulin resistance and lipotoxicity. Prog Lipid Res. 2006; 45:42–72.
95. Gault CR, Obeid LM, Hannun YA. An overview of sphingolipid metabolism: from synthesis to breakdown. AdvExp Med Biol 2010;688: 1-23.
96. Frangioudakis G, Garrard J, Raddatz K, Nadler JL, Mitchell TW, Schmitz-Peiffer C. Saturated-and n-6 polyunsaturated-fat diets each induce ceramide accumulation in mouse skeletal muscle: reversal and improvement of glucose tolerance by lipid metabolism inhibitors. Endocrinology 2010; 151(9): 4187-4196.
97. JeBailey L, Wanono O, Niu W, Roessler J, Rudich A, Klip A. Ceramide-and oxidant-induced insulin resistance involve loss of insulin-dependent Rac-

activation and actin remodeling in muscle cells. Diabetes 2007;56(2): 394-403.

98. Schwabe RF, Brenner DA. Mechanisms of Liver Injury. I TNF-alpha-induced liver injury: role of IKK, JNK, and ROS pathways. Am J PhysiolGastrointest Liver Physiol. 2006; 290:G583–9.
99. Brenner C, Kniep B, Maillier E et al. GD3–7-aldehyde is an apoptosis inducer and interacts with adenine nucleotide translocase. Biochemical and biophysical research communications 2010; 391(1): 248-253.