Non-alcoholic fatty liver disease (NAFLD): The link between hepatic steatosis and insulin resistance

Asha Abdulkadir

Abstract
NAFLD is linked with insulin resistance and the change of hepatokine secretion is observed to demonstrate the link between hepatic steatosis and insulin resistance in skeletal muscle through interorgan cross-talk. NAFLD is an hepatic manifestation of the metabolic syndrome in which there is an imbalance in lipid in and outflux of the liver. Hepatic steatosis is the first development of NAFLD and functional defects pathway such as overspill of fatty acid through a defected lipolysis mechanism of triglycerides in the adipose tissue, high dietary fat intake, a decrease in lipid oxidation by hepatic mitochondrial dysfunction, an increase in hepatic de novo lipid synthesis and a decreased production of hepatic very low density lipoprotein (VLDL) can occur. The liver secretes several hepatokines α2-HS-Glycoprotein and fibroblast decrease insulin resistance and Selenoprotein, Leukocyte Cell-derived Chemotaxin and Chemerin can promote insulin resistance. The increase of proinflammatory and decrease of protective hepatokines in a liver and blood stream, influencing insulin sensitivity of skeletal muscles through interorgan crosstalk. Detecting hepatic steatosis and insulin resistance with hepatokine might be easier and less invasive than liver biopsy that has a mortality rate of 0.01%. However, more research is needed understand the mechanism of how hepatokines can decrease insulin resistance and hepatic steatosis. Furthermore, identifying more hepatokines for the different NAFLD stages could further improve the analyzation of the severity and increase prevention. The only conclusion that can be made with this narrative review is that high dietary fat increase the risk of hepatic steatosis.
Introduction

Over the past couple of decades, nonalcoholic fatty liver disease (NAFLD) is the most prevalent liver disease in western countries. The prevalence of NAFLD has doubled during the last 20 years and NAFLD is connected mostly to visceral obesity, impaired glucose tolerance and/or type two diabetes mellitus. People in the population that suffer from diabetes and/or obesity in western countries, have an estimated NAFLD prevalence between 75 to 90% \(^2\). Additionally, the general population has an estimated NAFLD prevalence between 20-30% in European countries in which 20-40% in western European countries. With an worldwide increasing prevalence of 400 million in diabetes and obesity and an direct and indirect increase of 26% in medical cost in the coming 5 years globally, understanding and preventing insulin resistance that is caused by NAFLD is crucial \(^3,4\).

NAFLD is defined as the presence of excessive fat accumulation in the liver exceeding 5% of hepatocytes after exclusion of excessive alcohol consumption of more than 20 gram per day for men and 10 gram per day for women \(^5,6\). NAFLD can be divided in four stages: 1) fatty liver (hepatic steatosis), 2) non-alcoholic steatohepatitis (NASH), 3) fibrosis and 4) cirrhosis \(^5,7\). Hepatic steatosis is when excessive fat accumulates in the liver without causing any inflammation. Hepatic steatosis occurs mostly in obese people and influences the type of hepatokines secreted by the liver during interorgan cross-talk with adipose tissue. Changed hepatokine secretion might play an important role in the development of insulin resistance in obese and diabetic patients. Furthermore, hepatokines could be used as a less invasive biomarker to diagnose and prevent NAFLD earlier than a liver biopsy.

In this narrative review the aim is to demonstrate the link between hepatic steatosis and insulin resistance in skeletal muscle through interorgan cross-talk. This can be achieved by observing the change of hepatokine secretion during the development of hepatic steatosis in the liver and analyzing the development of insulin resistance in skeletal muscles that is caused by hepatokine secretion during the interorgan cross-talk.

Hepatic steatosis development

NAFLD is an hepatic manifestation of the metabolic syndrome in which there is an imbalance in lipid in and outflux of the liver, however the mechanism leading to NAFLD is still unclear so far \(^1,2\). Several functional defects can occur within the different pathways such as overspill of fatty acids from adipose tissue, dietary fat intake, mitochondria dysfunction, de novo lipid dysfunction and decreased or less functional VLDL triglycerides transporters can influence the development of hepatic steatosis \(^8\).

One of the ways that hepatic steatosis can occur is when there is an overspill of fatty acid through a defected lipolysis mechanism of triglycerides in the adipose tissue. The defect lipolysis mechanism of triglycerides increases the delivery of adipose fatty acid towards the liver. Adipose fatty acids delivery into the liver is more prone in obese compared to lean individual due to their higher level of fatty acids and insulin resistance in their adipose tissue. The defective lipolysis mechanism in adipose tissue causes anti-lipolytic effects such as insulin resistance and the release of triglyceride \(^2,9\). In the study of Donnelly (2005), it is demonstrated that 60% of the triglycerides in the liver comes from adipose tissue when adipose derived fatty acids in fasted state were tracer \(^10\). These results not only provide the link between obesity, insulin resistance and NAFLD, but also emphasize NALFD pathogenesis pathway.

Another possible pathway for hepatic steatosis to occur is through a high dietary fat intake as it is estimated that 20% of the triglycerides stored in the liver can be derived from dietary fat intake. The liver has an average triglyceride content of 40 grams and the average American diet of 100 grams per day would fill half of the total triglyceride storage capacity of the liver making people more susceptible to hepatic steatosis \(^11\). Van Herpen et al. (2011) have shown that after three in overweight men were randomly distributed into the high fat diet groups, intrahepatic lipid (IHL) increased by 17% while low fat diet groups had a decrease of 13% \(^12\). Furthermore high-fat feeding in rodent stimulated the increased development of hepatic steatosis within a couple of days in the liver. These studies indicate first of
mitochondrial dysfunction is unclear how they influence the mechanism for hepatic lipid oxidation and decrease hepatic steatosis, however fenofibrate and phytochemical quercetin can increase the development of steatosis. Additionally, statins, suggesting that mitochondria dysfunction could precede an reduced hepatic ATP concentration was found metabolically well-controlled T2DM were investigated, Furthermore, in the study of 2009 where patient with contribued by progressive mitochondrial dysfunction. Developed NAFLD and insulin resistance that was contributed by progressive mitochondrial dysfunction. Furthermore, in the study of Rector et al (2010) it was shown that non-hyperphagic control Long-Evans Tokushima Otsuka mice developed NAFLD and insulin resistance that was contributed by progressive mitochondrial dysfunction. Furthermore, in the study of 2009 where patient with metabolically well-controlled T2DM were investigated, an reduced hepatic ATP concentration was found suggesting that mitochondria dysfunction could precede the development of steatosis. Additionally, statins, fenofibrate and phytochemical quercetin can increase lipid oxidation and decrease hepatic steatosis, however how they influence the mechanism for hepatic mitochondrial dysfunction is unclear. On the other hand, the study of Buchner et al. (2011) indicate that hepatic mitochondrial dysfunction and lower numbers of hepatic mitochondrial might have been caused by the disruption of electron flow that depletes the mtDNA and increases production of reactive oxygen species (ROS). These studies have shown that mitochondrial dysfunction decreases insulin sensitivity and hepatic ATP concentration and increase the development of hepatic steatosis and NAFLD. However, further research is needed to understand the underlying mechanism causing mitochondrial dysfunction that is connect with hepatic steatosis.

Furthermore an increase in hepatic de novo lipid (DNL) synthesis in which fatty acids are derived from non-lipid sources, could play a pivotal role in the development of hepatic steatosis. DNL is synthesis from the oxidation of 2-carbon precursors derived glucose, fructose and amino acids and is stimulated by sterol regulatory element binding protein 1c (SREBP-1c) and carbohydrate responsive element binding protein (ChREBP). SREBP-1c and ChREBP co-ordinate the increased expression of many genes in the fatty acid biosynthetic pathway such as acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS) and mitochondrial glycerol 3-phosphate acyltransferase (GPAT). Additionally, ChREBP also improves the provision of substrate for FFA synthesis through glycolytic flux. In the study of Donnelly et al (2005) it was shown that 26% of liver triglycerides are derived from DNL synthesis. Moreover DNL rates in NAFLD patients is increase up to 3 fold when compared to BMI-matched controls, indicating that more fatty acid is produced in NAFLD patients. Interestingly lower NAFLD in women suggests that estrogen might have a protective effect on the DNL pathway, however, further research is needed for confirmation. The study of Donnelly suggest that 26 percent of triglycerides in the liver is caused by DNL which indicate that 26% of hepatic steatosis might be caused by DNL. Furthermore NAFLD patient have a higher DNL rates indicating that DNL dysfunction causes further increase of triglycerides storage in the liver.

Finally, hepatic steatosis can be caused by a decreased secretion of lipids though VLDL by the lack and or absence of apolipoprotein B, microsomal TG transfer protein (MTTP) and adequate supply of lipids. Although there is an increased secretion of lipids in patients with hepatic steatosis, secretion rates of triglycerides is not adequate enough to reverse hepatic steatosis due to reaching the maximal excretion levels. Chen et al. (2000) study in mice producing apo B38.9 enhances hepatic secretion efficiency, however, the transportation of triglyceride capacity of the apo protein is decreased in fatty livers. In a cross sectional study population of 1193 subjects, fasting serum lipid, insulin and non-esterified fatty acids were assessed and transformed to insulin resistance index HOMA-IR and Adipo-IR. Five common microsomal triglyceride transfer protein (MTTP) polymorphisms were conducted with the TaqMan assay and the results evidenced the MTTP polymorphisms could modulate the lipid homeostasis to decrease serum apoB containing lipoproteins and increase the risk of NAFLD formation. The results in the studies of Chen et al. and Hsiao P-J et al. indicate that less triglycerides are transporter from the liver to other cell in the body by the lack of apo B protein on VLDL transporters which is needed to be secreted by the liver into the bloodstream. Furthermore lower transport of triglycerides in each VLDL transporter further decreases the amount of triglycerides leaving the liver.
**NAFLD linked to insulin resistance**

Insulin resistance is a complex metabolic disorder that is closely linked to changes in fatty acid uptake, lipogenesis and energy expenditure and can affect the ectopic lipid deposit in the liver and muscles. Increased lipid deposit can cause cellular changed leading to impaired insulin resistance. Insulin resistance is strongly linked with hepatic steatosis indicating a causal role of hepatic steatosis in the development of insulin resistance. In the study of Koska (2008) where fifty-three obese pima Indians were observed, liver fat content was the only independent predictor of reduced peripheral and hepatic insulin action, indicating that increased fat accumulation is a predictor of insulin resistance. Furthermore, Stefan et al (2008) studied 314 subjects that were divided in four groups in which fats in the liver and skeletal muscle were measured with proton MR spectroscopy. The total body and visceral fat were higher in the overweight and obese groups compared to the normal-weight group, in which the obese insulin sensitive group had 54% less fat accumulation in the liver than in the obese insulin resistance group, which indicates that liver fat accumulation is a key determinant of insulin resistance development and differing between metabolically benign and malignant obesity. What is more, mice that were fed high fat diet in the study of Turner et al from 3 days to 16 weeks developed insulin resistance after 1 week of high fat diet which was initiated by impaired hepatic insulin action and has been associated with the accumulation of specific bioactive lipid species. These research studies in human and mice indicate that high fat diet increases fat content in the liver within one week and obese benign humans which had less fat in the liver were more insulin sensitive than obese malignant which had 54% more fat accumulated in the liver.

**Hepatokines**

Hepatokines are regulatory proteins secreted by the liver that affect the lipid and glucose metabolism similarly to adipocytokines. Here are several proinflammatory hepatokines that play an important role in the development of NAFLD and protective hepatokines that inhibit NAFLD development. Protective hepatokines such as FGF21 promote lipid oxidation, improve insulin resistance and inhibit hepatic steatosis, while proinflammatory regulatory proteins such as LECT2, SeP and Chemerin cause the opposite effect.

**Fibroblast Growth Factor 21 (FGF21)**

FGF21 consists of 209 amino acids peptide. FGF21 mainly functions as a hepatokine but also plays an important role as a myokine and an adipokine. FGF21 promotes lipid oxidation, improves insulin resistance that is induced through obesity and inhibits hepatic steatosis. FGF21 secretion into the bloodstream is increased by a high-fat/low carbohydrate ketogenic diet or by fasting conditions. Increased FGF21 triggers lipolysis in white adipose tissue while knockdown of the FGF21 leads to fatty liver and dyslipidemia.
induced suppression of CHOP has been shown to suppress metabolic genes through the disrupting the function of C/EBPα, leading to fatty acid oxidation and lipoprotein secretion. Furthermore FGF21 mechanistically linked to the ER homeostasis and influences ER stress-induced apoptosis in cultured cells 36. Additionally, FGF21 knocked out mice on methionine and choline-deficient had far more severe steatosis, fibrosis and inflammation than mice without FGF21 knock out. Furthermore FGF21 knocked out mice had reduced hepatic fatty acid activation and beta-oxidation which increase the free fatty acids. Moreover, infusion of FGF21 for 4 weeks in FGF21 knocked out mice reduced the steatosis and peroxidative damage, indicating that FGF21 regulatory protein is a protective against a fatty liver37. These studies have shown that FGF21 is a protective hepatokine against hepatic steatosis and when FGF21 gene is knocked out in mice the severity of hepatic steatosis increased more than when FGF21 gene isn't knocked out in mice. Furthermore more research need to be done to understand the mechanism FGF21 has on the homeostasis with ER as this is connected with Apo B proteins being connected with VLDL transporters that are secreted by the liver.

**Leukocyte Cell-Derived Chemotaxin 2 (LECT2)**

LECT2 is a 16-kDA protein that is mainly expressed in hepatocytes and is related to the homeostasis of hepatic natural killer T cells and hepatic inflammatory signaling 27,35,38. The relationship between LECT2 and obesity has been described with an positive correlation between LECT2 and BMI. Furthermore, high-fat diet in animals show higher LECT2 expression and mice with LECT2 knockout have an improved insulin sensitivity in skeletal muscles 39. Moreover, LECT2 expression is reduced via the AMPK activation caused by exercise and starvation of 60 hours in wildtype and LECT2 knockout mice showed a decreased LECT2 secretion indicating that LECT2 is involved in metabolic disorder39. In the study of Saito et al (2004) LECT2 knocked out mice had an increased number of hepatic NKT cells that increase the production of IL-4 and FasL that are involved in the pathogenesis of hepatitis or other inflammatory diseases such as NASH of NAFLD 38. Finally patient with obesity and NAFLD have shown significantly higher LECT2 levels in their serum plasma 27. These studies indicate that LECT2 is a proinflammatory hepatokine and can give an indication of the development of hepatic steatosis and NASH. Furthermore exercise and starvation of more than 60 hours decrease LECT2, indicating that fasting and exercise can inhibit hepatic steatosis development.

**Selenoprotein P (SeP)**

SeP is a 60kDa glycoprotein that is mainly produced in the liver and is released into the plasma into the tissues such as the testes and brain 35,40. SeP caused insulin resistance by inhibiting the AMPK activation in the liver when mice were administrated SeP 41. Furthermore, depletion of SeP in mice improved insulin sensitivity and glucose tolerance, while palmitate treatment increased SeP expression . NAFLD, T2DM and visceral obesity have shown increased SeP circulation in which HOMA-IR, hsCRP, VFA and several components of metabolic syndrome in NAFLD patients were increased and are positively related to SeP 42. In another study where subjects with NAFLD, high Sep serum level also showed increased levels of HOMA-IR, hsCRP, VFA and several metabolic syndrome while levels of adiponectin and HDL cholesterol were decreased. From these studies we can conclude that SeP is a proinflammatory hepatokine that inhibits AMPK activation and induces HOMA-IR hsCRP and VFA in the liver and can predict the development of NAFLD and T2DM which indicate that is might be a novel biomarker for analyzing these diseases faster 42.

**Chemerin**

Chemerin is linked to obesity and high levels of chemerin are found in patients with NAFLD 27,35,43. Chemerin contributes to impaired glucose homeostasis and is more expressed due to high cholesterol in diet and NAFLD 44. Also lack and or defect of methionine and choline increased chemerin expression levels in the liver. In liver tissue samples of 47 subjects the concentration of chemerin was measured and was significantly elevated in patients with NAFLD. Hepatic chemerin mRNA expression is independently associated with liver fibrosis, steatosis, inflammation and hepatocyte ballooning 45. In the study of Ernst et all (2010), the expression of chemerin and its receptors chemokine-like receptor 1, chemokine (C-C motif) receptor like 2 and G-protein-coupled receptor 1 are altered in the liver tissue of obese/diabetic mice.

---

Narrative Reviews 2017

5
Chemerin administration in obese and diabetic mice influences glucose homeostasis, serum insulin level and glucose uptake. These research studies indicate that chemerin is a proinflammatory hepatokine and is connected with the development of hepatic steatosis and hepatocyte ballooning by impairing glucose homeostasis through altering chemokine-like receptor 1, 2 and G-protein-coupled receptor 1.

**Conclusion**

Hepatic steatosis is linked with insulin resistance through the change of secretion of the hepatokines. The change of hepatokine secretion is mainly caused by dietary intake pathway in which 20% of dietary fat is stored into the liver. Proinflammatory hepatokines production of LECT2, SeP, Fetuin A and chemerin are increased in patient with hepatic steatosis. On the other hand, the production of protective hepatokines such as FGF21 are decreased. The increase of proinflammatory and decrease of protective hepatokines in a liver and blood stream, influencing insulin sensitivity of skeletal muscles through interorgan crosstalk. At the moment, hepatic steatosis and insulin resistance are detected with liver biopsy that has a mortality rate of 0.01%. Developing a less invasive detection method through the change in hepatokine secretion might be easier. However, more research is needed to understand the mechanism of how hepatokines can decrease insulin resistance and hepatic steatosis. Furthermore, identifying more hepatokines for the different NAFLD stages could further improve the analyzation of the severity and increase prevention. The only conclusions that can be made with this narrative review is that high dietary fat increase the risk of hepatic steatosis, fasting more than 60 hours decrease the proinflammatory hepatokine Fetuin A and increase the protective hepatokine FGF21.

**Literature**


