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Review Article

Metabolic Liver Inflammation in Obesity

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Abstract

Nonalcoholic fatty liver disease (NAFLD) has become a common disorder growing in line with the obesity epidemic. Simply fatty liver can be a completely benign condition but can raise the risk of heart disease, diabetes, cirrhosis and liver cancer, due to metabolic inflammation and scarring. In this mini-review pathogenesis, pathophysiology, course and treatment of NAFLD are discussed.

Introduction

About 30% to 40% of American adults have a condition that has no visible signs and rarely causes symptoms but can raise the risk of heart disease, diabetes, cirrhosis and liver cancer. It is called non-alcoholic fatty liver disease (NAFLD) and as American waistlines continue to expand the prevalence of this condition is growing as well [1,2]. A fatty liver is the result of excess fat in liver cells. Fatty tissue slowly builds up in the liver when a person's diet exceeds the amount of fat his or her body can handle. A person has a fatty liver when fat makes up at least 5% of the liver [3]. Simply fatty liver can be a completely benign condition and usually does not lead to liver damage. However, once there is a buildup of simpe fat, the liver becomes vulnerable to further injury, which may result in inflammation and scarring of the liver [4].

Like fat accumulates in the rest of the body, this condition is often related to obesity, poor diet and sedentary lifestyle. Excess weight also seems to make genetic risk factors worse. People with a high- risk variant of PNPLA3 gene were more likely to have fatty liver disease if they were obese than if they were thin [5]. While fatty liver is much more common in people who are overweight and obese, people with normal body weight can have what is known as "lean fatty liver" too. Fatty liver tends to affect certain groups of people who tend to have lower BMIs than typical Western populations, like peope of Asian descent with an "l". Given the abscence of traditional risk factors, it tends to remain underrecognised. The metabolic profiles of lean NAFLD are frequently comparable to those of obese NAFLD patients [6].

Fatty liver has already been observed in the fetuses of obese pregnant monkey mothers exposed to high fat, high sugar diets during pregnancy, predisposing children to obesity, metabolic and cardiovascular disorders later in life [7]. NAFLD is the most common cause of elevated liver enzymes in children in the United States [8]. In this mini-review pathogenesis, pathophysiology, course and treatment of this common disorder will be discussed.

Pathogenesis NAFLD

Insulin resistance, the metabolic syndrome or type 2 DM and genetic variants of PNPAL3 or TM6SF2 seem to play a role in the pathogenesis of NAFLD. The pathological progression of NAFLD follows tentatively a "three hit" process, namely steatosis, lipotoxicity and inflammation. The presence of steatosis, oxidative stress and inflammatory mediators like TNF-alpha (tumor necrosis factor alpha) and IL-6 (interleukine-6) has been implicated in the alterations of nuclear factors such as CAR, PXR, PPAR-alpha in NAFLD. These factors may result in altered expression and activity of drug metabolizing enzymes (DMEs) or transporters. Existing evidence suggests that the effect of NAFLD on CYP3A4, CYP2E1 and MRP3 is more consistent across rodent and human studies. CYP3A4 activity is down-regulated in NASH (non-alcoholic steatosis hepatis) whereas the activity of CYP2E1 and the efflux transporter MRP3 is upregulated. The alterations associated with NAFLD could be a potential source of drug variability in patients and could have serious implications for the safety and efficacy of xenobiotics [9].

Pathophysiology of NAFLD

NAFLD is associated with a wide pathological spectrum ranging from indolent liver fat storage associated with a benign clinical course, to progressive cardiovascular metabolic and liver and kidney diseases with high cancer risks. Insulin resistance (IR) plays a pivotal role in the pathogenic switch of fatty liver. IR as a hallmark of metabolic syndrome and stems from the complex dimensional interplay among inflammation and key circulating mediators, organs and tissues, genetic background and major conditioning factors such as lifestyle (i.e. diet and physical activity). Circulating lipids, released compounds from adipose, muscle and liver tissues and pancreatic and gut hormones in relation to lifestyle and inflammation play a role.

Circulating Lipids in NAFLD

Free Fatty Acids (FFAs)

Circulating FFAs, which represent the major source of hepatic fat accumulation in patients with NAFLD are mainly derived from adipose tissue lipolysis and partly from lipoprotein spill over and are the major fuel substrate for all tissues, except the brain during fasting. Thus, their plasma levels are high during fasting and decline after feeding because of the anti-lipolytic action of insulin. In the presence of adipose tissue insulin resistance, FFA levels are high despite high levels of circulating insulin, because of the resistance to the anti-lipolytic action of this hormone [10,11]. FFAs are involved in the pathogenesis of different metabolic disorders associated with insulin resistance and different forms of FFAs have different implications in cardio-metabolic disorders ranging from protective to harmful effects [12-16].
Plasma FFAs are reabsorbed in various organs where if not oxidized they accumulate under the form of triglycerides within intracytoplasmic lipid droplets, and some lipid intermedates, such as diacylglycerols (DAGs), promoting lipotoxicity and mitochondrial dysfunction. Hepatic FFAs can be exported as very low density lipoproteins (VLDL), which can contribute to high ciculating TGs and are involved in hepatic insulin resistance [24]. Low density lipoprotein (LDL), reduced high density lipoproteins (HDL) are an increased risk of atherosclerosis [17].

Elevated plasma FFA levels, affected also by diet and exercise and resulting from obesity or high-fat feeding can cause insulin resistance as well as low-grade inflammation [18]. Recently, the activation of the c-Jun-terminal kinase (JNK) pathway by saturated fatty acids(SFA) was demonstrated in vivo, contributing to the development of hepatic steatosis and insulin resistance, as well as activation of pro-inflammatory M1 macrophages [19]. Other in vitro studies showed that palmitate may induce endoplasmic reticulum (ER) and oxidative stress in hepatocytes and trigger the inflammasome via the activation of macrophages through TLR2/1 dimerization [20,21]. On the contrary, the contribution of unsaturated fatty acids (e.g. oleate, lineolate) to insulin resistance is still debated. They seem unable to affect the cell, but can impact triglycerides(TGs) storage [22]. Finally, FFAs are the source of diacyl glycerol (DAG), TGs and other metabolites such as ceramides which are synthesized in the ER of hepatocytes from long-chain SFAs, as a substrate [23]. Ceramides were shown to be lipotoxic to pancreatic cells and involved in hepatic insulin resistance [24]. Direct evidence of their pro-apoptotic role on hepatocytes is missing [25]. Increased hepatic ceramides and saturated TGs and FFas were found in patients with NAFLD [26]. ER stress contributes to NASH progression and saturated FFAs were shown to induce an ER stress response in hepatocytes and increased levels of ER stress in patients with NAFLD and NASH [27].

The above mentioned effect of FFAs on insulin resistance and low-grade inflammation can explain the link between FFAs and NAFLD and NASH. Recent in vitro and in vivo studies support the hypothesis that FFAs, which are not esterified and stored in lipid droplets, may induce irreversible cell damage and trigger pro-inflammatory signalling pathways either alone or in combination with other lipid metabolites [28-30]. In addition, other in vitro and in vivo studies have shown that inhibiting hepatic TG synthesis results in an amelioration of hepatic steatosis, but exacerbates liver cell damage due to an increased accumulation of FFAs [31]. All together these observations suggest a possible protective role for increased hepatic TG synthesis against FFAs mediated cell toxicity.

Cholesterol and NAFLD

Lipodomic analyses of NAFLD have demonstrated that apart from triglycerides, there is also an accumulation of free cholesterol without a similar increase in cholesterol esters in both NAFLD and NASH [32]. The cholesterol-related pro-inflammatory mechanisms involved in vascular damage have also been linked to cholesterol-mediated liver damage in NASH. Along this line multiple and complex alterations occur in the pathways of cholesterol homeostasis in both NAFLD and NASH [33]. Consequently, statn use has been associated with possible protection from hepatic damage and fibrosis in NAFLD [34].

Adiponectin and NAFLD

Adiponectin is a cytokine that is mostly produced by adipocytes being primarily determined by adipocyte size and insulin sensitivity, with larger, insulin-resistant adipocytes being less productive [35,36]. It is a "protective" adipocytokine involved in the regulation of glucose and lipid metabolism, as well as in inflammation inhibiting NF-kB and TNF-alpha production in macrophages. Consistent with these data its serum concentrations are inversely related to obesity and diabetes [37]. Adiponectin levels are inversely related to insulin resistance and are lower in obese subjects and patients with established insulin resistance e.g. in type2 diabetes, NAFLD and NASH and hypertension. Adiponectin levels are elevated in classic chronic inflammatory autoimmmune diseases unrelated to increased adipose tissue such as rheumatoid arthritis, SLE, inflammatory bowel disease (IBD) and type1 DM [38]. Due to the insulin-sensitizing and anti-inflammatory activity of adiponectin its plasma levels are decreased in patients with NAFLD and are associated with fat content [39]. After treatment with thiazolidinediones adiponectin values increase in NASH as a sign of improvement of hepatic steatosis. necro-inflammation and most importantly fibrosis [40].

Leptin and NAFLD

Leptin is a cytokine that is primarily secreted from adipose tissue with a critical role in the regulation of body weight and fat mass. In obese mice, leptin causes weight loss, increasing energy expenditure and fatty acid oxidation, reducing appetite and TG synthesis and counteracting the lipogenic action of insulin [41]. Its role in humans is less clear-cut. Only patients with lipodystrophy have a benificial effect when treated with leptin, while obese subjects do not lose weight. Circulating leptin is strongly associated with both subcutaneous and visceral fat and different studies have hypothesized that obesity might induce a state of leptin resistance [42]. High leptin levels are associated with reduced insulin secretion, increased gluconeogenesis and reduced glucose uptake, leading to hyperglycaemia and ultimately contributing to insulin resistance [43-45]. Leptin may negatively affect the cardiovascular system by exerting potential atherogenic, thrombotic and angiogenic activities, as well leading to cardiac hypertrophy [46].

Leptin may exert pro-inflammatory activity by the impairment of NO-related vascular relaxation via increased oxidative stress and by increased endothelin expression [37,47]. Leptin potentiates the effect of angiotensin-2 which in turn increases leptin synthesis by inducing pro-inflammatory cytokines (e.g.TNF-alpha, IL-6 and MCP1 receptor), by increasing the expression of adhesion molecules (e.g. VCAM1, ICAM1 and E-selectin). These features could explain why hyperleptinemia is observed in many chronic inflammatory

states such as atherosclerosis and how it can participate in damage [48,49]. A recent meta=analysis indicates that circulating leptin levels are higher in patients with NAFLD than in controls and higher serum leptin levels were associated with an increased severity of NAFLD [50].

Insulin and NAFLD

Insulin promotes de novo lipogenesis (DNL) and glyceroneogenesis [30]. Both pathways are increased in NAFLD, even in non-diabetic patients, contributing to the synthesis of hepatic TGs and the promotion of hepatic steatosis [51]. In addition, patients with NAFLD have increased hepatic synthesis of palmitate through DNL, and this increases the risk of lipotoxicity and cell damage [30,52]. Finally, insulin in the context of insulin resistance, prompts fibrogenesis by stellate cells [53,54]. Most patients with NAFLD have normal fasting glucose levels but high levels of fasting insulin and high hepatic insulin resistance. Thus it is not surprising that NAFLD is a major risk factor for the development of type2 diabetes.

Glucagon and NAFLD

Since glucagon stimulates lipolysis and reduces lipogenesis, glucagon was proposed as a therapy option for hepatic steatosis [55,56]. Similarily, it was thought the reduction of glucagon signalling i.e. via the use of glucagon receptor antagonists, might lead to the accumulation of lipids in the liver [57,58]. However, more recent studies have shown that glucagon receptor knockout mice have reduced hepatic lipid contents compared with wild-type mice [58]. The impact of glucagon on NAFLD has not been elucidated. Junker *et al.* have shown that patients with NAFLD have fasting hyperglucagonemia, independent of their glucose status [59]. This finding suggests that NAFLD might be involved in the generation of hyerglucagonemia in type2 DM, which is supported by several animal studies [60].

Gut Released Hormones and NAFLD

GLP-1

Glucagon-like peptide(-1(GLP-1) is an incretin produced mainly by the L-cells of the gut in response to food intake. GLP-1 has an important role in the regulation of glucose metabolism, since it potentiates insulin secretion and inhibits glucagon release [61,62]. GLP-1 exerts its effect through binding to GLP-1 receptors, which are mainly expressed in the pancreas and brain, but also in the heart, liver, colon and kidney [61]. Other effects of GLP-1 include the control suppression of appetite and the induction of satiety by delaying gastric emptying [61,63]. Other than these classic activities, GLP-1 seems to be able to modulate the function of different key organs by interacting with GLP-1 receptors present in the lung, stomach, liver, colon, kidney and heart. Consistent with these data, growing evidence suggests a direct protective effect of GLP-1 on the cardiovascular system [61,63]. In human livers of subjects with NASH both the expression and protein content of the GLP-1 receptor are decreased compared to subjects without NASH [64,65]. In subjects with hepatic steatosis, open-label studies have shown that exenatide may improve liver enzymes and decrease steatosis when assessed by magnetic resonance spectroscopy and even improving histology [66-68]. A recent study by Armstrong *et al.* (LEAN study) has shown that after 48 months of double-blind treatment with liraglutide versus placebo 39% of patients receiving liraglutide vs 9% of those receiving placebo had a resolution of nonalcoholic steatohepatitis with no worsening in fibrosis [69]. Among the mechanisms that

lead to the improvement in liver histology were significant weight loss, reduced FFA flux to the liver, reduced hepatic DNL and anti-inflammatory activities [70]. All together these findings qualify the GLP-1 receptor agonists as a potential candidate for the treatment of NAFLD.

Ghrelin and NAFLD

Ghrelin is a hormone that is mainly derived from the stomach and duodenum, with a key role in growth hormone release and in food intake control by inducing appetite and controlling energy expenditure [71]. Ghrelin exerts anti-inflammatory activity by reducing the production of anti-inflammatory cytokines such as IL-1, IL-6 and TNF-alpha, via suppression of NF-kB [72]. The anti -inflammatory properties of ghrelin are consistent with the evidence from murine models that ghrelin prevents diabetes and has a protective cardiovascular effect [72]. These anti-inflammatory properties prompt ghrelin as a promising new target for the treatment of NASH [72].

Whether ghrelin levels are altered in NAFLD is still controversial because several investigators found high as well as low levels of ghrelin in NAFLD compared to controls [73,74]. However, the effects of ghrelin on energy and lipid metabolism, insulin resistance, inflammation and apoptotic cell death, which are common to both obesity and NAFLD, highly suggests it to interplay with NAFLD and NASH'pathogenesis [75].

Muscle Released Compounds and NAFLD

Irisin

Irisin is a recently discovered myokine encoded by the FNDC5 gene, it is implicated in the regulation of energy homeostasis and metabolism and the interaction between skeletal muscle and other tissues. Irisin can induce the differentiation of white adipose into brown adipocytes, along with upregulation of uncoupling protein 1(UCP1) expression and an increase in heat production [76,77]. Accordingly, circulating irisin can increase total energy expenditure, thus reducing obesity and insulin resistance [76,77]. Lower irisin levels were associated with higher hepatic TG content [81]. However, in a recent study by Polyzos et al. irisin levels were slightly higher in patients with NAFLD and significantly higher in patients with portal inflammation [82]. Contrasting data on higher or lower serum irisin levels could be mostly due to the inaccuracy and lack of standardization of commercially available ELISA assays. Mechanisms underlying the protective metabolic effect are not well understood and seem mostly related to higher energy expenditure and not to anti-inflammatory activities, such as NF-kB inactivation [78-80].

Liver Released Compounds in NAFLD

Selenoprotein P

Selenoprotein P, (SeP, encoded by SEPP1 in humans) is a secretory protein produced mainly by the liver that functions as a selenium transporter from the liver to the rest of the body [83,84]. SeP functions as a hepatokine that contributes to insulin resistance in type2 diabetes [83]. Importantly,the RNA interference-mediated knockdown of SeP improves insulin resistance and hyperglycemia in a mouse model of type 2 diabetes,suggesting the suppression of SeP in the liver [85,86]. SeP was found to be increased in NAFLD

patients [85-89]. However, the role of SeP in NAFLD remains to be ellucidated, despite its ability to modulate inflammatory response and insulin resistance. In addition, different evidence suggests that metformin improves systemic insulin sensitivity through the regulation of SeP production, suggesting a novel potential therapeutic approach to treating type 2 diabetes [90].

Fetuin-A and NAFLD

Fetuin-A is a glycoprotein principally produced in the liver and adipose tissue. Fetuin- A is a hepatokine and works as a natural inhibitor of insulin receptors in the liver and skeletal muscle, Serum Fetuin-A levels have been shown to correlate with the metabolic syndrome [91-93]. Increased Fetuin-A has been reported in obese children and lean adults with NAFLD. In patients with NAFLD, Fetuin-A leves were associated with the severity of steatosis. There was no correlation observed between hepatic inflammation and serum Fetuin-A levels in patients with NAFLD. Fetuin-A could affect NAFLD/NASH because it is implicated in the development of insulin resistance and accelerated atherogenesis associated with fatty liver [94-97].

Course and Treatment of NAFLD

Natural history and outcomes

Over the past two decades' studies have reported the natural history of patients with NAFLD [98]. There is growing evidence that patients with histological NASH, especially those with some degree of fibrosis, are at higher risk for adverse outcomes such as cirrhosis and liver-related mortality [98]. These studies showed also:

-Patients with NAFLD have increased overall mortality compared to matched control populations without NAFLD [99,100].

-The most common cause of death in patients with NAFLD is cardiovascular disease (CVD), independently of other metabolic conditions.

-Although liver-related mortality is the 12th leading cause of death in the general population, it is the second or third cause of death in subjects with NAFLD [101].

-Cancer-related mortality is among the top 3 causes of death in subjects with NAFLD [102].

-Patients with histological NASH have an increased liver mortality rate [102,103].

-In a recent meta-analysis, liver specific and overall mortality rates among NAFLD and NASH were determined to be 0,77 per 1000(range 0,33-1,77) and 11,77 per 1000 person years (range7,10-19,53) and15,44 per 1000(range 11,72-20,34) and 25,56 per 1000 person years (range 6,29-103,80), respectively [104].

-The most important histological feature associated with long-term mortality is fibrosis: specifically, zone3 sinusoidal fibrosis plus periportal fibrosis (stage2), progressing to advanced bridging fibrosis (stage3) or cirrhosis, stage4. These are independent predictors of liver-related mortality [105,106].

-NAFLD is now considered the third most common cause of hepatocellular carcinoma (HCC) in the U.S. Given the growing epidemic of obesity the incidence of NAFLD-related HCC has been shown to increase at a 9% annual rate [107,108].

-It is important to recognize that most patients with cryptogenic cirrhosis may have what is considered "burned out" NAFLD [109,110].

Treatment of NAFLD

Lifestyle interventions

Lifestyle modifications consisting of diet, exercise and weight loss have been advocated to treat patients with NAFLD. Weight loss generally reduces hepatic steatosis, achieved either by hypocaloric diet alone or in conjunction with increased physical activity. A combination of a hypocaloric diet (daily reduction by 500-1000 kcal) and moderate intensity exercise is likely to provide the best likelihood of sustaining weight loss over time [98].

Weight loss of at least 3% to 5% of bodyweight appears necessary to improve steatosis, but a greater weight loss (7%-10%) is needed to improve the majority of the histopathologic features of NASH, including fibrosis [98].

Exercise alone in adults with NAFLD may prevent or reduce HS, but its ability to improve other aspects of liver histology remains unknown [98].

Pharmacotherapy of NAFLD

Pharmacological treatments aimed primarily at improving liver disease should generally be limited to those with biopsy-proven NASH and fibrosis [98].

Insulin Sensitizers

Metformin

Although several studies have shown an improvement in serum aminotransferases and insulin resistance, metformin does not signifcantly improve liver histology. Two publshed meta-analyses conclude that metformin therapy did not mprove liver histology in patients with NAFLD and NASH [111,112].

Thiazolidinediones

Proglitazone improves liver histology in patients with and without type2 DM with biopsy-proven NASH. Therefore,it may be used to treat these patients. Until further data supports its safety and efficacy pioglitazone should not be used to treat NAFLD without biosy-proven NASH [98].

SGLT2-Inhibitors

Recently, empagliflozin showed benefits in a small, uncontrooled trial (n=50; sE-Lift trial) in reducing fat and liver enzymes. Its role in the treatment of NASH/NAFLD has yet to be determined.

GLP-1 Agonists

There has been an interest in investigating the role of GLP-1 agonists as therapeutic agents in patients with NAFLD and NASH, as described above. In a recently published randomized trial consisting of 52 patients with biopsy-proven NASH, liraglutide administered subcutaneously once daily for 48 weeks was associated with greater resolution of SH and less progression of fibrosis [69]. However, it is premature to consider GLP-1 agonists to specifically treat liver disease in patients with NAFLD or NASH [98].

Vitamin E

Oxidative stress is considered a key mechanism of hepatocellular injury and disease progression in subjects with NASH. Vitamin E is an antioxidant and has been investigated as a treatment in NASH. Vitamin E administered at a daily dose of 800 IU/day improves liver histology in nondiabetic adults with biopsy-proven NASH and may be considered for this patient population. Until further data supporting its effectiveness become available. Vitamin E is not recommended to treat NASH in diabetic patients, NAFLD without liver biopsy, NASH, cirrhosis or cryptogenic cirrhosis [98].

Bariatric surgery

Foregut bariatric surgery can be considered in otherwise obese eligible individuals with NAFLD or NASH. It is premature to consider foregut bariatric surgery as an established option to specifically treat NASH. The type, safety and efficacy of foregut bariatric surgery in otherwise eligible obese individuals with established cirrhosis attributed to NAFLD are not established. In otherwise eligible patients with compensated NASH or cryptogenic cirrhosis, foregut bariatric surgery may be considered on a case-by-case basis by an experienced bariatric surgery program [98].

Conclusion

The last two decades brought a great deal of new insights into the complex interplay of mechanisms and mediators of fatty liver disease (NAFLD). Genomic, meta-genomic and metabolic profiling technologies are well suited for the study of metabolic syndrome and NAFLD. Redefining risks and prognosis, as well as identifying new diagnostic criteria, are needed. The role of new bio-markers of disease progression has to be settled. New endpoints of clinical trials, which are until now scarce, have to be defined. Lifestyle modification by diet and exercise is now the first therapeutic option. Pharmacotherapy and bariatric surgery have yielded prudent preliminary hopeful results.

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