ADIPOSE TISSUE, METABOLIC SYNDROME, AND NON-ALCOHOLIC FATTY LIVER DISEASE - A SHORT REVIEW

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ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease globally, and it is expected to rise even further as a result of the increase in obesity and related risk factors. This short review summarises current evidence on the role of adipose tissue and insulin resistance in NAFLD and the interrelationship between NAFLD and the metabolic syndrome (MetS), considering central adiposity is a major feature of both the MetS and NAFLD, and that NAFLD has been previously described as the hepatic manifestation of the MetS. In addition, genetic studies of NAFLD with relation to adiposity and insulin resistance are reviewed, and up-to-date diagnostic and therapeutic tools are also discussed.

Keywords: NAFLD, adipose tissue, insulin resistance, metabolic syndrome, genetics of NAFLD.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is considered the most common chronic liver disease worldwide, covering a wide range of diseases from simple steatosis to non-alcoholic steatohepatitis (NASH), with and without fibrosis.[1,2] The liver cell damage that is observed in patients with NASH can lead to cirrhosis or even end-stage liver disease.[3,4] However, estimates of the magnitude of progression to cirrhosis and subsequent transplantations for NASH-related cirrhosis have not been well established.[5,6] In the US, NASH accounted for around 5.5% of the total liver transplants over the period 2001-2009, as either the primary or the secondary indication, with the latter including mostly cryptogenic cirrhosis cases.[6]

NAFLD has been linked to obesity, dyslipidaemia, diabetes mellitus, and hypertension-associated cardiovascular disease, while it also contributes to the overall morbidity and mortality of the latter.[1,2,7] As indicated by its name, the disease is diagnosed in patients who do not have a history of alcohol abuse and have an excessive accumulation of fat in their liver parenchyma.[7] The histopathological diagnosis of NAFLD is actually very similar to alcoholic liver disease, making the diagnosis of NAFLD challenging since differentiation is based on self-reported alcohol consumption.[8]

Normally, only a small amount of fat from dietary fat and carbohydrates is stored in the liver. Lipolysis (in adipose tissue) and *de novo* hepatic lipogenesis also contribute to the stored mass of hepatic fat but counteracting fat removal processes keep this mass low. However in cases of prolonged excess energy supply, where adipose tissue cannot store more free fatty acids, the liver becomes the site of storage, thus resulting in higher fat accumulation.[9] Additionally, changes in the rate of fat synthesis and fat removal by oxidation of fatty acids or by secretion of triglyceride-rich lipoproteins from the liver also contribute to this imbalance.[2,7] In cases of over-nutrition, obesity, and insulin resistance, the balance between fat accumulation and fat disposal is disturbed, resulting in a fatty liver.[2] This excess accumulation of fat in adipose tissue and in the liver induces inflammation and metabolic stress,[9] highlighting

the central role of adiposity in the pathology of NAFLD.

ADIPOSE TISSUE AND ITS ROLE IN NAFLD

Adipose tissue consists of brown and white adipose tissue.[10] Brown tissue (BAT) has a metabolic and a thermogenic role and is more prominent in neonates and infants; however, evidence suggests that it also persists in adulthood, controls energy expenditure, thus protecting against adiposity,[11-12] and exhibits an inverse relationship with Body Mass Index (BMI).[13] Yilmaz et al.[14] have also reported that a reduction in BAT is also associated with an increased risk of NAFLD. The suggested explanations for this finding concentrate on the ability of BAT to reduce hyperlipidaemia[15] and on its thermogenic role, which is mediated by the actions of uncoupling protein 1 (UCP1).[16] On the other hand, white adipose tissue (WAT), which makes up most of the adipose tissue in adulthood, has been better studied and has been shown to be the major site of energy surplus storage in the form of triglycerides that contain intracellular droplets.[17]

In the last two decades, especially after the discovery of the first adipokine - leptin - a lot of attention has been focused towards the dual role of adipose tissue as an endocrine organ, in addition to energy storage. WAT is involved in metabolic activities by releasing adipokines, which are hormone-like proteins, thus promoting adipocyte differentiation and contributing to energy homeostasis and insulin sensitivity. In addition, it releases inflammatory cytokines and anti-inflammatory factors which contribute to the inflammatory processes.[10]

Adiponectin is the most important adipokine, secreted from adipose tissue, found to be associated with insulin resistance. More specifically, decreased levels of adiponectin activity have been associated with obesity or complications of obesity, including insulin resistance and diabetes, as well as with NAFLD.[10,18] It acts through binding to one of its three receptors (AdipoR1, AdipoR2 and T-cadherin), resulting in the activation of a cascade that eventually leads to glucose uptake, decreased entry of free fatty acids into the liver, increased free fatty acid oxidation, and prevention of insulin resistance and inflammation. Expression of adiponectin is inhibited by inflammatory factors secreted by adipose tissue, including tumour necrosis factor alpha (TNF-α) and interleukins IL-6 and IL-18, as well as by oxidative stress and a high fat diet, that act through different signalling pathways.[18] Adiponectin exhibits its effect on insulin resistance mainly through the sensitisation of insulin. Clinical evidence suggests that in people with type 2 diabetes, adiponectin levels are lower than normal,[19] while in patients with NASH, adiponectin was found to be significantly lower compared to a group of patients with simple steatosis.[20]

In addition to adiponectin's central role in insulin sensitivity, leptin and resistin have also been linked to insulin resistance.[21] Leptin controls the pancreatic islet cells, and its levels are usually proportional to insulin levels. However, when there is a decreased signalling of leptin, hyperinsulinaemia may be observed.[10] Resistin has also been linked to insulin resistance (owing its name to that), however, despite experimental evidence in mice where resistin has been shown to be involved in the pathogenesis of diabetes, its role in humans remains unclear. Other adipokines that are proportionally linked to insulin resistance include: apelin, which is raised when insulin levels are elevated, and also in the presence of obesity, in order to regulate insulin resistance by altering the concentration of adiponectin; and visfatin, which is secreted by lymphocytes in adipose tissue and lowers resistance to insulin.[10]

INSULIN RESISTANCE AND MITOCHONDRIAL DYSFUNCTION IN THE LIVER AND MICROBIOTA

Evidence suggests that the liver becomes insulin resistant after steatosis (fatty liver).[21-23] In a fatty liver, insulin can no longer inhibit the production of glucose, thus a high concentration of glucose is produced and released in the plasma (hyperglycaemia) triggering hyperinsulinaemia. At the same time, there is a higher production of very low-density lipoprotein (VLDL) cholesterol, followed by a decreased high-density lipoprotein (HDL) concentration.[21,23] If hyperinsulinaemia persists, then the pancreatic beta cells could be damaged, eventually leading to the development of type 2 diabetes.[24] Contrary to this, it has also been argued that steatosis and subsequent NAFLD are a consequence of insulin resistance. Insulin resistance enhances lipolysis in adipose tissue and stimulates the uptake of free fatty acids by hepatocytes and their accumulation in the liver.[2,7] Moreover, insulin

resistance is accompanied by hyperinsulinaemia, which in turn activates the formation of fatty acids in the hepatocytes by *de novo* lipogenesis and the deposition of triglycerides inside these cells, in a vicious cycle.

Mitochondrial dysfunction is another important characteristic observed in NAFLD.[7] More specifically, the over-production of reactive oxygen species (ROS) by adipocytes, due to hyperglycaemia or excessive energy intake, exerts an overload on the mitochondria, resulting in insulin resistance accompanied by cell damage and mutations in the mitochondrial DNA (mtDNA), as well as a reduction in adipocyte's oxygen consumption. This in turn prevents the oxidation of free fatty acids, thereby enhancing lipid accumulation.[17] Lipid peroxidation, cytokine induction, chemoattraction of inflammatory cells, activation of hepatic stellate cells, and fibrinogenesis are also outcomes of the mitochondrial ROS overload.[21]

In addition to insulin resistance and mitochondrial dysfunction, in the last few years there has been an emerging interest regarding the role of intestinal microbiota in the metabolism of the host,[23] especially with regards to obesity and the metabolic syndrome (MetS). Experimental evidence from mice has shown a change in the gut microbiota when fed a high-fat diet; these alterations triggered the release of pathogen or damage-associated molecular patterns, which increased the intestinal permeability and activated innate immune responses causing inflammation and severe fibrosis.[1,22] The causal association between microbiota composition and development of obesity was established experimentally in mice, by transplanting faecal microbiota from lean and obese mice in germ-free mice.[25] Mice which received the microbiota from the obese mice had a greater fat mass increase. Similar studies have been extended to humans. In a double-blinded, randomised, controlled trial by Vrieze et al.[26] patients with MetS who had received faecal material from lean donors showed a decrease in their fasting triglycerides and improvement in peripheral and hepatic insulin sensitivity.[27] Additional studies have linked gut microbiota with intestinal permeability, and have also linked NASH[15] and microbiota contribution to the lipid metabolism in the liver and development of NAFLD, independent of obesity.[28] Although evidence from human studies is promising, it is inconclusive and clinical trials on the use of probiotics are still needed.

THE METABOLIC SYNDROME AND NAFLD

Development of NAFLD results from the interaction between environmental factors - including obesity, a high calorie diet, and sedentary lifestyle - and genetic predisposition through liver crosstalk with gut, adipose tissue, and the pancreas.[1] Despite being the most common liver disease, the underlying mechanisms of its pathogenesis are not well understood, however it has been suggested that there is an association with insulin resistance and mitochondrial dysfunction.[7] The temporal pathway is still unclear so it is not known whether insulin resistance precedes NAFLD or is just an epiphenomenon. Since NAFLD has previously been associated with several features of MetS including obesity, type 2 diabetes, atherogenic dyslipidaemia, and hypertension, and is characterised by insulin resistance, it has been suggested that this disease may actually be the hepatic manifestation of MetS.[29] Epidemiological data from European populations show that the prevalence of NAFLD among people with type 2 diabetes ranges between 42.6-69.5%, underlining the association between the two diseases.[30]

MetS describes a spectrum of disorders that may contribute to visceral obesity, insulin resistance, hyperglycaemia, dyslipidaemia, and hypertension.[1,10] There has been some evidence that NAFLD promotes the development of MetS and of type 2 diabetes in predisposed individuals[22] - although whether one precedes the other is not yet quite clear - as well as an increased prevalence of MetS in patients with NAFLD, varying with their obesity levels (18-67%).[21,31] Despite its association with MetS and type 2 diabetes, NAFLD can also be observed in non-obese and non-diabetics, thus, it should be referred to as a manifestation of MetS, independent of obesity and plasma glucose.[32]

In obese people, adipocytes, activated macrophages, and Kupffer cells secrete TNF-α, which interacts with its receptors causing systemic and hepatic inflammation.[33] This proinflammatory cytokine (TNF-α), in turn, targets the adipocytes and causes a reduction in adiponectin and an increase in leptin levels. Furthermore, the increase in TNF-α and IL-6, and the subsequent decrease in adiponectin levels, is responsible for insulin resistance in the muscles and the liver.[10,33] However, inflammation and insulin resistance do not always develop in obese people, as every individual has a different capability of

expanding its adipose tissue, thus these effects will only be experienced by the ones that cannot store the excess fat in their adipose tissue.[17]

Regardless of the effects of adipose tissue and MetS on liver histology, several prescribed drugs used for treatment or prevention of other disorders have also been associated with increased risk of NAFLD, especially in people with MetS. More specifically, Tamoxifen, used for the treatment of oestrogen-receptor positive breast cancer, has been associated with increased risk of NAFLD in women MetS,[34] as well as with increased hepatotoxicity in women with pre-existing liver steatosis and breast cancer, regardless of their BMI.[35] Other listed medications that exhibit a similar hepatotoxic effect include antidepressants[36] and corticosteroids.[37] All of the above may do so by exacerbating MetS components like insulin resistance, central obesity, and hypertriglyceridemia.[37] Polypharmacy and/or drug accumulation may also contribute, and careful observation after drug administration is needed to prevent serious liver injury.

GENETICS AND NAFLD

Nutrition and lifestyle choices have been known to affect an individual's probability of developing NAFLD. However, since the first small family studies indicated the potential of familial clustering of NAFLD cases,[38,39] a significant amount of evidence has accumulated, highlighting the importance of the genetic component of the disease as well. Variations of NAFLD in different ethnic populations have also suggested the probability of genetic susceptibility;[40] however, some of those differences could also be explained by the difficulty of actually diagnosing NAFLD without a conclusive liver biopsy. While this may be true, in the controlled setting of the US SCALE study, African Americans had a lower risk of presenting with NAFLD compared to Hispanics, who appeared to be the most susceptible.[40] Subsequent reports by the same group also focused on the increased risk for siblings (59%) and for parents (78%) of probands compared to siblings and parents of non-probands (17% and 37%, respectively).[41] The genetics of NAFLD are therefore quite complex, presenting with a polygenic pattern of susceptibility and with genetic factors participating in many metabolic pathways, including lipid and glucose metabolism, oxidative stress, immune response, and apoptosis.[42] Here we have focused on genetic studies and methods that have looked at the association between adiposity/MetS and NAFLD.

Older studies had shown an association between simple genetic variants with NAFLD, but findings about a common genetic ground between visceral adiposity and liver fat have not been consistent.[43-45] More recently, Speliotes et al.[46] confirmed a previously described genetic association with the PNPLA3 gene, and have identified additional genetic variants (single-nucleotide polymorphisms [SNPs]) in genes associated with both computed tomography (CT) hepatic steatosis and histologically proven NAFLD (rs2228603-NCAN, rs12137855-LYPLAL1, rs780094-GCKR). Among them only the NCAN and GCKR genes were associated with metabolic traits like serum and liver lipids and also glucose traits. Adding to previous reports,[45] these findings suggest that the pathophysiology of NAFLD may result from distinct abnormalities, visceral adiposity and glucose resistance being considered as two of them.

Another important pathway that has been implicated with progression of the disease is the combination of variants of ENPP1/PC-1 membrane protein (ectoenzyme nucleotide pyrophosphate phosphodiesterase 1/plasma cell antigen) and variants of the IRS-1 (insulin receptor substrate-1), which affects insulin receptor signalling in the liver. The Lys121Gln polymorphism in the ENPP1/PC1 combined with the Gly972Arg variant in the IRS-1 genes have been recently and significantly associated both with the features of MetS and with a more progressive state of NAFLD, independently of confounding factors.[47]

Another set of evidence that supports the presence of a common genetic ground between adiposity/MetS and NAFLD comes from studies that had focused on adiponectin, secreted by adipose tissue. Via its receptor, AdipoR2, which is expressed primarily in the liver, adiponectin increases free fatty acid oxidation and inhibits insulin resistance, while it has also been reported to suppress fibrosis and inflammation.[18,48] Genetic variants in the adiponectin gene have been found to be more prevalent in cases of severe fibrosis compared to milder manifestations of NAFLD (+45T>G) and also in patients versus controls (+276G>C and -11377G>C).[49,50] Moreover, genetic polymorphisms that affect adiponectin levels have already been hypothesised to be implicated in the variability seen in the NAFLD phenotype.[51]

Some examples include the UCP1 -3826 A>G polymorphism, which has been associated with severe hepatic steatosis even in the absence of MetS,[52] and the 161C>T polymorphism in the peroxisome proliferator-activated receptor-gamma (PPAR-γ) gene, which has been reported to be associated with NAFLD status in a case control study in a Chinese population.[53] More recently, additional SNPs that have been previously implicated in the adiponectin pathway have been examined for association with NAFLD as well, with reports that a combination of the APPL1-C/APPL2-A alleles significantly increased the risk of NAFLD (OR=2.50; 95% CI:1.45 to 4.32) as well as the probability of severe steatosis compared to the major allele combination (OR=3.88; 95% CI:1.582 to 9.531).[54] A meta-analysis of eight case-control studies, however, failed to confirm an association between the PPAR-γ2 Pro12Ala polymorphism and NAFLD.[55]

DIAGNOSTIC TOOLS AND TREATMENT OPTIONS FOR NAFLD

An important first step in the diagnosis of NAFLD is the exclusion of significant consumption of alcohol, with a consensus threshold of <21 drinks per week for men and <14 drinks per week for women (≈2 years before the examination) being used.[56] Along with absence of other causes of chronic liver disease like the use of steatogenic medication, hereditary disorders, Wilson disease, severe malnutrition, and Hepatitis C, NAFLD can be diagnosed using histology or imaging for the quantification of hepatic steatosis.[57] Histology assessment following invasive, expensive, and sometimes serious complication-inducing liver biopsy is considered the only definite test, but several non-invasive diagnostic methods have also been developed for NAFLD. These include plasma liver aminotransferase measurements, ultrasound imaging (US), CT, magnetic resonance imaging (MRI), and transient elastography, as well as plasma cytokeratin-18 fragment levels.[58]

In a recent review, Festi et al.[59] highlighted the strengths and weaknesses of the diagnostic methods that are currently in practice and proposed an algorithm of non-invasive tests that could facilitate physicians and improve diagnostic accuracy while eliminating the need for liver biopsy. US was confirmed as the most appropriate screening method, and although already widely adopted,[60] it is still being evolved; the most recent example is the development of the Controlled Attenuation Parameter (CAP) application, which is based on ultrasound attenuation by liver fat measured by FibroScan® (Echosens, France).[61] Early reports regarding the validity of this method compared to the 'gold standard' liver biopsy, highlight its ability to assess steatosis in a simple fashion and in an operator-independent manner.[62,63] In a similar fashion, liver stiffness measurement (LSM) has been used to evaluate the stage of fibrosis[64] but, mainly due to its high negative predictive value, LSM has been proposed as a tool that could cost-effectively exclude fibrosis and cirrhosis of the liver.[65]

Imaging techniques can always be coupled with biochemical markers, while approaches like cytokeratin-18 plasma levels, CT, and MRI can be utilised in case of conflicting results.[59] However, the challenge lies in the quantification of fibrosis, and a number of biomarker combinations have been examined towards this goal, including the officially recommended[57] NAFLD fibrosis score (NFS),[66] the FibroTest,[67] the Original European Liver Fibrosis (OELF) panel,[68] and the FibroMeter.[69] Each of them has proven to be quite useful;[59,70] however, comparisons between them are not easy since their results are based on heterogeneous populations.[70]

Some novel non-invasive approaches include the use of an oral chlorine tolerance test and the utilisation of the terminal peptide of procollagen III (PIIINP). Using the oral chlorine tolerance test, it was shown that fasting plasma free choline (fCh) levels with a threshold value of 0.16 mg/dL could lead to the early detection of NASH patients with a sensitivity of 80.1% and a specificity of 82.6%,[71] while the terminal peptide of procollagen III was able to distinguish patients with advanced fibrosis.[72] Furthermore, a recent report on cell death biomarkers used in NAFLD diagnostics stated that both caspase-cleaved and uncleaved cytokeratin-18 performed better in diagnosing lower levels of fibrosis compared to only caspase-cleaved cytokeratin-18 fragments,[73] while a separate group in Germany has recently developed and validated a cost-effective fibrosis scoring system called Koeln-Essen-index (NIKEI). This alone, or especially when combined in a stepwise mode with the FIB-4 test, could exclude advanced fibrosis in NAFLD patients.[74]

Up-to-date clinical practice for treating NAFLD involves both dietary and physical activity

interventions. Although randomised controlled trials (RCTs) that had assessed the effect of lifestyle interventions on NAFLD were subject to limitations, evidence suggests an overall positive effect for the patients, with such interventions as weight loss being reported to lead to a significant reduction (10-51%) in the percentage of liver fat.[58] In addition, strong evidence suggests that physical activity coupled with diet modification has a significant effect on several parameters of NAFLD, including reduction of aminotransferase and intra-hepatic fat levels, as well as improved insulin sensitivity.[75] A number of recent reviews and meta-analyses have compared the differential effect of the low-carbohydrate diet versus the most widely adopted low-calorie diet on several NAFLD metrics; these reported that waist circumference, which is a proxy of abdominal fat, was significantly reduced by the low carbohydrate diet as opposed to the low-calorie diet.[76-79] The importance of such findings is further highlighted by independent studies reporting a significant relationship of visceral adipose tissue (VAT) with increased risk of fatty liver disease[51,80] as opposed to subcutaneous adipose tissue.[81] Furthermore, of special interest for clinicians and nutritionists worldwide is the effect of the amount of alcohol consumed on the risk of developing NAFLD. Although high alcohol consumption is definitely associated with liver toxicity, a recent meta-analysis reported that moderate alcohol consumption (<40 g/day) is associated with a protective effect, especially in women, and with a 50% reduction in the risk of the disease progressing to more advanced stage (NASH) compared to abstainers.[82]

Overall, a successful lifestyle intervention should not only target VAT and evaluate the amount of alcohol consumed, but should also include a diet with appropriate macronutrients, as recent studies have identified discrepancies between diet composition and improvements in fatty liver disease.[76,83] Long-term clinical trials on the dietary recommendations for NAFLD are still needed.[83]

In patients for which lifestyle intervention has not resulted in long term improvement or stabilisation of the disease, bariatric surgery may be suggested, which would result in a relevant improvement in steatosis, inflammation, and fibrosis.[84] Although there is no pharmaceutical compound specifically administered for NAFLD or NASH, several products are being used or investigated for their effects on several pathways or manifestations of the disease. Insulin sensitisers (like pioglitazone) are well studied, and evidence supports that a significant reduction of insulin resistance, liver inflammation, and fibrosis can be achieved with their use.[85-87] Statins have also been found to reduce the risk of hepatic steatosis,[88] while antioxidants like vitamin E, a relatively cheap choice, can also be recommended and have been shown to be comparable to pioglitazone in a comparative study.[89] Low levels of such antioxidants have been related to the development of the disease,[90] however a well-designed study failed to show a statistically significant effect.[91] Future studies should concentrate on revealing the true nature of antioxidants on NAFLD progression and also evaluate the potential health risks from increased doses of such synthetic compounds, as recent evidence, although weak, suggests the possibility of vitamin E being positively associated with oncogenesis.[92]

On the other hand, obeticholic acid, a semi-synthetic farnesoid X receptor (FXR) agonist, recently under investigation by Intercept Pharmaceuticals (double-blind, placebo-controlled FLINT trial) for its potential effect on NAFLD, appears to be a promising candidate for the treatment of nonalcoholic steatohepatitis. Although relevant publications on the FLINT trial are not yet available, the company has officially announced that the trial has been stopped as interim results have confirmed the efficacy of the compound (significant reduction in the NAFLD Activity Score of at least two points in the treated group compared to the placebo group). A number of animal studies have also demonstrated the positive effect of obeticholic acid on liver histology, with reported reductions in profibrotic growth factors, liver inflammation, and oxidative stress.[93] The only other human study looking at obeticholic acid also reported significant improvements in insulin sensitivity and reduction in markers of liver fibrosis.[94] An antihyperglycaemic agent, metformin, is another well studied candidate for NAFLD pharmaceutical intervention as animal[95,96] and human studies[97-99] indicated its safety and effectiveness in treating NAFLD. However, small sample sizes and conflicting results from other studies[100] highlight the need for larger and well planned human studies.

Central adiposity is a major feature of both MetS and NAFLD, and NAFLD has been described as

the hepatic manifestation of MetS, while in patients with both NAFLD and type 2 diabetes, rapid progression of both diseases, accompanied by an increased number of complications, are the end result. Current research on pharmaceutical agents for NAFLD seems promising, with new compounds expected to be available soon. However, given the worldwide rise in obesity prevalence, an ensuing rise in the prevalence of MetS and NAFLD is to be expected, with a relevant increase in related morbidity and mortality. This further highlights the importance of both primary and secondary lifestyle modifications in reducing the incidence of NAFLD and managing its symptoms.

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