Review

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Pathogenesis of non-alcoholic fatty liver disease

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Introduction

Non-alcoholic fatty liver disease (NAFLD) represents a spectrum of disease ranging from hepatocellular steatosis through steatohepatitis to fibrosis and irreversible cirrhosis. The prevalence of NAFLD has risen rapidly in parallel with the dramatic rise in obesity and diabetes,[1,2] and is rapidly becoming the most common cause of liver disease in Western countries.[3] Indeed, NAFLD is now recognized to be the aetiology in many cases previously labelled as cryptogenic cirrhosis.[4]

In Western populations, estimates of NAFLD prevalence vary between 20 and 30%,[5,6] rising up to 90% in morbidly obese individuals.[7] The more severe, and clinically significant form of NAFLD, non-alcoholic steatohepatitis (NASH) is less common, affecting an estimated 2–3% of the general population,[8] and up to 37% of the morbidly obese.[7] Of particular concern, and with significant implications for future disease burden, is the increasing prevalence of NAFLD in children and young adults. Studies have reported a 3% prevalence of NAFLD in the general paediatric population, rising to 53% in obese children.[9,10] NAFLD has a strong association with type 2 diabetes, with steatosis present in 70% of type 2 diabetics screened with ultrasound,[11] and thus it is now recognized to represent the hepatic manifestation of the metabolic syndrome.

NAFLD occurs in all ethnic groups although it appears to have a lower prevalence in African-Americans compared with Hispanic and European Americans. This difference remains even after controlling for obesity and insulin resistance (IR)[5,12] and may be related to ethnic differences in lipid homeostasis.[5]

There are no laboratory, imaging or histological findings which can accurately distinguish between NAFLD and alcohol-induced steatosis or steatohepatitis, and the diagnosis can therefore only be made in the absence of a history of significant alcohol intake. Other specific causes of steatosis need to be considered and include metabolic disorders e.g. lipodystrophy and abetalipoproteinaemia, nutritional causes such as rapid weight loss, jejuno-ileal bypass and total parenteral nutrition, and drug-induced. Commonly implicated agents include glucocorticoids, methotrexate, amiodarone, synthetic oestrogens, tamoxifen, diltiazem and highly active anti-retroviral drugs.[13–15] Steatosis also commonly occurs in association with hepatitis C, particularly genotype 3, and has an increased prevalence in women with polycystic ovary syndrome, when it is usually associated with IR.[16]

In the great majority of patients NAFLD develops in association with features of IR and the metabolic syndrome. The metabolic syndrome comprises a cluster of clinical and biochemical features, namely IR, glucose intolerance or diabetes, central obesity, hypertension and dyslipidaemia and is

associated with significant cardiovascular morbidity and mortality.[17–19]

Whilst simple steatosis in the absence of significant fibrosis is considered to be a relatively benign condition,[20] the presence of fibrosis predicts both disease progression and liver-related complications over a subsequent 10-year period.[21] Decreased survival in this sub-group is due to predominantly cardiovascular causes, although there is a significant increase in liver-related deaths.[21] NASH also carries an increased risk of hepatocellular carcinoma (HCC)[21] and thus the observation of increased incidence of HCC in type 2 diabetics[22] is likely to be due to their high prevalence of NASH.[21] In a recent US study, NASH was found to account for at least 13% of overall cases of HCC.[23]

There are as yet few proven therapies available for patients with NASH, and current strategies are directed towards improving aspects of the metabolic syndrome. Ultimately when such measures fail, liver transplantation remains the only option for patients with end-stage cirrhosis.

Although the pathogenesis of NAFLD/NASH is not yet fully understood, much progress has been made in recent years in elucidating the mechanisms of progression from steatosis to more advanced liver inflammation and fibrosis. In this review, we discuss the current understanding of NAFLD pathogenesis, and anticipate that such knowledge will eventually translate into the development of novel treatment strategies for this increasingly important disease.

NAFLD pathogenesis

The '2-hit hypothesis'

Initial theories for the pathogenesis of NASH were based on a '2-hit hypothesis' (Figure 1a). The 'first hit', hepatic triglyceride accumulation, or steatosis, increases susceptibility of the liver to injury mediated by 'second hits', such as inflammatory cytokines/adipokines, mitochondrial dysfunction and oxidative stress, which in turn lead to steatohepatitis and/or fibrosis.[24,25] However, there is increasing recognition of the role that free fatty acids (FFA) play in directly promoting liver injury, which has led to modification of this theory (Figure 1b). In obesity and IR there is an increased influx of FFA to the liver. These FFA either undergo β-oxidation or are esterified with glycerol to form triglycerides, leading to hepatic fat accumulation. There is now substantial evidence that FFA can directly cause toxicity by increasing oxidative stress and by activation of inflammatory pathways,[26] therefore hepatic

triglyceride accumulation may be a protective mechanism by preventing the toxic effects of unesterified FFA.[27] Additionally, a further component, or 'third-hit' has been added to reflect inadequate hepatocyte proliferation (Figure 1c).[28] In the healthy liver, cell death stimulates replication of mature hepatocytes which replace the dead cells and reconstitute normal tissue function.[28] However oxidative stress, a central feature of NAFLD pathogenesis, inhibits the replication of mature hepatocytes which results in expansion of the hepatic progenitor cell (oval cell) population.[29] These cells can differentiate into hepatocyte-like cells, and both oval cell and intermediate hepatocyte-like cell numbers are strongly correlated with fibrosis stage, suggesting that cumulative hepatocyte loss promotes both accumulation of progenitor cells and their differentiation towards hepatocytes.[29] Activation of these cells has also been implicated in hepatocellular carcinogenesis.[29] In chronic liver injury, the development of fibrosis/cirrhosis is dependent on the efficacy of hepatocyte regeneration, and therefore cell death with impaired proliferation of hepatocyte progenitors represents the proposed 'third hit' in NAFLD pathogenesis.[28]

Lipid accumulation/steatosis

NAFLD is characterized by the accumulation of triglycerides, which are formed from the esterification of FFA and glycerol within the hepatocyte. FFAs arise in the liver from three distinct sources; lipolysis (the hydrolysis of FFA and glycerol from triglyceride) within adipose tissue, dietary sources, and *de novo* lipogenesis (DNL).[30] In contrast, FFA may be utilized either through β-oxidation, re-esterification to triglycerides and storage as lipid droplets, or packaged and exported as very low density lipoprotein (VLDL). Hence hepatic fat accumulation can occur as a result of increased fat synthesis, increased fat delivery, decreased fat export, and/or decreased fat oxidation (Figure 2).[30]

To establish the relative contribution of lipid accumulation in patients with NAFLD, Donnelly *et al.* used a multiple-stable-isotope method, demonstrating that approximately 60% of liver triglyceride content derived from FFA influx from adipose tissue, 26% from DNL, and 15% from diet.[31] This contrasts with healthy individuals in whom DNL contributes <5% of hepatic triglyceride formation.[32,33]

Triglyceride can also be exported from the liver in VLDL particles, which are formed by the incorporation of triglyceride into apolipoprotein B (apoB) by microsomal transfer protein (MTP).[34] Aberrant alterations of MTP/apoB synthesis and secretion
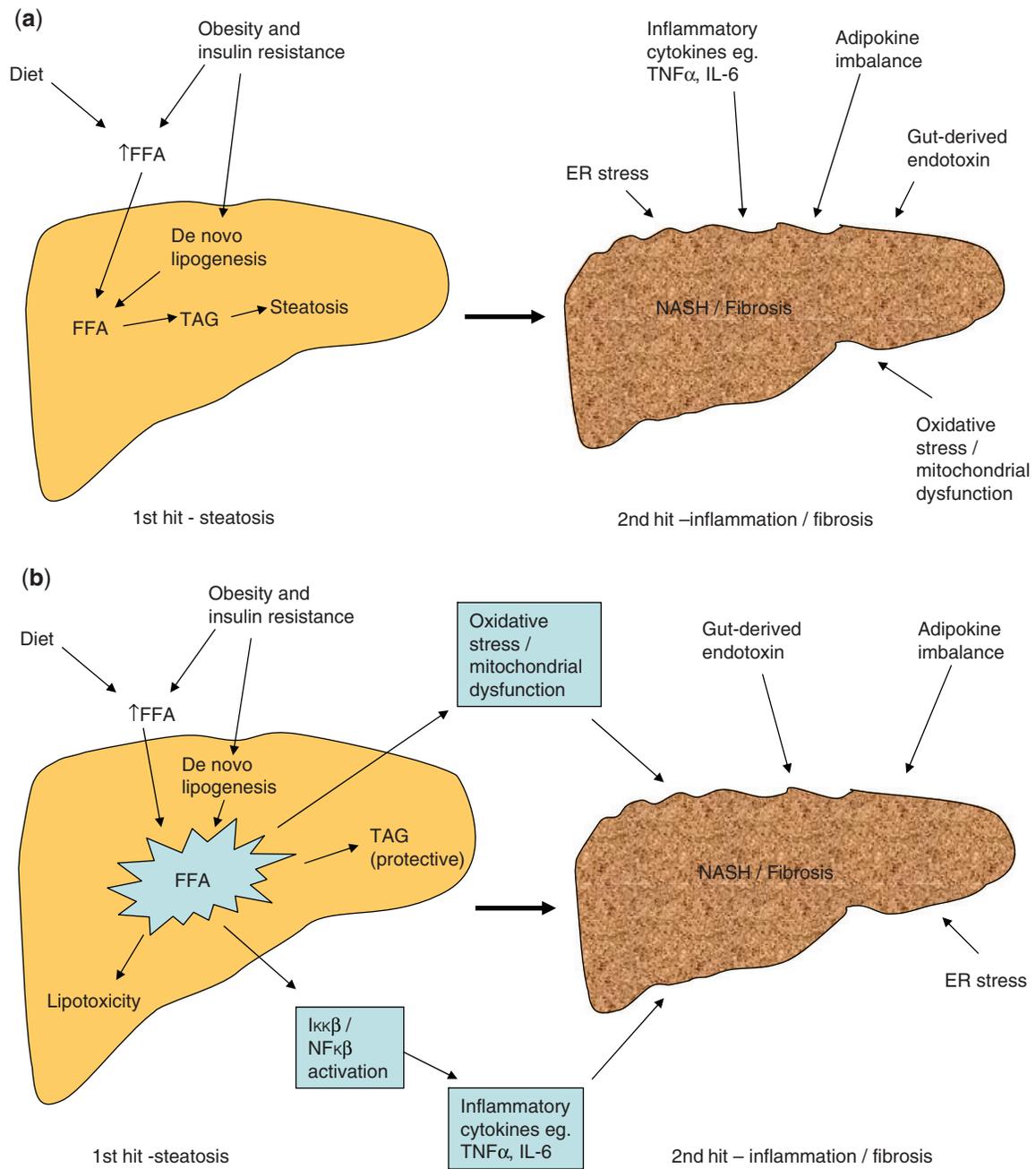
Figure 1. (**a**) The traditional 2-hit hypothesis: steatosis represents the 'first hit', which then sensitises the liver to injury mediated by 'second hits', such as inflammatory cytokines, adipokines, oxidative stress and mitochondrial dysfunction, leading to steatohepatitis and fibrosis. The presence of high levels of oxidative stress reduces the ability of mature hepatocytes to proliferate, resulting in reduced endogenous liver repair. (**b**) Modified 2-hit hypothesis: the accumulation of FFA alone has been suggested to be sufficient to induce liver damage, without recourse for a second hit. Indeed, rather than being harmful, triglyceride accumulation in the form of steatosis may actually be protective by preventing FFA-induced inflammation and oxidative stress. (**c**) The 3-hit hypothesis: oxidative stress reduces the ability of mature hepatocytes to proliferate, resulting in the recruitment of other pathways of liver regeneration, such as HPCs. These cells have the capability of differentiating into both cholangiocytes and hepatocytes and contributing to liver repair. It has been suggested that an inability to mount such a ductular response, as is seen in patients transplanted for NASH who have denervated livers, may be responsible for a more progressive pattern of liver damage. Thus, impaired proliferation of hepatocyte progenitors represents the proposed 'third hit' in NAFLD pathogenesis.[28]
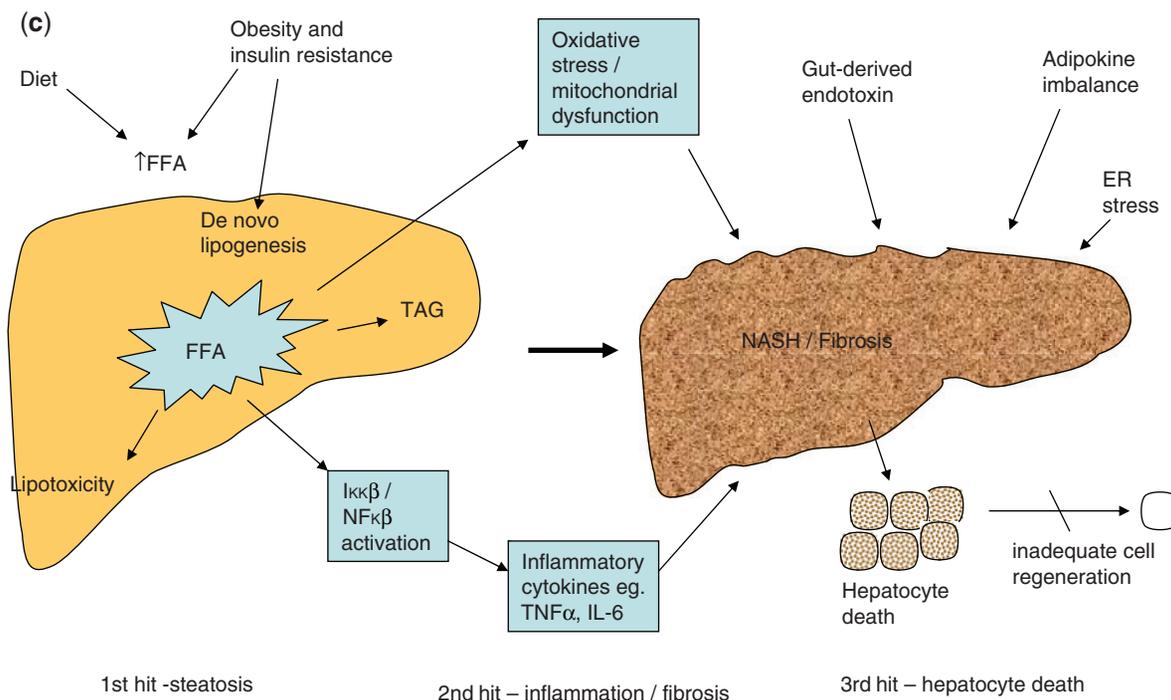
(c)

1st hit -steatosis

2nd hit – inflammation / fibrosis

3rd hit – hepatocyte death
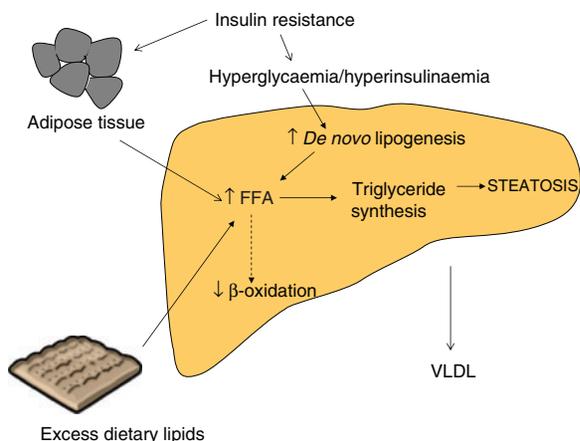
Figure 1. Continued

Figure 2. Mechanisms of hepatic fat accumulation.

have been proposed as potential mechanisms underpinning the pathogenesis of NAFLD leading to a decreased capacity for lipid export.[35,36]

Insulin resistance

In healthy individuals, binding of insulin to its receptor leads to phosphorylation of several substrates including insulin receptor substrates (IRS)-1, -2, -3 and -4, which propagate the insulin signal.[30,37] Insulin stimulation of IRS-1 and -2 leads to activation of intracellular PI3K (phosphoinositide 3-kinase) and AKT/PKB (protein kinase B) pathways, which are intimately involved in mediating the metabolic effects of insulin.[30] Ultimately, AKT/PKB activation results in translocation of glucose transporter, GLUT4, containing vesicles to the plasma membrane, thus facilitating glucose uptake. In addition, the expression of key lipogenic genes is increased, with a concomitant decrease in gluconeogenic gene expression via its regulation of forkhead (FOXO) transcription factor activity.

Insulin has a potent action to suppress adipose tissue lipolysis. However, in situations of IR, such as NAFLD, this suppression is impaired resulting in an increased efflux of FFA from adipose tissue.[38] The hyperinsulinaemia associated with IR leads to: (i) up-regulation of the transcription factor sterol regulatory element binding protein-1c (SREBP-1c), which is a key transcriptional regulator of genes involved in DNL,[15] and (ii) Inhibition of β-oxidation of FFA thus further promoting hepatic lipid accumulation.[30]

Many of the abnormalities reported in NAFLD interfere with the insulin signalling cascade, and thus contribute to IR. These include FFAs, tumour necrosis factor-alpha (TNF-α), nuclear factor kappa B (NF-κB), ceramide, jun N-terminal kinase 1 (JNK1), SOCS (suppressors of cytokine signalling) and cytochrome CYP2E1.[39,40] Increased lipid metabolites such as diacylglycerol (DAG) have been implicated in a protein kinase Cε (PKCε) dependent mechanism, to interfere with insulin signalling through inhibition of insulin
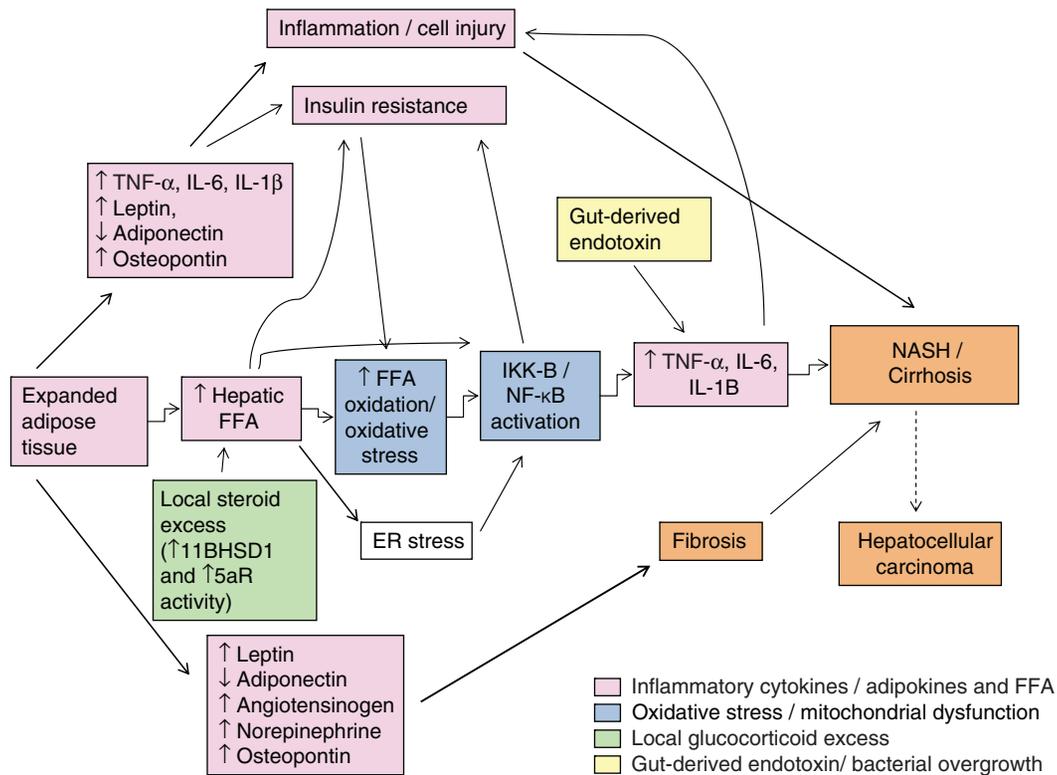
Figure 3. Proposed pathogenesis of NASH. The likelihood of progression to advanced NASH/cirrhosis results from a complex interplay between genetic predisposition and the mechanisms described earlier.

receptor activity and modulation of IRS-2 phosphorylation.[30] Similar processes occur in skeletal muscle cells, leading to a more generalized state of IR.

Inflammation/steatohepatitis

Inflammatory cytokines and FFA

The presence of steatosis is tightly associated with chronic hepatic inflammation,[41] an effect in part mediated by activation of the Iκκ-β/NF-κB signalling pathway. In murine models of high-fat diet (HFD)-induced steatosis, increased NF-κB activity is associated with elevated hepatic expression of inflammatory cytokines such as TNF-α, interleukin-6 (IL-6) and interleukin 1-beta (IL-1β), and activation of Kupffer cells.[41] Liver-specific NF-κB inhibition prevents HFD-induced inflammatory gene expression, whereas HFD-induced hyperglycaemia and IR can be reproduced by selective over-expression of constitutively active Iκκ-β in hepatocytes.[41] The Iκκ-β/NF-κB pathway in hepatocytes can also be activated directly by FFA, providing a further mechanism by which central obesity with consequent increased hepatic FFA supply can contribute to inflammation (Figure 3).[26] Furthermore, the conversion of FFA to hepatic triglyceride may serve as a

protective measure to prevent direct hepatic lipotoxicity. This is endorsed by a murine model of NAFLD, where inhibition of DGAT2, the enzyme that catalyzes the final step in triglyceride synthesis, resulted in improvement of hepatic steatosis and IR but exacerbation of injury and fibrosis.[27,42]

Both serum and hepatic levels of TNF-α are elevated in patients with NASH,[43,44] and levels correlate with histological severity.[45] In addition to its proinflammatory effects, TNF-α promotes IR.[46] Conversely, inhibition of TNF-α signalling improves IR and histological parameters of NASH.[47–49] Similarly, serum IL-6 levels are also elevated in both animal and human models of IR and NAFLD,[43,50,51] and levels correlate with increasing liver inflammation and fibrosis.[52] The key role of hepatocyte cytokine production in the progression of steatosis to NASH is supported by studies demonstrating that cytokines can replicate all of the histological features associated with NASH, including neutrophil chemotaxis, hepatocyte apoptosis/necrosis, Mallory body formation and stellate cell activation.[25] Additionally, data suggests that inflammation and NF-κB activation can promote carcinogenesis,[53] and that the chronic inflammatory state associated with hepatic steatosis may also play a key role in HCC development.[25]

Adipokines

Adipose tissue is not just an inert site of energy storage, but an actively secreting endocrine organ. The functional role of adipocyte-derived cytokines (adipokines), is now increasingly recognized, with leptin and adiponectin amongst the most well described. Leptin is a 16 kDa hormone produced mainly by mature adipocytes whose actions include the regulation of energy intake and expenditure,[54] regulation of the immune system,[55,56] and promotion of inflammation and fibrogenesis.[56,57] Higher leptin levels are observed in obese patients and those with NAFLD,[54,58–60] which are commonly regarded as states of leptin resistance.[58] It remains plausible that leptin may have a functional role to play in the pathogenesis of NAFLD.

In contrast to leptin, secretion and circulating levels of adiponectin are inversely proportional to body fat content,[61] and are reduced in patients with NAFLD.[44,62] Adiponectin is anti-inflammatory and increases insulin sensitivity,[63] and the administration of recombinant adiponectin improves hepatomegaly, as well as the biochemical and histological parameters of NAFLD in a murine model.[64,65] Adiponectin antagonises the effects of TNF-α, which itself suppresses adiponectin production.[61] The importance of adiponectin in NAFLD is supported by studies showing that serum adiponectin levels can help to distinguish NASH from simple steatosis.[44,66,67] Other adipose tissue derived factors found in excess in NAFLD include TNF-α, IL-6, angiotensinogen and resistin, all of which antagonise the lipogenic effects of insulin,[28] but their precise role in the pathogenesis of NAFLD remains to be determined (Figure 3).

Oxidative stress and mitochondrial dysfunction

The role of oxidative stress and mitochondrial dysfunction in NASH is well-established, with more advanced disease correlating with greater degrees of oxidative stress.[68–71] β-oxidation within the normal liver takes place in the mitochondria, but in the context of NAFLD[72] this process can become overwhelmed as a result of increased FFA load, giving rise to reactive oxygen species (ROS).[71] ROS induce oxidative stress, with subsequent activation of inflammatory pathways,[73] and also mitochondrial damage. Structural mitochondrial abnormalities[71] and a reduction in mitochondrial respiratory chain activity have been observed in human studies of NASH.[74] Elevated expression and activity of the hepatic microsomal fatty acid oxidizing enzyme cytochrome P450 2E1 (CYP2E1) has been observed in human and animal models of NASH and represents a potent source of ROS.[75,76] Importantly, transgenic over-expression of CYP2E1 activity is associated with oxidative stress, IR and hepatic fat accumulation.[77]

ER stress and bacterial overgrowth

Other mechanisms implicated in NASH pathogenesis include endoplasmic reticulum (ER) stress and gut-derived endotoxinaemia.[25] ER stress can be caused by a variety of biological stresses, including hyperinsulinaemia and hyperlipidaemia,[25,78] and can result in activation of various pathways leading to IR, inflammation, apoptosis and mitochondrial dysfunction. ER stress is known to be important in alcohol-induced steatohepatitis and further study of its role in NASH is warranted.[25]

Evidence is also emerging for a role of bacterial overgrowth in the pathogenesis of NASH. Bacterial overgrowth results in production of ethanol[79] and release of bacterial lipopolysaccharides, both of which can activate TNF-α production in Kupffer cells and thus induce hepatic inflammation.[80] Small intestinal bacterial overgrowth and increased gut permeability have been found more frequently in patients with NASH when compared with controls.[81,82] This has led to the suggestion that this may explain the onset of NASH and liver fibrosis as a complication of jejunoileal bypass surgery.[83,84] This hypothesis is further supported by evidence that alteration of gut flora with antibiotics[85] and probiotics[49,86,87] can reduce hepatic inflammation in both animals and humans.

Glucocorticoids

GCs from both exogenous and endogenous sources are a well-recognized cause of NAFLD. Patients with Cushing's syndrome, who have increased circulating GC levels develop a characteristic metabolic phenotype of central obesity, IR and diabetes. Importantly, a significant proportion of these patients will also develop hepatic steatosis.[88] The mechanisms by which GCs promote hepatic fat accumulation include inhibition of fatty acid β-oxidation and promotion of hepatocyte DNL. However most patients with NAFLD have normal circulating cortisol levels, suggesting that tissue-specific mechanisms are driving the metabolic dysfunction.

This has led to emerging interest in two enzyme systems which play a key role in local GC metabolism and consequent GC availability to bind and activate the GC receptor. 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) converts inactive cortisone to the active GC cortisol,[89] and thus increases local GC levels and amplifies GC action. Inhibition

of 11βHSD1 has been shown to lower body weight and lipid levels and improve glucose tolerance in animal models,[90] and increase hepatic insulin sensitivity in humans.[91,92]

In parallel, the A-ring reductase enzymes, 5-α- and 5-β reductase, are responsible for the metabolism of cortisol to its inactive tetrahydrometabolites. Increased hepatic 5-α reductase (5αR) activity has been demonstrated in patients with IR[89] and NAFLD,[93] which may represent a compensatory mechanism to decrease local GC availability in an attempt to prevent development or progression of NAFLD. In animal models, both pharmacological and transgenic inhibition of 5αR activity has been shown to increase susceptibility to development of IR and fatty liver.[94] Hence reduction of local hepatic GC exposure by modulation of 11βHSD1 and the A-ring reductases may represent a potential therapeutic intervention for preventing the development and progression of NAFLD.

Fibrosis

Fibrosis, and its more advanced form cirrhosis, represents the final common pathway of almost all chronic liver diseases including NASH. Advanced fibrosis results in liver failure and portal hypertension with its associated complications of ascites and life-threatening variceal bleeding, as well as an increased risk of HCC. The pathogenesis of fibrosis is not within the remit of this review and is well covered elsewhere.[95]

There are certain aspects of liver fibrosis and repair which are relatively specific to NASH and are worth considering here. In most conditions of liver injury repair arises by replication of mature hepatocytes,[96] however the presence of ongoing injurious factors such as NASH or viral infection, is associated with high levels of oxidative stress which reduce the ability of these mature hepatocytes to proliferate. In this situation, other pathways of liver regeneration, hepatic progenitor cells (HPCs), are recruited. HPCs are transit-amplifying cells which reside in the Canal of Hering, and which on proliferation form a complex of small ductules and cholangiocytes known as a ductular reaction. This term was first used to identify the expanded population of epithelial cells at the interface of the biliary tree and the hepatocytes, and refers to proliferation of pre-existing ductules, progenitor cell activation and appearance of intermediate hepatocytes.[97] Subsequently these cells have the capability of differentiating into both cholangiocytes and hepatocytes and contributing to liver repair. An inability to mount such a ductular response, as is seen in patients transplanted for NASH who have denervated livers, may be responsible for a more progressive pattern of liver damage.

Controversy continues to exist in the literature about the interplay of the ductular reaction and fibrosis in NAFLD. Initial work demonstrated a close association between the expansion of HPCs and the ductular reaction in liver biopsy specimens of NASH. The extent of ductular reactions in turn strongly correlated with the degree of fibrosis, suggesting that HPC expansion/ductular reaction may be responsible for stimulating a progressive periportal fibrosis.[98] Possible mechanisms for this include the secretion of profibrogenic cytokines (TGF-β, IL6, IL8 and MCP1) by the ductular reaction,[99] as well as direct epithelial mesenchymal transition of the cholangiocytes to myofibroblasts.[100] This hypothesis is attractive in that it provides a rationale for the generation of portal fibrosis in NAFLD which is a key feature of progressive disease. More recent work in the CDE model of murine NAFLD has suggested that liver fibrosis precedes the proliferation of HPCs, suggesting that fibrosis does not occur purely as a result of HPC expansion.[101] The timing of the presence of the differing histological findings is less clear on further evaluation,[102] raising the possibility of an altogether more complex interaction between fibrosis and HPC expansion, in which both cellular processes can stimulate each other respectively. Indeed, the creation of a progenitor cell niche, with deposition of matrix, may act not only as a migratory pathway for HPCs to migrate along from the portal tract into the parenchyma, but also provide trophic factors for the survival of these cells.[103]

Genetic predisposition

Although hepatic steatosis is common in patients with obesity and IR only a minority progress to NASH and cirrhosis, suggesting an important interplay between genetic predisposition and environmental factors.[72] Polymorphisms in genes related to lipid metabolism, IR, oxidative stress, cytokines/adipokines and fibrogenesis may all increase susceptibility to NASH development.[104] Several studies have identified single nucleotide polymorphisms (SNPs) which influence fibrosis development in other liver diseases, particularly chronic hepatitis C.[105–107] Studies in NASH have so far demonstrated polymorphisms in the angiotensinogen and TGF-β1 genes to be associated with advanced hepatic fibrosis in obese patients.[108] In addition, SNPs in the angiotensin II type 1 receptor are associated with an increased risk of NAFLD and NAFLD-related fibrosis.[109] Further studies are needed to identify more candidate genes which will undoubtedly be

informative not only as to the pathogenesis and prognosis of the disease, but also may represent novel treatment targets.

Current and emerging therapies

Current therapies

Despite an increasing understanding of the mechanisms of NAFLD pathogenesis, there are few effective therapies available. Current treatments are primarily directed towards improving the metabolic parameters which contribute to disease pathogenesis, such as weight loss and exercise, reducing IR and improving diabetic control.

In addition to lifestyle changes, current therapies utilized for patients with NAFLD include insulin sensitizers, e.g. metformin and the thiazolidinediones, weight loss drugs, e.g. orlistat and sibutramine, and consideration of bariatric surgery for morbidly obese patients. Liver transplantation remains the only curative treatment option for end-stage cirrhosis.

The urgent need for specific treatments for NAFLD has rendered this a critical area of research, with particular focus on developing treatments which can reverse or prevent the more advanced and clinically relevant stages of NASH. The presence of fibrosis predicts likelihood of liver related complications and therefore therapies which can prevent or reverse fibrosis are important goals.

New and emerging therapies

Therapies currently undergoing evaluation in NASH include antioxidants, such as vitamins C, E[110,111] and betaine,[112] iron depletion,[113] ursodeoxycholic acid,[114,115] statins[116–118] and pentoxifylline.[47,119] Whilst none of these treatments has yet shown convincing evidence of benefit, further trials are ongoing.

Glucagon-like peptide-1 (GLP-1)-based therapy may represent a novel therapeutic option for slowing the progression of NAFLD. In diabetic patients GLP-1 analogues, such as Exenatide, have been shown to increase insulin secretion, suppress glucagon secretion, slow gastric emptying and increase satiety in association with modest weight loss,[120] and in animal models GLP-1 agonists reduced IR, markers of oxidative stress and hepatic steatosis.[121]

Antifibrotic therapies

Hepatic fibrosis is the product of hepatic myofibroblasts which predominantly arise from activation of hepatic stellate cells (HSCs), that reside in the space of Disse.[122] HSCs express the nuclear peroxisome proliferator activated receptor gamma (PPARγ). Studies have demonstrated that PPARγ ligation by PPARγ agonists, such as the thiazolidinediones (Rosiglitazone and Pioglitazone), leads to reduced HSC activation. Encouraging results with these agents have been demonstrated in patients with NAFLD with improvements in both liver biochemistry and histology.[123–125] However side-effects including congestive cardiac failure,[126] osteoporosis[127] and weight gain[125] are of concern. Benefits also appear to be reversed on discontinuation of therapy.[128]

Angiotensin has been shown to promote myofibroblast survival and liver fibrosis,[129] and thus the beneficial effects of ACE inhibitors and angiotensin receptor blockers (ARBs) are likely to include antifibrotic properties. Several clinical studies of antioxidants such as vitamin E, which have been shown to suppress fibrosis *in vitro*, have so far failed to demonstrate that dietary supplementation of vitamin E improves histological fibrosis in humans.[110,111] A large number of cytokines are intimately involved in the proliferative, contractile and fibrogenic activities of HSCs, antagonism of which represents another potential target for antifibrotic therapies.[130] Potential candidates include platelet-derived growth factor (PDGF), transforming growth factor beta-1 (TGFβ-1),[130] connective tissue growth factor (CTGF),[131] endothelin-1 (ET-1), thrombin, vascular endothelial growth factor (VEGF), fibroblast growth factor and insulin-like growth factor, which also exert their effects through tyrosine-kinase receptors.[130] Consistent with this, studies in animal models of liver fibrosis have demonstrated antifibrotic effects using tyrosine kinase inhibitors such as imatinib[132,133] which is currently licensed for use in chronic myeloid leukaemia and gastrointestinal stromal tumours.

Other potential targets alluded to earlier include inhibiting ER stress,[134] and modulation of the gut liver axis using pre- and probiotics.[49,85–87,135]

Conclusions

NAFLD now represents one of the commonest causes of liver disease in the Western world, and the rising levels of obesity, diabetes and metabolic syndrome will ensure that it remains a major cause of morbidity and mortality. Although simple steatosis carries a relatively benign prognosis, a significant proportion of patients will progress to NASH and later cirrhosis with risk of HCC.

The traditional '2-hit' hypothesis of NAFLD pathogenesis has been modified several times; in most patients however NAFLD does appear to begin

with lipid accumulation, or steatosis, which is in turn driven by obesity and IR. Progression to steatohepatitis and fibrosis depends on additional factors such as FFAs, inflammatory cytokines and adipokines, oxidative stress and mitochondrial dysfunction in a complex interplay with genetic predisposition.

Current treatment strategies for NASH focus on improving components of the metabolic syndrome, such as obesity and IR, with no liver-specific agents yet available. However, modulation of any of the multiple mechanisms involved in NASH pathogenesis could provide useful targets to prevent the development of fibrosis and its associated complications. This knowledge, and the significant advances that continue to be made in our understanding of the pathogenesis of NASH, are required to inform the development of novel therapeutic strategies for this increasingly important condition.

Funding

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