Non-alcoholic fatty liver disease from pathogenesis to management: an update.

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Obesity Comorbidities

Non-alcoholic fatty liver disease from pathogenesis to management: an update

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Summary

Non-alcoholic fatty liver disease (NAFLD), the most common chronic liver disease in the Western world, is tightly associated with obesity and metabolic syndrome. NAFLD entails an increased cardiometabolic and liver-related risk, the latter regarding almost exclusively non-alcoholic steatohepatitis (NASH), the progressive form of NAFLD. Pathogenetic models encompass altered hepatic lipid partitioning and adipokine action, increased oxidative stress, free fatty acid lipotoxicity. On this basis, lifestyle-, drug- or surgically induced weight loss, insulin sensitizers, antioxidants, lipid-lowering drugs have been evaluated in NAFLD/NASH. Most trials are small, of short duration, nonrandomized, without histological end points, thus limiting assessment of long-term safety and efficacy of proposed treatments.

All NAFLD patients should be evaluated for their metabolic, cardiovascular and liver-related risk. Liver biopsy remains the gold standard for staging NAFLD, but non-invasive methods are under intense development. Weight loss through lifestyle intervention is the initial approach, because of established efficacy on NAFLD-associated cardiometabolic abnormalities, and to emerging benefits on necroinflammation and overall disease activity in NASH. Bariatric surgery warrants further evaluation before it can be routinely considered in morbidly obese NASH.

Larger- and longer-duration randomized trials assessing safety and benefits of drugs on patient-oriented outcomes are needed before pharmacological treatment can be routinely recommended for NASH.

Keywords: Management, NAFLD, NASH, treatment.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in the Western world. We will discuss the epidemiology of NAFLD, recent diagnostic and pathogenetic acquisitions, the evidence for current therapeutic approaches and unresolved issues. Online medical databases and abstracts from National and

International meetings were searched for: non-alcoholic steatohepatitis (NASH), NAFLD, non-alcoholic, fatty liver, liver fat, steatosis, metabolic liver disease. Methodological quality of original human studies through July 2009 was scored according to Downs and Black checklist independently and in duplicate by two authors (G. M., M. C.): studies scoring >16 points (range 0–27) were analysed to assess the natural history, pathogenesis, prognosis and treatment of NAFLD (1). Meta-analyses following the Guidelines for Meta-Analyses of Observational Studies in Epidemiology and the Cochrane Handbook for systematic Reviews of Interventions were also included (2).

The flow of study selection is reported in Fig. S1

Definition

The NAFLD is defined by a hepatic fat infiltration >5% hepatocytes, as essessed by liver biopsy (LB) or magnetic resonance spectroscopy [MRS; sensitivity of 98% for steatosis; (3)], in the absence of excessive alcohol intake, defined by two standard drinks (20 g ethanol) daily for men and one standard drink (10 g ethanol) daily for women (4).

The NAFLD was traditionally categorized as 'primary' or 'secondary' depending on the underlying aetiology. 'Primary' NAFLD occurs most commonly, its pathogenesis is unknown, is tightly associated with insulin-resistance and metabolic syndrome, and will be the focus of this review. Before formulating a diagnosis of primary NAFLD, other competing aetiologies of hepatic steatosis ('secondary' NAFLD) with specific treatments and different prognoses should be ruled out (Table 1). Currently, the term 'secondary' NAFLD is discouraged and preferred nomenclature includes the known causative factor and the resultant pathology, e.g. total parenteral nutrition-induced, drug-induced steatosis/steatohepatitis.

The NAFLD encompasses a histological spectrum ranging from simple steatosis to non-alcoholic steatohepatitis (NASH), defined by steatosis, hepatocellular damage and lobular inflammation (4). The distinction between these two histological subtypes can currently be made only by LB, and has prognostic significance, as NASH can progress to cirrhosis (see below).

Epidemiology and natural history

Depending on the sensitivity of the method used to detect hepatic steatosis, NAFLD has a prevalence in the general western adult population of 30% by MRS (sensitivity of 98% for steatosis), of 14–26% by ultrasound (sensitivity ranging 49–94%, insensitive for steatosis involving <30% hepatocytes, (5) and of 6–12% by liver enzyme elevation (6,7). Notably, serum transaminases can be normal in up to 75% of cases of NAFLD (3,4).

Beyond different case definitions, ethnicity and metabolic factors affect disease prevalence. The prevalence of NAFLD is 14–16% in Asians, 31–33% in African–Americans and Caucasians and 45% among Hispanics, differences partly explained by different visceral adiposity distribution (8). Metabolic disorders increase the risk of NAFLD, occurring in 65–70% of obese and diabetic and in up to 96% of morbidly obese subjects(5,9). Another emerging association is between NAFLD and obstructive sleep apnoea syndrome (OSAS), which can be found in >50% of obese patients with NAFLD (10,11).

Beside overall risk of NAFLD, metabolic factors impact also the severity of liver disease: NASH affects 3% of the general population, 20–30% of obese and diabetic and 35–40% of morbidly obese subjects (4,9). The presence of OSAS, as well, increases by fourfold the risk of NASH in morbidly obese patients, independently of overweight (12): whether chronic intermittent hypoxia promotes liver injury warrants further prospective investigation.

Overall, NAFLD is a slowly progressive disease, with morbidity and mortality affecting a minority of patients. (13–15). In the Third National Health and Nutrition Examination Survey (NHANES-III), NAFLD had higher overall (OR 1.038, 95% CI: 1.036–1.041, $P < 0.0001$) and liver-related mortality (OR 9.32, 95% CI: 9.21–9.43, $P < 0.0001$) than the general population over 8.7 years, after adjusting for age, gender, race, body mass index (BMI), hypertension and diabetes(7). Cardiovascular disease (CVD), malignancy and liver disease were the leading causes of death in NAFLD. Mortality was particularly increased among the 45–54 age group, with a standardized mortality ratio of 4.40 (95% CI: 1.27–13.23) for all-causes and of 8.15 (95% CI: 2.00–33.20) for CVD. Further evidence connects NAFLD to cardiometabolic complications:

1. Diabetes: in community- and population-based prospective studies, NAFLD predicted incident diabetes independently of traditional risk factors, including obesity, insulin resistance, metabolic syndrome and C-reactive protein (16–21).

Table 1 Types of non-alcoholic fatty liver disease (NAFLD)

Type	Association
Primary	Obesity, insulin resistance and metabolic syndrome, diabetes, dyslipidemia
Secondary	
Viral infection	Hepatitis B, C, Epstein-Barr
Autoimmune or hereditary diseases	Autoimmune hepatitis, systemic erythematosus lupus*, Wilson disease, hereditary hemochromatosis, alpha-1-antitrypsin deficiency, celiac disease
Drugs	Amiodarone, tamoxifen, corticosteroids, corticosteroids, methotrexate, Calcium channel blockers, valproate, highly active antiretroviral therapy
Toxins	Organic solvents, mushroom toxins
Endocrine-metabolic conditions	Hypobetalipoproteinemia, lipodystrophy, hypopituitarism, Weber-Christian syndrome, Cushing syndrome, Reyes syndrome, pregnancy, hypothyroidism
Nutritional	Total parenteral nutrition, rapid weight loss, starvation, jejuno-ileal bypass

*Low titer antinuclear antibodies (≤1 : 160) or anti-smooth muscle actin antibodies ≤1 : 40 can be seen in up to 33% of patients with NAFLD.

2. CVD: a meta-analysis of five community-based and two hospital-based cross-sectional studies found an independent association between NAFLD and subclinical carotid atherosclerosis (OR: 3.13, 95% CI: 1.75–5.58, $P < 0.0002$) (22); other cross-sectional and prospective studies confirmed an independent association of NAFLD with different cardiovascular outcomes, including systemic and coronary endothelial dysfunction, myocardial dysfunction and clinical events (6,15,23–31).

Whereas cardiovascular risk seems increased in all patients with NAFLD, without significant differences between NASH and simple steatosis (32), the hepatological prognosis differs between histopathological subtypes (13,14): while steatosis progresses to fibrosis in <5% of cases, NASH develops progressive fibrosis in 30% of cases over 3–4 years and cirrhosis in 25% of cases over 8–10 years; once cirrhosis develops, median survival approaches 6 years, with an overall mortality rate 1.5- to 1.75-fold higher than steatosis (13–15,33). Consistently, most patients with cryptogenic cirrhosis come from unrecognized NASH, which lost the histological feature of hepatic steatosis with disease progression (34).

Pathogenesis: the two-hit hypothesis revisited

According to the original 'two-hit' model, hepatic fat accumulation is the first hit and is the pre-requisite for liver injury to develop. The fatty liver subsequently becomes vulnerable to 'second hits' leading to hepatocyte injury, inflammation and fibrosis. This view has been challenged by recent findings, suggesting the same noxae may determine both fat infiltration and necroinflammation/fibrosis, and that hepatic triglyceride (TG) accumulation may actually protect hepatocytes from free fatty acid (FFA) toxicity.

Mechanisms underlying hepatic fat accumulation

The NAFLD develops when FFA uptake and synthesis exceed oxidation and resecretion into the blood. Mechanisms potentially contributing to hepatic steatosis include: adipose tissue-derived and dietary FFA overflow to the liver, hepatic *de novo* lipogenesis (DNL), impaired hepatic FFA elimination through oxidation and secretion into very low density lipoprotein triglycerides (VLDL-TG). Stable isotope technique demonstrated that 59% of hepatic TG derive from adipose tissue FFA spill-over, 26% from DNL and 15% from dietary fat in NAFLD (35). DNL, as opposite to healthy subjects, is constitutively elevated in NAFLD and fails to suppress in the fasting state. Hepatic VLDL secretion rates in NAFLD are inadequate to the increased hepatic TG availability: while in healthy subjects VLDL-TG secretion rate increases linearly with increasing intra-hepatic TG disposal, in NAFLD it reached a plateau as

hepatic TG infiltration exceeded 10% (36). VLDL-TG synthesis and secretion are more compromised in NASH than in simple steatosis, thus further aggravating hepatic lipid retention (37). Therefore, increased uptake of adipose tissue-derived FFA, an inappropriately elevated DNL and an inadequate export of TG from the liver are responsible for hepatic steatosis in NAFLD.

Adipokines

Visceral adipose tissue is believed to play a pivotal role in the pathogenesis of NAFLD and current therapeutic approaches aim at reducing visceral adipocyte-derived adipokine and FFA overflow to the liver by weight loss and insulin-sensitizers. The role of single adipokines in the pathogenesis of NAFLD and insulin resistance is described in Table 2 and reviewed elsewhere (38). Most adipokines, including tumor necrosis factor-α (TNF-α), resistin, angiotensin II, induce insulin resistance and low-grade inflammation through activation of stress-related protein kinases, i.e. c-Jun NH2-terminal kinase-1 (JNK-1), and of the inhibitor kappa kinase beta (IKKβ)/nuclear factor kappa B (NF-κB) pathway. Activated JNK-1 phosphorylates insulin receptor substrate-1 protein, thereby inactivating insulin receptor signalling. IKKβ activation leads to translocation of the transcription factor NF-κB into the nucleus, resulting in a feed-forward loop enhancing synthesis of proinflammatory cytokines, including IL-6, TNF-α, IL-1β and monocyte chemoattrattant protein-1.The net result is a state of hepatic and systemic low-grade chronic inflammation and insulin resistance (39–41).

Leptin, TNF-α and IL-6 promote also hepatic fibrogenesis through hepatic stellate cells (HSC) activation, both directly by binding to HSC receptors and indirectly through transforming growth factor-β secretion by Kupffer cells (38,42).

Consistently, TNF-α pathway antagonism reversed hepatic insulin resistance and liver injury in ob/ob mice and in human NASH (38,43).

Contrary to other adipokines, adiponectin protects against the development of NAFLD, and hypoadiponectinemia has been consistently linked with liver disease in experimental and human NAFLD, even in the presence of normal plasma levels of other adipokines, suggesting it may be an early event in the pathogenesis of NAFLD (44). Consistently, administrations of recombinant adiponectin markedly improved NASH in ob/ob mice and the histological improvement observed in NASH with thiazolidinediones (TZDs) correlate tightly with enhanced adiponectin secretion and adipocyte insulin sensitivity than with visceral fat mass changes, suggesting functional impairment of adipocyte is central to the pathogenesis of NASH (45,46). Retinol binding protein 4 (RBP4) was identified in the adipose tissue of mice rendered insulin resistant by adipose-

specific inactivation of the glucose transporter GLUT4 (47). Hepatic, adipose tissue and plasma levels of RBP4 correlate with the degree of obesity, insulin resistance and liver fat content (48,49). Mechanisms whereby RBP4 interferes with insulin action are incompletely understood, but may involve hepatic retinol metabolism (50).

FFA lipotoxicity

An emerging concept is that hepatic TG accumulation is not *per se* toxic, but rather would protect the hepatocyte by buffering the accumulation of toxic FFA: inhibiting hepatic

TG synthesis improved hepatic steatosis, but exacerbated liver injury, necroinflammation and fibrosis in obese mice with NASH (51). In this model, the severity of liver injury paralleled the extent of hepatic FFA accumulation, of cytochrome P450 2E1 activation and of lipid peroxidation. Conversely, in humans hepatic FFA uptake inhibition with acipimox acutely improved hepatic injury and insulin sensitivity, without affecting liver TG stores (52). FFA lipotoxicity involves the following mechanisms:

1. JNK-1-mediated hepatocyte apoptosis activation (53). Despite a similar ability to cause steatosis, saturated (SFA) seem substantially more cytotoxic than unsaturated

Table 2 An overview of main mechanisms involved in the pathogenesis of NAFLD

Membrane fatty acid transporters involved in NAFLD

	Activators	Inhibitors	Action	Clinical implications
FATP5			Enhances facilitated hepatic FFA uptake	Experimental FATP5 inhibition reverses NAFLD
FABP4, FABP5			Enhances facilitated hepatocyte and adipocyte FFA uptake	
FAT/CD36			Enhances facilitated hepatic FFA uptake	

Nuclear receptors involved in NAFLD

	Activators	Inhibitors	Action	Clinical implications
PPAR-α (Present in hepatocytes, muscle and heart miocytes)	PUFA, fibrates, eicosanoids, adiponectin		Increases FAT/CD36-mediated FFA uptake Increases mitochondrial and peroxisomal β-oxidation Inhibits NF-κB pathway	Mediates PUFA and fibrate beneficial effects on human and murine NAFLD
PPAR-γ (Present in adipocytes, hepatocytes and hepatic stellate cells, macrophages, pancreatic β-cells, endothelium)	TZD		Enhances insulin action, FFA oxidation, adiponectin secretion, hepatocyte FFA uptake by FAT/CD36 Inhibits macrophage and hepatic stellate cell activation Inhibits secretion of proinflammatory cytokines by adipocytes and macrophages	TZD improve NAFLD in animals and humans
SREBP-1c (present in adipocytes and hepatocytes)	Insulin, endocannabinoids (CB-1 agonists, LXR-α, SOCS-3, Homocystein, endoplasmic reticulum stress SFA, TFA	Leptin, glucagon, PUFA, AMPK	Enhances transcription of lipogenic enzymes ACC, FAS, SCD-1	PUFA and metformin inhibit lipogenesis
LXR-α	Oxysterols, glucose		Enhances transcription of lipogenic enzymes ACC, FAS, SCD-1; Activates (SREBP)-1c transcription; enhances FAT/CD36-mediated FFA uptake	Exogenous antagonist 22-S-HC inhibits lipogenesis in human muscle cells (162,163).
ChREBP	Glucose, LXR-α		Enhances transcription of lipogenic enzymes ACC, FAS	ChREBP deletion improved steatosis, hyperlipidemia, hepatic and muscle insulin resistance (164)

Table 2 *Continued*

Adipokines involved in NAFLD

	Activators	Inhibitors	Action	Clinical implications
Leptin	Overfeeding, obesity, glucocorticoids, glucose, insulin	Fasting, sustained exercise, cold exposure, weight loss	Central anorexigenic, insulin sensitizer, increasing mitochondrial FFA oxidation by malonyl-CoA synthesis inhibition, stimulates AMPK, which activates ATP-producing catabolic pathways, such as β-oxidation, glycolysis and inhibits ATP-consuming anabolic pathways; fibrogenic action by hepatic stellate cell activation	Obese NAFLD patients, are leptin resistant and have elevated leptin levels. Leptin administration reduces body mass index, hepatic fat content and histological steatohepatitis in lipoatrophic patients
TNFα			Induces insulin resistance by reducing GLUT-4 expression and LPL activity in adipocytes and hepatocytes; induces inflammation in the liver by activating stress-related kinase JNK-1 and NF-κB pathway	Experimental NAFLD is significantly improved by hepatic TNF-α antagonism. Pentoxifylline, a weak TNF-α inhibitor, is currently tested in human NASH
Resistin	TNF-α, IL-6, IL-1, lipopolysacharride		Promotes insulin resistance by AMPK activation and down-regulation of GLUT-4 in adipocytes; Induces nuclear translocation of NF-κB and TNF-α secretion from macrophages; Resistin levels are increased in NAFLD patients and correlate with histological severity and with impaired hepatic glucose metabolism	Resistin expression is markedly reduced by thiazolidinediones, and anti-resistin antibodies improve blood glucose and insulin action in mice with diet-induced obesity
Adiponectin	Weight loss	TNF-α, IL-6, resistin, insulin, glucocorticoids	It stimulates mitochondrial β-oxidation by activating AMPK and inhibits lipogenesis by down-regulating SREBP-1c; hepatic anti-inflammatory/fibrogenic properties by antagonizing TNF-α action and inducing hepatic stellate cells apoptosis.	Recombinant adiponectin markedly improves experimental NASH. It mediates TZDs action on liver histology in human NASH
Retinol binding protein 4			Impairs insulin-stimulated glucose uptake in muscles, enhances hepatic glucose production and interferes with insulin signalling in adipocytes, although the mechanism is not fully clear. RBP4 correlates with hepatic steatosis independently of obesity and insulin resistance in chronic hepatitis C, suggesting other mechanisms may link RBP4 to steatosis, possibly hepatic retinol metabolism	
SOCS-3	TNF-a, IL-6 and leptin	Adiponectin	Enhances *de novo* lipogenesis by up-regulating hepatic SREBP-1c; Promotes insulin resistance by phosphorylation of insulin receptor substrate-2 (165)	
Interleukin-6	SFA		Inhibits adiponectin secretion and lipoprotein lipase; Stimulates SOCS-3 secretion; Hepatic proinflammatory activity by Kupffer cell activation.	Anti-IL-6 monoclonal antibody (tocilizumab) proved beneficial in other models of human pathology and warrant investigation in NASH (166)

22-S-HC, 22-S-hydroxycholesterol; ACC, acetyl CoA carboxylase; AMPK, AMP-activated protein kinase; ChREBP, carbohydrate response element binding protein; FABP, fatty acid binding protein; FAS, fatty acid synthase; FAT, fatty acid traslocase; FATP, fatty acid transport protein; FFA, free fatty acid; IL, interleukin; LPL, lipoprotein lipase; LXR-α, liver X receptor-α; NAFLD, non-alcoholic fatty liver disease; NF-κB, nuclear factor kappa B; NASH, non-alcoholic steatohepatitis; PPAR, peroxisome proliferator-activated receptors; SCD, stearoyl-CoA desaturase; SFA, saturated fatty acids; PUFA, polyunsaturated fatty acid; SOCS-3, suppressor of cytokine signalling-3; SREBP, sterol-regulatory element-binding protein; TFA, trans-fatty acids; TNFα, tumor necrosis factor-α; TZD, thiazolidinediones.

fatty acids; consistently, the liver of subjects with NASH is characterized by a relative depletion of unsaturated FFA in favour of SFA. Whether this is a consequence of the peroxidative process or may have a pathogenetic role in NASH warrants investigation (54).

2. Adipokine-independent hepatocyte IKKβ-mediated NF-κB pathway activation (55). Consistently, IKK inhibition reversed experimental NASH (56); NF-κB pathway inhibition by salsalate improved glycemia and inflammatory markers in obese humans, but the effects of this approach on human NAFLD are unknown (57). Whether the inhibition of the final effector of most proinflammatory stimuli, i.e. NF-κB, is more effective and safer than a strategy antagonizing selective adipokine pathways, needs careful evaluation in future studies.

3. The SFA promote endoplasmic reticulum stress or UPR (Unfolded Protein Response), a stress response to various stimuli which is dys-regulated in NAFLD, and that, when persistently activated, triggers hepatocyte apoptosis, a critical step in the progression from simple steatosis to NASH and cirrhosis (58–60). Apoptotic hepatocytes are phagocytized by adjacent macrophages, eventually leading to hepatic stellate cell activation and fibrogenesis. The role of apoptosis in the progression of liver injury has been highlighted in a recent proof-of-concept study, where caspase inhibition improved hepatic fibrosis in an animal model of NASH (61).

The relevance of FFA in the pathogenesis of liver injury prompted research on mechanisms regulating FFA entry and partitioning in hepatocyte. A total of 90% of the hepatic uptake of circulating long-chain FA is mediated by three saturable transmembrane carrier families: fatty acid transport proteins (FATPs, six isoforms with different tissue distribution), fatty acid translocase (FAT/CD36) and fatty acid binding proteins (FABPs, nine different isoforms) (60). The most extensively studied isoforms in NAFLD are FATP5, FAT/CD36, FABP-1, FABP-4 and FABP-5: their overexpression promotes steatosis, insulin resistance and dyslipidemia in mice fed a high-fat diet, while their functional deletion ameliorated these disorders (60,62,63). The extension of these findings to human NAFLD is complex: dietary and hormonal regulation of these proteins, the phenotype deriving from the interaction among different transmembrane carriers and the functional significance of genetic variants are largely unknown (64–66).

Once in hepatocyte, SFA are unsaturated to monounsaturated fatty acids by stearoyl-coenzyme A (CoA) desaturase-1 and subsequently esterified to TG by acyl CoA:Diacylglycerol acyltransferase 1: while a high activity of these enzymes is associated with higher hepatic TG content in animal and human NAFLD, a reduced activity promotes accumulation of intermediate SFA and diacylglicerol that trigger hepatocyte apoptosis and insulin resistance (67–69). If confirmed, the protective role of these lipid partitioning enzymes in the prevention of liver injury may have relevant therapeutic implications.

The other type of FFA that has been linked to the pathogenesis of NAFLD are trans fatty acids, a form of unsaturated fats relatively rare in nature, deriving mostly from partial hydrogenation of vegetable oils, that can be found in fast food and other processed foods. Compared with an isocaloric high-fat diet, trans fat feeding resulted in higher hepatic insulin resistance and necroinflammation and enhanced hepatic interleukin-1β and TNF-α gene expression (70–73).

Nuclear transcription factors

Another growing research field involves the modulation of hepatocyte glucose and lipid metabolism by nuclear transcription factors, a superfamily of nuclear receptors that upon ligand binding interact with DNA response elements and co-ordinately regulate transcription of genes involved in multiple metabolic and inflammatory pathways, at hepatic and extrahepatic level. The nuclear transcription factors linked to NAFLD (60) are reported in Table 2.

Role of genetics in NAFLD

The pathogenesis of NAFLD appears multifactorial, involving both genetic and enviromental factors. Two recent cohort studies and one community-based study in different ethnicities estimated the heritability of NAFLD to approach 35–40% of the total predisposition, even after adjusting for age, gender, race and BMI (74–76). Several genes modulating crucial steps of hepatic glucose and lipid metabolism, inflammation and fibrogenesis, that have been addressed in the pathogenesis section, have been candidated by small cohort studies and are reviewed elsewhere (77). Recently, two independent genome-wide association population-based studies and three smaller cohort studies found the missense variant rs738409 (encoding I148M) in the Patatin-like Phospholipase 3 (PNPLA3) gene encoding adiponutrin is associated with hepatic steatosis across different ethnic groups and independently of obesity and diabetes status (78–82). Adiponutrin is a transmembrane protein with phospholipase, TG lipase and acylglycerol transacylase activity (transfers fatty acids to mono- and di-acylglycerol), expressed in liver and adipocytes, whose biological significance is largely unknown, that is induced during adipocyte differentiation and in response to fasting and peroxisome proliferator-activated receptor-γ (PPAR-γ) activation and is down-regulated by insulin and TNF-α (83).

Future confirmatory studies are needed to elucidate the physiopathological mechanism(s) underlying this and other

genetic associations before they can be used to predict individual risk of steatosis, steatohepatitis and fibrosis and to develop genetic molecular therapeutic strategies

Diagnostic issues

Diagnosing non-alcoholic

The threshold for defining significant alcohol consumption has been recently debated, as obese and diabetic individuals may develop steatosis with ≥1 daily alcohol drink and, conversely, modest alcohol consumption may protect against NAFLD (84,85). When history is unreliable or in subjects with associated metabolic risk factors, it is hard to differentiate alcoholic and non-alcoholic fatty liver. Conventional variables, including mean corpuscular volume (MCV), gammaglutamyltransferase and aspartate/alanine aminotransferase (AST/ALT) ratio are inaccurate. Serum carbohydrate-deficient transferrin has 81% sensitivity with 98% specificity in detecting chronic alcohol abuse (86), but is not routinely measurable. The recently developed 'ALD/ NAFLD Index' (ANI) combines 6 readily accessible parameters (MCV, AST, ALT, height, weight and gender) into a score (87): an ANI score >0 favours alcoholic liver disease (ALD) whereas a value <0 predicts NAFLD with sensitivity and specificity of 87.8% and 97.4%, respectively. As most alcoholic patients in this cohort had high model for end-stage liver disease scores, ANI score is likely to be more applicable to patients with advanced liver disease and cirrhosis, while its accuracy for ambulatory patients remains to be determined.

Diagnosing steatosis

As previously discussed, the diagnostic accuracy of liver enzymes and ultrasonography for steatosis is suboptimal and computed tomography (CT) and magnetic nuclear resonance imaging (MNR) imaging are not routinely feasible. Using multivariate regression analysis Kotronen *et al.* developed and validated in 470 subjects the NAFLD liver fat score, which combines five readily available variables into a score to give the probability of increased liver fat as assessed by MRS (Table S1) (82). The same authors also developed an equation for estimating individual liver fat content. Although promising, this score need independent validation in different populations.

Assessing liver disease severity

As different histological subtypes of NAFLD have radically different prognoses, it is mandatory to identify patients with progressive liver disease, who are at risk of poor outcome (13–15). LB, currently the gold standard to stage NAFLD, is not feasible in all NAFLD patients, is costly, risky (procedural mortality 0.01%), invasive, and suffers from sampling and intra- and inter-observer variability [κ coefficient of agreement of 0.55 for diagnosis of NASH and 0.69 for fibrosis staging, (88)]. Therefore, non-invasive radiological and biochemical methods for assessment of the severity of liver disease are under development. Transient elastography, measuring liver stiffness as an index of hepatic fibrosis, has limited accuracy in the presence of steatosis and obesity (89); MR elastography is still investigational and hardly accessible. Scoring systems combine clinical and biochemical parameters to individuate two histological features within NAFLD: NASH and advanced fibrosis (Table S2). Identifying advanced fibrosis would allow screening for complications of liver disease (hepatocellular carcinoma, oesophageal varices), while detecting NASH would allow earlier treatment of a progressive disease. As treatments with differential efficacy on necroinflammation and fibrosis will become available, both histological features will have to be identified.

All these scores need independent and prospective validation in large, ethnically different cohorts before they can replace LB to stage NAFLD and to monitor disease progression and response to treatment (90,91). The usual experts recommendation of referring patients with clinical risk factors for advanced liver disease (age ≥ 45 years, obesity, diabetes, hypertriglyceridemia, metabolic syndrome) to gastroenterologists for LB is hardly feasible, implying that 66% of obese subjects should get a gastroenterological evaluation. These figures highlights the inadequacy of current screening tools and the urgent need for adequate, cost-effective and easy-to-use methods to screen obese NAFLD patients for progressive liver disease.

Treatment

Histological evaluation of proposed treatment is essential, as liver enzymes and steatosis spontaneously fluctuate over time and often improve as necroinflammation and fibrosis progress. Unfortunatey, few well-designed randomized controlled trials (RCTs) with histological end points are available.

Weight reduction through lifestyle modifications or drugs (Table S3)

Because most NAFLD patients are overall or abdominally obesity, weight reduction has been advised on the basis of the benefits on multiple cardiometabolic risk factors. This recommendation has long relied on small nonrandomized trials, few with post-treatment histology, all of short duration (3–12 months), showing a weight loss of 4–23% was safe, improved biochemical and histological NAFLD/

NASH to a different extent, with improvement in necroinflammation and fibrosis being associated with higher weight losses (92–100).

These concepts were confirmed in several well-designed RCTs targeting weight loss through lifestyle interventions similar to the Look AHEAD programme or drugs like orlistat (101–105). In these RCTS, a weight loss ≥7% was safe and achieved significant improvement in hepatic steatosis, necroinflammation and NAS (NAFLD activity score, a composite of steatosis and necroinflammation indicating overall disease activity). Categorizing the patients according the degree of weight loss, a weight loss ≥5% improved steatosis insulin sensitivity and associated cardiometabolic risk factors, but only a weight loss ≥7% improved necroinflammation and NAS (97,101). Orlistat was safe and well-tolerated with minor adverse gastrointestinal complaints not requiring discontinuation of therapy.

On the basis of cross-sectional evidence for a protective role of regular exercise against NAFLD, at least partly through abdominal fat reduction (106–108), three RCTs assessed the effects of physical activity *alone* on NAFLD: beyond the known benefits on metabolic abnormalities, 1–6 months of regular moderate-intensity aerobic exercise dose-dependently improved biochemical and MRS-detected hepatic steatosis, even in the absence of body-weight reduction, independently of changes in total or visceral fat mass (109–111).

These preliminary data suggest aerobic exercise may reduce hepatic steatosis, possibly through hepatic AMPK activation (60), and should be strongly promoted for the management of NAFLD, the benefits of which are not exclusively contingent upon weight loss.

The effects of different doses of aerobic exercise on NAFLD are being assessed by the trial ClinicalTrials.gov identifier NCT00771108.

The optimal nutrient composition of the diet for NAFLD is unknown: in the absence of prospective head-to-head comparisons with histological assessment, some indications can de drawn from available data (Table S4).

In summary, RCTs with post-treatment histology suggest weight loss improves liver disease and associated metabolic abnormalities in NAFLD. Importantly, while a 5% weight loss improves steatosis and NAFLD-associated cardiometabolic risk factors, recent RCTs suggest a >7% weight loss would be required to improve necroinflammationa and overall disease activity in NASH. The issue of a higher threshold of weight loss necessary to improve the progressive histological features of NASH needs further investigation. Long-term compliance is also a concern: even within short-term trials, adherence to prescribed regimen did not exceed 40% of patients, which raises doubts about the durability of these benefits. This is even more concerning since longer duration of these interventions may be required to reverse hepatic fibrosis.

Bariatric surgery

NAFLD, as a component of the metabolic syndrome, recognizes ectopic fat accumulation as a key pathogenetic event and is likely to benefit from surgery. The first bariatric procedure, the jejuno-ileal bypass, worsened liver disease because of severe malabsorption and bacterial overgrowth, and was replaced by Roux-en-Y gastric bypass, laparoscopic adjustable banding, vertically banded gastroplasty and biliopancreatic diversion. The efficacy and safety of these techniques on NAFLD has been recently reviewed (9). Steatosis improved or resolved in 91.6% of cases, steatohepatitis in 81.3%, fibrosis in 65.5%. NASH completely resolved in 69.5% of cases. Nevertheless, most available studies were observational or retrospective, some showing limited or no improvement in moderate-to-advanced fibrosis (112); a recent 5-year prospective study on morbidly obese subjects without advanced liver disease showed a significant worsening of mild fibrosis 5 years after surgery (113). Therefore, although promising, long-term effects of surgery need to be evaluated in homogeneous well-designed multi-center trials.

Insulin sensitizers: metformin and TZDs (Table S3)

Metformin improves insulin resistance by decreasing hepatic glucose production and increasing skeletal muscle glucose uptake. It also reduces hepatic expression of TNF-α, a mediator of hepatic insulin resistance and necroinflammation, increases FFA oxidation and suppress lipogenesis through AMP kinase activation (38). Following few fairly encouraging small uncontrolled trials (114,115), safety and efficacy of metformin in NAFLD was evaluated in four RCTs (two with post-treatment histology). (116–119). Overall, metformin was safe and well-tolerated in these studies, with no cases of lactic acidosis and gastrointestinal intolerance as the most common side effect, not generally requiring discontinuation of therapy. Despite its weight-losing properties (4–7% of initial body weight), metabolic benefits and a significant improvement in transaminases (112), metformin did not significantly improve ultrasonographic NAFLD (113) or histological steatosis, necroinflammation, NAS or fibrosis.(114,115).

The TZDs are agonists of the PPAR-γ. After withdrawal of troglitazone for hepatic toxicity, the second generation TZD pioglitazone and rosiglitazone significantly improved steatosis, necroinflammation and fibrosis in two uncontrolled trials, with 54% of patients not meeting histologic criteria for NASH after 48 weeks (120,121).

Subsequently, three RCTs evaluated pioglitazone and one RCT assessed rosiglitazone in NASH. Pioglitazone significantly improved steatosis and necroinflammation; fibrosis score also improved in one RCT (122–124). Rosiglitazone significantly improved steatosis, but not other histological features, although there was a significant

delay in progression of necroinflammation and fibrosis in the rosiglitazone arm (125).

Tiikkainen randomized drug-naive diabetic subjects to rosiglitazone or metformin for 16 weeks (126): body weight decreased with metformin but not with rosiglitazone. Despite similar improvements in HbA1c, insulin, serum FFA and hepatic insulin sensitivity, steatosis and ALT levels decreased (by 51% and by 31%, respectively) only with rosiglitazone, closely correlating with the 123% serum adiponectin increase.

In summary, TZDs showed significant benefits on metabolic profile, steatosis and necroinflammation, while the effects on liver fibrosis are inconsistent, possibly to the short trial duration. NASH and associated metabolic abnormalities relapse 1 year after discontinuation of TZDs, suggesting long-term treatment may be required (127). This issue raises major safety concerns related to weight gain, bone loss in postmenopausal women and elderly diabetics and to an apparently increased risk of cardiovascular events. Finally, the efficacy of TZD in diabetic subjects warrants further assessment, as most RCT enrolled non-diabetic subjects and diabetes predicted lack of histological response in one RCT (121). Further larger and longer duration RCTs enrolling diabetic subjects are warranted.

Lipid-lowering drugs

Atherogenic dyslipidemia is often associated with NAFLD as part of the metabolic syndrome. Therefore, the potential therapeutic role of lipid-lowering drugs in NAFLD has been explored. A controlled trial with the fibrate gemfibrozil 600 mg daily for 4 weeks showed some biochemical improvement in NAFLD, while clofibrate produced no biochemical or histological improvement (128,129). Probucol, a lipid-lowering agent with strong antioxidant properties, improved liver enzymes in NASH in a small RCT (130).

In uncontrolled trials HMG-CoA reductase inhibitors pravastatin and atorvastatin were safe, improved serum aminotransferases in NAFLD and showed also beneficial effects on necroinflammation in two studies (131–134). A recent RCT randomized 186 hyperlipidemic NAFLD patients to atorvastatin, fenofibrate, or both (135). All arms received lifestyle intervention. After 1 year, weight loss in all arms averaged 11–13%, but biochemical plus ultrasonographic regression of NAFLD was significantly higher with atorvastatin alone or in combination, than with fenofibrate. A weight loss >4% and concomitant use of orlistat or metformin independently predicted response to treatment. In another small placebo-controlled RCT, 1 year of simvastatin was safe but did not improve any histological feature in NASH (136).

An important issue regarding the use of statins in-patients with NAFLD is their potential hepatotoxicity. After analysing available evidence, a consensus panel stated concluded that patients with NAFLD are not at significantly increased risk of severe hepatic toxicity with standard doses of statins and these drugs can be safely used in-patients with NAFLD (137).

ω-3 polyunsaturated fatty acid (PUFA) supplementation improved biochemical and ultrasonographic steatosis in several uncontrolled trials (138–140). In two RCTs, evaluating PUFA plus diet vs. diet alone, PUFA significantly improved liver enzymes and ultrasonographic steatosis was reversed in an average of 23% patients. However, weight loss was nearly double in the treatment arm compared with controls in one RCT and was not reported in the other RCT, making difficult to assess the role of PUFA in the observed improvements (141,142). Safety and efficacy of PUFA in NAFLD are being evaluated in RCT ClinicalTrials.gov NCT00845845, NCT00323414, NCT00819338, NCT00760513, NCT00681408, NCT00230113.

Antioxidants and ursodeoxycholic acid (UDCA)

Because oxidative stress is believed to be the second hit leading to inflammation in NASH, antioxidant vitamins E and C have been evaluated in animal and human models, yelding controversial results. An analysis on an intention-to-treat basis of six RCTs concluded that despite the significant improvements in liver enzymes and minor adverse events, radiological and histological data are too limited to support or repudiate the use of antioxidants in-patients with NAFLD (143). In a 24-month RCT, the combination of vitamin E and UDCA for 24 months significantly improved steatosis, but not other histological features, compared with either agent alone, in NASH (144).

The UDCA exerts its effect by reducing the portion of hydrophobic bile acids contributing to oxidative stress. Several clinical trials, of which only one assessed histology and had a low-bias risk, have been conducted in NAFLD (145). No significant differences in the degree of steatosis, inflammation or fibrosis could be found between the treated and placebo arms. Because of the heterogeneity with respect to inclusion criteria, sample size, duration of treatment and outcome, the Cochrane analysis concludes that the data are insufficient to use UDCA in treatment of patients with NAFLD (146).

Endocannabinoid receptor antagonists

Rimonabant antagonizes cannabinoid type 1 (CB1) receptors in the central nervous system and the liver, reducing food consumption and caloric intake and inhibiting hepatic DNL and stellate cell activation (60).

In a subgroup of abdominally obese dyslipidemic patients from the An International Study of Rimonabant in Dyslipidemia With AtheroGenic Risk In Abdominally Obese Patients (ADAGIO)-Lipids trial, rimonabant significantly improved hepatic steatosis and all cardiometabolic

risk factors compared with placebo (147). Patients with a history of depression were excluded from the trial. Adverse effects leading to discontinuation of the drug included gastrointestinal, depressive (2.0% vs. 1.3% of placebo) and anxiety disorders (2.2% vs. 1.0%). Concern about the risk of suicide associated with depressive and anxiety disorders led food and drug administration (FDA) to deny approval of rimonabant in the USA.

Angiotensin II type 1 receptor blockers

Preclinical studies demonstrated a marked decrease in hepatic, insulin resistance, steatosis and fibrosis and stellate cell activation with angiotensin II type 1 receptor blockers (ARBs). Telmisartan and irbesartan possess also PPAR-γ agonism activity, thus improving insulin sensitivity experimentally (148,149).

In humans, angiotensin II type 1 receptor polymorphisms have been associated with the presence and severity of NAFLD (150).These findings and the frequent coexistence of hypertension with NAFLD prompted evaluation of ARBs in NAFLD.

Following the encouraging results of two uncontrolled trials with losartan (151,152), 54 hypertensive patients with NASH were randomized to telmisartan or valsartan (153). After 20 months, both agents improved blood pressure and steatosis to a similar extent, but telmisartan improved plasma lipids, insulin sensitivity, steatosis, necroinflammation and fibrosis more consistently than valsartan. Larger- and longer-duration RCTs will assess the role of ARB with PPAR-γ activity in the treatment of NASH.

Other agents

Given the role of TNF-α in necroinflammation and insulin resistance, anti-TNF therapy may be effective in the treatment of NASH. Pentoxifylline, a TNF-α inhibitor, significantly improved liver enzymes and insulin resistance in uncontrolled trials (154) and consistently reduced histological steatosis and necroinflammation in NASH in a recent RCT (43).

NASH is associated with small intestinal bacterial overgrowth and increased gut permeability (155). Bacterial endotoxins can stimulate hepatic inflammatory cytokine production and increase oxidative stress leading to liver injury. Probiotics improved aminotransferases and markers of lipid peroxidation in human NAFLD (156) and are being evaluated in ClinicalTrials.gov NCT00099723, NCT00808990, NCT00870012.

Incretin glucagon-like protein1 (GLP-1) analogues improve insulin secretion by stimulating pancreatic β-cell growth and insulin release. This agents improved hepatic steatosis and insulin resistance in ob/ob mice (157). Exenatide, a GLP-1 analogue, significantly improved transaminases in three RCTs enrolling diabetic subjects (158),

and its effect on liver histology in NASH are being tested in ClinicalTrials.gov NCT00529204 and NCT00650546.

Liver transplantation

Indications for liver transplantation in NASH-related cirrhosis are the same as other aetiologies. Compared with the latter, patients with NASH showed a trend for higher 1-year mortality, especially if having age ≥60 years, obesity, diabetes or hypertension (159). NASH recurs in 50% of patients within 4 years, depending on higher insulin resistance or steroid use, post-transplantation weight gain, diabetes, highlighting the importance of controlling metabolic risk factors to reduce disease relapse (160).

Concluding remarks

Future research should clarify cellular and molecular mechanisms regulating hepatocyte lipid partitioning and metabolism and underlying altered hepatic insulin action and lipoprotein metabolism and the increased low-grade inflammation/oxidative stress. Delucidating such mechanisms would provide the basis for understanding the progression from steatosis to NASH and the increased cardiometabolic risk of NAFLD.

Currently, a diagnosis of NAFLD should prompt a thorough metabolic and cardiovascular risk assessment to correct associated cardiometabolic risk factors, but there is no evidence that improving NAFLD would benefit cardiometabolic and liver-related morbidity and mortality in the long term.

There is also urgent need for effective and easy-to-use non-invasive methods to assess the severity of liver disease in NAFLD: while simple steatosis has a benign hepatological prognosis and may be managed with measures aiming at reducing cardiometabolic risk, NASH may progress to end-stage liver disease and requires early hepatological referral for experimental treatment and tight follow-up.

Despite the large number of drugs used to treat NASH, most studies are small-sized and of short duration without post-treatment histology. Therefore, there is no currently established treatment for NASH. Lifestyle intervention should be first-line treatment for all NAFLD patients: a ≥5% weight loss benefits steatosis and associated cardiometabolic risk factors, while more consistent weight loss may also improve necroinflammation and overall disease activity in NASH. Preliminary data show also that the combination of lifestyle changes with drugs (i.e. with TZDs or statins) may achieve sustained histological remission even after drug discontinuation (161).

Future adequately powered RCTs with histological end points, of appropriate duration will clarify long-term safety and efficacy of proposed treatments.

Conflict of interest statement

No conflict of interest was declared.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Figure S1. The flow of study selection regarding epidemiology, pathogenesis, diagnosis and treatment of NAFLD/NASH.

Table S1. Liver fat score and liver fat equation for the prediction and quantitation of hepatic steatosis ((82) of main text).

Table S2. Biomarker panels for non-invasive assessment of the severity of liver disease in NAFLD.

Table S3. Therapeutic trials of weight loss and insulin sensitizers in NAFLD.

Table S4. Optimal nutrient composition in-patients with NAFLD.

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