# NON-ALCOHOLIC FATTY LIVER DISEASE: AN EMERGING DRIVING FORCE IN CKD

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## Abbreviations:

AGE, advanced glycation end-products

AMPK, adenosine monophosphate-activated protein kinase

Ask-1, apoptosis signal-regulating kinase 1 (Ask-1 is also known as mitogen-activated protein kinase 5)

CETP, cholesterol ester transfer protein

CCL2, chemokine (C-C motif) ligand 2 (CCL2 is also referred to as monocyte chemoattractant protein 1 (MCP1))

CCR, chemokine receptor

FGF, fibroblast growth factor

FXR, farnesoid X receptor

GLP-1, glucagon like peptide 1

IL-6, interleukin 6

JNK, C-Jun-N-terminal kinase

LPS, lipopolysaccharide

mTOR, mechanistic target of rapamycin**/** mammalian target of rapamycin

NEFA, non-esterified fatty acids

NF-kB, nuclear factor-kB

Nrf 2, nuclear factor (erythroid-derived 2)-like 2

PPAR, peroxisome activated proliferated receptor

PAI-1, plasminogen activator inhibitor-1

PUFA, polyunsaturated fatty acids

PYY, peptide YY (PYY is also known as peptide tyrosine or pancreatic peptide YY 3-36)

ROS, reactive oxygen species

SCFA, short chain fatty acids

SREBP, sterol regulatory element binding protein

TGF-β, transforming growth factor-β

TMA, trimethylamine

TMAO, trimethylamine oxide

TNF-α, tumour necrosis factor-α

## 1. ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) is a lipid-related liver condition that may progress over time to increase the risk of cirrhosis, end-stage liver disease and hepatocellular carcinoma. The prevalence of NAFLD is increasing rapidly due to the global epidemic of obesity and type 2 diabetes mellitus (T2DM), and it has been predicted that NAFLD will become the most important indication for liver transplantation over the next decade. It is now increasingly clear that NAFLD not only affects the liver but also affects risk of developing other extra-hepatic diseases that have a considerable impact on health care resources. These extra-hepatic diseases include T2DM, cardiovascular disease and chronic kidney disease (CKD), and the “cross talk” between each affected organ or tissue with these diseases has the potential to further harm function and worsen patient outcomes. The aim of this review article is to discuss the diagnostic tests for confirming NAFLD, the epidemiology linking NAFLD to CKD, and the pathogenic mechanisms underpinning the link between NAFLD and CKD. This review will also discuss potential treatments for NAFLD as well as a pragmatic algorithm for case finding and diagnosing the severity of NAFLD, in patients with CKD.

## 2. INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) has become the most common chronic liver disease in high-income countries, affecting up to one third of the general adult population[1-3]. In addition, NAFLD is now the third most common indication for liver transplantation in the United States and is on a trajectory to become the most common[4]. Similarly, NAFLD is also the most rapidly growing indication for simultaneous liver kidney transplantation with poor renal outcomes[5].

Over the last 15 years, it has become increasingly evident that NAFLD is associated not only with liver-related mortality and morbidity, but there is now growing evidence that NAFLD is a multisystem disease that affects multiple extra-hepatic organ systems, including the cardiovascular system[6]. In recent years, recognition of the importance of NAFLD and its strong relationship with the metabolic syndrome has stimulated considerable interest in its putative prognostic impact on the risk of chronic kidney disease (CKD)[7]. CKD is a disease that causes high morbidity, mortality, and health care costs across the globe[8]. CKD is becoming increasingly common and in the United States, for example, more than 10% of the adult population (about 26 million people) and more than 25% of individuals older than 65 years have CKD[9].

NAFLD and CKD share multiple risk factors (abdominal obesity, insulin resistance, atherogenic dyslipidemia, hypertension and dysglycemia) and mechanistic pathways in their pathogenesis[7,10,11]. The existence of mechanistic pathways linking the liver and kidneys is also supported by the presence of the hepato-renal syndrome, which may develop in cirrhotic patients with portal hypertension.

Here, we review the accumulating body of clinical and experimental evidence supporting the existence of a link between NAFLD and CKD. We discuss the diagnostic tests for confirming NAFLD, the epidemiology linking NAFLD to CKD, and the pathogenic mechanisms underpinning the link between NAFLD and CKD. We also discuss potential treatments for NAFLD and an algorithm for case finding and diagnosing the severity of NAFLD in patients with CKD.

## 3. DIAGNOSIS AND EPIDEMIOLOGY OF NAFLD

### 3.1. Diagnosis

NAFLD is a clinico-pathological spectrum of liver diseases that encompasses simple fatty infiltration in more than 5% of hepatocytes (simple steatosis), fatty infiltration *plus* inflammation (non-alcoholic steatohepatitis, NASH), advanced fibrosis and, ultimately, cirrhosis that may progress to hepatocellular carcinoma[1,3].

Diagnosis of NAFLD is based on the following criteria: (1) hepatic steatosis on either imaging or histology, (2) no excessive alcohol consumption (a threshold of 20 g/day for women and 30 g/day for men is conventionally adopted), and (3) no competing causes for hepatic steatosis (e.g., virus, drugs, iron overload, autoimmunity)[1,3]. Liver biopsy remains the reference standard for diagnosing NASH and staging fibrosis in patients with NAFLD. However, this procedure is invasive, potentially risky, patient-unfriendly, and subject to sampling error; therefore, liver biopsy is not suitable for patient monitoring or for diagnosis in large cohorts of individuals[1,3].

Liver ultrasonography is the recommended first-line imaging modality for detecting NAFLD in clinical practice[1,3]. On ultrasonography, hepatic steatosis produces a typical diffuse increase in echogenicity (the so-called “bright liver”). Ultrasonography has a good diagnostic accuracy to detect the presence of mild and moderate-to-severe hepatic steatosis, demonstrating a sensitivity and specificity, respectively, of approximately 85% and 95% (when liver fat infiltration is at least 20-30%)[12]. Moreover, ultrasonography is relatively inexpensive and may help clinicians to exclude other causes of liver disease and identify any early signs of cirrhosis or portal hypertension. To date, T1-weighted dual-echo magnetic resonance imaging and proton magnetic resonance spectroscopy have the best diagnostic accuracy in defining hepatic steatosis. Proton magnetic resonance spectroscopy enables quantitative assessment of hepatic triglyceride content, has excellent reproducibility and sensitivity, but is resource intensive and cannot reliably discriminate simple steatosis from NASH[1,3].

Most patients with NAFLD have no symptoms or clinical signs of liver disease at the time of diagnosis, although some patients report fatigue and a sensation of fullness or abdominal discomfort; moderate hepatomegaly may be the only physical finding in most patients. A large part of patients with NAFLD display the typical features of metabolic syndrome (i.e., abdominal obesity, atherogenic dyslipidemia, hypertension, insulin resistance, glucose intolerance or type 2 diabetes mellitus [T2DM])[1,3]. The presence of mildly to moderately elevated levels of serum liver enzymes (serum aminotransferases

and gamma-glutamyltransferase) are the most common and often the only laboratory abnormality found in patients with NAFLD; other laboratory abnormalities (e.g., thrombocytopenia, increased bilirubin and prothrombin time) may be found in patients with more advanced forms of NAFLD (cirrhosis)[1,3]. However, serum liver enzyme levels are not reliable indicators for the screening and diagnosis of NAFLD and, therefore, they should not be used alone in clinical practice. Patients with fairly normal serum liver enzyme levels may display the full pathological spectrum of NAFLD[1,3].

A common clinical concern in patients with NAFLD is whether patients have simple steatosis or NASH and, more importantly, what the stage of hepatic fibrosis is and whether the level of fibrosis has increased over time. Such clinical concern is based on the fact that NAFLD patients with advanced fibrosis are at the greatest risk of developing complications of end-stage liver disease[1,3]. Although non-invasive methods require further validation, the various non-invasive biomarker tests could be useful for selecting those patients with NAFLD, who will require a liver biopsy. The sensitivity and specificity of these non-invasive tests for the assessment of advanced hepatic fibrosis have recently been described[13]. The NAFLD fibrosis score and the fibrosis (FIB)-4 score (that include in their equations routine clinical and laboratory variables, such as age, serum aminotransferases, serum albumin, platelet count, body mass index or diabetes status) are examples of validated nonproprietary clinical scores for estimating advanced liver fibrosis. The Enhanced Liver Fibrosis (ELF) test and the Fibrotest are examples of proprietary techniques that have also been proposed for the non-invasive assessment of advanced liver fibrosis based on panels of specific serum biomarkers[13].

Hepatic fibrosis can be also staged using the ultrasonography-based transient elastography (FibroScan®), which measures the velocity of a low-frequency elastic shear wave propagating through the liver. This velocity is directly related to tissue stiffness. The stiffer the tissue, the faster the shear wave propagates. The main limitation of ultrasonography-based transient elastography in clinical practice is its failure to obtain reliable liver stiffness measurements (approximately 20% of cases, mainly in obese patients), which diminishes its application in NAFLD[13,14].

Several other liver-elasticity-based imaging techniques are being developed, including 2D acoustic radiation force impulse imaging, shear-wave elastography and 3D magnetic resonance elastography[14].

**Figure 1** shows a possible pragmatic algorithm for the diagnosis and management of NAFLD in 'high-risk' individuals with metabolic risk factors or CKD. It is important to emphasize that there is currently an intense debate on these aspects, and that a validated, widely accepted, algorithm for the diagnosis and management of NAFLD in this group of 'high-risk' individuals does not exist yet. Therefore, this algorithm is a synthesis of the available evidence and guidelines[3,13] together with the authors' personal opinions.

### 3.2 Epidemiology

Estimates of NAFLD prevalence vary by the population that is studied (for example, studies in patients with different ethnicities, sex and comorbid conditions) and the sensitivity of the method used for diagnosis of the disease (i.e., biochemistry, imaging or histology).

A recent systematic review and meta-analysis of 86 studies (involving a total of about 8 million subjects from 22 countries) has estimated that 25% of the adult population in the world has NAFLD as diagnosed by imaging[15]. Although NAFLD was highly prevalent in all continents, the highest prevalence rates were reported from South America (31%) and the Middle East (32%), whereas the lowest prevalence was reported from Africa (14%). This meta-analysis has also confirmed previous findings of similarity in the prevalence of NAFLD between the Europe and United States (24%)[15]. The prevalence of NAFLD was much higher among 'high-risk' patient populations, such as patients with T2DM or severe obesity (in which NAFLD occurs in up to 80-90% of these patients). Another interesting finding of this meta-analysis was that the pooled regional prevalence estimates for NASH among patients with NAFLD who had an indication for biopsy were 64% for Asia, 69% for Europe, and 61% for North America, respectively[15]. On the other hand, NASH prevalence estimates among NAFLD patients without an indication for biopsy were approximately 7% for Asia and 30% for North America. Because of the small number of studies that contained data on NAFLD incidence, the results of this meta-analysis were obtained only for China, Japan and Israel. The pooled regional NAFLD incidence estimates for Asia and Israel were approximately 52 per 1000 and 28 per 1000 person-years, respectively[15].

## 4. EPIDEMIOLOGICAL STUDIES LINKING NAFLD TO RISK OF CKD

NAFLD is strongly associated with abdominal obesity, T2DM and other clinical features of metabolic syndrome[1,3,6]. Given these strong associations, it is therefore not surprising that

cardiovascular disease (CVD) is the leading cause of death in patients with NAFLD[3,6], and that there is also a link between NAFLD and CKD[7].

In the last 5 years, a number of cross-sectional community-based and hospital-based studies have consistently demonstrated that NAFLD, as diagnosed either by imaging[16-24] or by histology[25-29], is associated with an increased prevalence of CKD (defined as presence of decreased estimated glomerular filtration rate [eGFR] or abnormal albuminuria or overt proteinuria) in patients with NAFLD. In these studies, the prevalence of CKD in patients with NAFLD ranged from approximately 20% to 55% compared to 5%-30% in those without NAFLD[16-29]. Notably, in most of these studies the association between NAFLD and CKD was independent of common cardio-renal risk factors across a wide range of patient populations. The finding of a significant and independent association between NAFLD and early kidney dysfunction has also been confirmed in a single cohort of 596 children with overweight or obesity of whom 268 had NAFLD[30]. Recognition of this abnormality in the childhood or adolescence is clinically important because the treatment to reverse this process is most likely to be effective if applied earlier in the disease process. Finally, some smaller case-control studies that used liver biopsies to diagnose NAFLD have also shown that there is a significant, graded relationship between the histological severity of NAFLD (principally the hepatic fibrosis stage) and the presence of either decreased kidney function or abnormal albuminuria[25-29]. However, it is important to underline that none of these studies have used renal biopsy to examine the pathology of their CKD (so, it is currently unknown if NAFLD is associated with a specific type of kidney disease).

Although the cross-sectional associations between NAFLD and CKD are strong and consistent across different patient populations, the data on whether NAFLD *per se* is a new 'driving force' for the development and progression of CKD remains an issue of intense debate. The validation of NAFLD as an independent risk factor would have direct potential relevance for the primary prevention of CKD. For example, in a community-based cohort of nearly 8,400 nondiabetic and non-hypertensive South Korean men with normal kidney function and no overt proteinuria at baseline, who were followed-up for a mean period of 3.2 years, NAFLD (diagnosed by ultrasonography) was associated with an increased incidence of CKD (adjusted hazard ratio 1.60; 95% confidence interval [CI] 1.3 to 2.0) [31]. This finding was present after adjustment for body mass index, hypertension, insulin resistance, plasma C-reactive protein, baseline eGFR and other potential

confounding factors for CKD. Also, in the Valpolicella Heart Diabetes Study of 1,760 T2DM patients with preserved kidney function who were followed over a 6.5-year period, there was an increased incidence of CKD (defined as eGFR<60 ml/min/1.73 $m^2$ or overt proteinuria) in patients with NAFLD (diagnosed by ultrasound) (hazard ratio 1.69; 95% CI 1.3 to 2.6)[32]. This finding was present after adjustment for age, sex, body mass index, waist circumference, blood pressure, smoking, duration of diabetes, glycosylated hemoglobin, lipids, baseline eGFR, microalbuminuria and use of antihypertensive, hypoglycemic, antiplatelet and lipid-lowering medications[32].

In agreement with these findings, in a follow-up study of 261 type 1 diabetic patients with preserved kidney function and no overt proteinuria at baseline, who were followed for a mean period of 5.2 years, the presence of NAFLD on ultrasonography was associated with an increased incidence of CKD (hazard ratio 2.85, 95% CI 1.6 to 5.1). Adjustments for age, sex, duration of diabetes, hypertension, glycosylated hemoglobin and baseline eGFR did not appreciably attenuate this association. The results remained unchanged even after excluding those patients who had microalbuminuria at baseline. Notably, addition of NAFLD to traditional risk factors for CKD significantly improved the discriminatory capability of the regression models for predicting incident CKD[33].

A recent systematic review and meta-analysis of thirty-three studies (involving a total of nearly 64,000 individuals; 20 cross-sectional and 13 longitudinal studies) has examined the association between NAFLD and risk of CKD[34]. In this meta-analysis were included observational studies diagnosing NAFLD by biochemistry, imaging or histology, and defining CKD as either eGFR <60 ml/min/1.73 $m^2$ or proteinuria. Meta-analysis of the data from the cross-sectional studies indicated that NAFLD was associated with a two-fold increased prevalence of CKD (odds ratio 2.12, 95% CI 1.7 to 2.7). Meta-analysis of data from the longitudinal studies indicated that NAFLD was associated with a nearly two-fold increased risk of incident CKD (hazard ratio 1.79, 95% CI 1.7 to 1.9). Although only a few studies used biopsies to diagnose NAFLD, the presence of NASH was associated with a higher prevalence (odds ratio 2.53, 95% CI 1.6 to 4.1) and incidence (hazard ratio 2.12, 95% CI 1.4 to 3.2) of CKD than simple steatosis. Similarly, advanced hepatic fibrosis was associated with a higher prevalence (odds ratio 5.20, 95% CI 3.1 to 8.6) and incidence (hazard ratio 3.29, 95% CI 2.3 to 4.7) of CKD than non-advanced fibrosis. In all these analyses, the significant association between NAFLD and increased risk of CKD persisted after adjustment for diabetes status and other traditional risk factors for CKD[34].

Although the results of this updated meta-analysis provide robust evidence of a strong association between the presence and the severity of NAFLD with the risk of CKD, it is important to underline that the quality of published studies was not always high, and that causality remains to be proven in high-quality intervention studies. Moreover, it is also important to note that all these studies have used creatinine-based GFR estimating equations (which do not perform well in patients with obesity or cirrhosis), instead of direct GFR measurements to define CKD. Furthermore, no detailed information was available in these studies about specific renal pathology/morphology associated with NAFLD.

Further longer prospective and intervention studies in larger cohorts of patients with histologically confirmed NAFLD are needed to confirm these findings and determine whether NAFLD may selectively contribute to the pathogenesis of different types of kidney disease, and to elucidate whether improvement in NAFLD ultimately will prevent or delay the development and progression of CKD. Taken together, however, the published studies clearly suggest that patients with NAFLD are at high risk of having CKD and need more intensive surveillance and treatment to reduce their risk of developing CKD over time.

## 5. PATHOPHYSIOLOGIC MECHANISMS OF NAFLD AND CKD

When considering the pathophysiology of NAFLD and CKD, it is plausible that: a) there are common cardiometabolic risk factors that influence pathways in both the liver and kidneys; b) there are risk factors that influence pathways in the liver, and the consequent changes in the liver subsequently influence kidney structure and function; and c) there are risk factors that influence pathways in the kidneys, and the consequent changes in the kidneys subsequently influence liver structure and function. Each of these three possibilities is illustrated in **Figure 2**. This schematic figure highlights how diet, expanded ('dysfunctional') adipose tissue and intestinal dysbiosis may influence the liver and kidneys to cause NAFLD and CKD. The figure also illustrates the links between NAFLD and CKD; the relationships between T2DM and NAFLD; and the links between T2DM, CVD and CKD. **Figure 3** illustrates the potential cellular pathways, signalling molecules and factors influencing NAFLD and CKD. **Table 1** describes the properties and potential functions of molecules and pathways that are relevant to NAFLD and CKD. The table also describes the key potential effects of these molecules and pathways on NAFLD and CKD and also

describes the effects of modification (of these molecules and pathways) on NAFLD and CKD.

Although many different factors and pathways are illustrated in these figures and in table 1, a low-grade inflammatory state underpins the patient phenotype in many individuals with NAFLD, CKD, T2DM and CVD. However, that said, there is currently no convincing evidence that a low-grade inflammatory state initiates the development of each of these diseases. Many different factors are associated with a low-grade inflammatory response, and it is often difficult to know whether these different factors arise as a consequence of inflammation, or cause the inflammation. For example, in NAFLD, various factors may contribute to the development of liver lipid accumulation (that defines NAFLD). As the liver disease progresses, additional factors also occur e.g., insulin resistance and endothelial cell activation, and it is plausible that these additional factors (rather than the liver lipid) could be responsible for causing the hepatic inflammation. With deterioration in the liver condition, there is also the potential for the liver condition to more strongly influence extra-hepatic pathways and structure and function in other organs and systems. For example, with progression of NAFLD from simple steatosis to NASH, deterioration in the liver condition may further influence the development of CKD, T2DM and CVD. With NASH there is increased cytokine production, reactive oxygen species generation, and production of inflammatory mediators, lipopolysaccharide, insulin resistance, endothelial dysfunction and a tissue inflammatory infiltrate; all of which could influence CKD. It is beyond the remit of this review to discuss each of these factors and pathophysiological processes in detail; and therefore, we have focussed on those factors and pathways that the authors consider important in linking NAFLD and CKD. This section begins with a brief discussion of key factors involved in the pathogenesis of hepatic steatosis and NASH in NAFLD and proceeds to discuss factors linking NAFLD with CKD.

### 5.1 Pathophysiology of NAFLD

***Predisposing factors contributing to hepatic steatosis***

In NAFLD, several factors may contribute to liver lipid accumulation and the most common cause of liver lipid accumulation is an increased caloric intake exceeding the rates of caloric expenditure, with a consequent spillover of extra-energy in the form of non-esterified fatty acids (NEFA) from expanded visceral adipose tissue into ectopic fat depots, such as the liver.

Many different lipids can be found in NAFLD, but liver triglyceride accumulation is the lipid that is used to define the condition. Liver triglyceride accumulates when the rate of hepatic triglyceride synthesis exceeds that of hepatic triglyceride catabolism and triglyceride export as very low-density lipoprotein particles[3,6]. Approximately 60% of hepatic lipid derives from increased peripheral lipolysis of triglycerides (due to adipose tissue insulin resistance and failure to adequately suppress peripheral triglyceride lipolysis), while dietary fats and sugars contribute approximately 35-40%. The liver can also contribute to steatosis producing lipid from dietary carbohydrates by *de novo* lipogenesis[6]. The contribution of *de novo* lipogenesis to liver fat content is less than 5% in healthy individuals and may increase to approximately 25% in patients with NAFLD. For example, compared with individuals who have low liver fat, those with high liver fat have a marked increase in the fractional contribution from *de novo* lipogenesis to very low density lipoprotein (VLDL) fatty acid, with a much higher (approximately 3 fold) rate of production of VLDL-triglycerides derived from *de novo* lipogenesis[35].

Accumulating evidence suggests that heritability also plays a major role in determining the inter-individual variability in the susceptibility to NAFLD[1-3]. It has been estimated that genetic factors account for about of half of the variability in hepatic fat content, and fibrosis tends to be co-inherited with steatosis. Recent genome-wide association studies have begun to reveal the specific common genetic determinants of NAFLD (e.g., the p.I148M loss-of-function variant of the patatin-like phospholipase domain-containing protein 3 [*PNPLA3*] gene). With the presence of the *PNPLA3* I148M variant, individuals are at increased risk of NASH, cirrhosis and hepatocellular carcinoma[1-3].

***Predisposing factors contributing to NASH: diet, insulin resistance and adipose tissue inflammation***

Increased dietary fructose intake from increased sugary drinks in particular has become a major public health issue. Increased consumption of dietary fructose may not only increase hepatic *de novo* lipogenesis[36], increasing risk of NASH, but may also increase serum uric acid concentrations[37]. Hyperuricemia may not only increase risk of gout but urinary excretion of uric acid may also damage the kidneys further in patients susceptible to CKD development[37]. With increased dietary calorie intake, and net positive energy balance, expansion of intra-abdominal visceral adipose tissue releases increased amounts of NEFAs and proinflammatory molecules into the vasculature[38,39]. Adipose tissue inflammation is one of the earliest events that can promote systemic insulin resistance.

Proinflammatory pathways converge on two main intracellular transcription factor signalling pathways, the NF-kB pathway and the C-Jun-N-terminal kinase (JNK) pathway[40], and results in animal studies indicate that JNK-1 activation may be involved in hepatic insulin resistance[41]. Accumulation of diacylglycerol intermediates in hepatocytes impairs hepatic insulin signaling and fuels gluconeogenesis, promoting hyperglycemia and predisposing to T2DM development[6]. Increased amounts of circulating and intracellular NEFAs are also associated with an increase in nuclear factor-kB (NF-kB), leading to increased hepatic transcription of multiple proinflammatory cytokines and dysregulation of adipokine production by the adipose tissue, such as decreased levels of adiponectin, which may also contribute to NAFLD progression[6]. The development of hepatic necro-inflammation (NASH) may result in the further release of several pathogenic mediators into the systemic circulation from the steatotic and inflamed liver. Such mediators include reactive oxygen species, advanced glycation end-products (particularly in T2DM that is very common in NAFLD), C-reactive protein, plasminogen activator inhibitor-1, transforming growth factor-beta and other proinflammatory and procoagulant factors (**Figure 2).**

### 5.2 Pathophysiologic mechanisms linking NAFLD with CKD

***Visceral obesity, insulin resistance, inflammation and dysbiosis***

NAFLD and CKD may be influenced by visceral obesity, insulin resistance, inflammation and intestinal dysbiosis. (The potential role of intestinal dysbiosis as a mediator linking NAFLD, CKD, T2DM and CVD is discussed in more detail in 5.3 below). An increase in visceral obesity or ectopic fat accumulation, together with insulin resistance, may favour the development of T2DM, which in turn increases the risk of developing liver disease, kidney disease and vascular disease (**Figure 2**). It has been suggested that insulin resistance plays a pathogenic role in kidney disease progression by worsening renal hemodynamics by activation of the sympathetic nervous system, sodium retention and down-regulation of the natriuretic peptide system[42], making insulin resistance a possible mechanistic link between NAFLD and CKD. NF-kB pathway activation in NASH increases the transcription of a variety of proinflammatory genes that can amplify systemic chronic inflammation[43, 44]. Hence, the increased intra-hepatic cytokine production that occurs in NAFLD/NASH is likely to play a pathogenic role also in the development of extra-hepatic complications, such as CVD and CKD.

**Figure 2** highlights how dietary factors, expanded visceral adipose tissue and intestinal dysbiosis may influence both NAFLD and CKD, as well as the relationships between T2DM and NAFLD, and between T2DM and CVD with CKD. Although the presence of T2DM undoubtedly increases the risk of CVD in patients with NAFLD, several studies have shown that potential mediators of vascular and renal damage occur more frequently in patients with NAFLD, regardless of whether or not they also have T2DM[45-48]. Increased oxidative stress, reactive oxygen species and the inflammatory response are thought to be all important pathogenic factors that are not only involved in the development of NASH, but are also pathogenic factors for the development and progression of CKD. In trying to establish whether it is the liver *per se* in NAFLD that mediates an increase in CKD, a study in patients with T2DM, with or without persistent hepatic inflammation (due to chronic hepatitis B virus infection), suggests that it is the presence of liver inflammation that is a key mediator of increased risk of CKD. In this study patients with T2DM and chronic hepatitis B virus infection were more likely to develop end-stage renal disease than patients without hepatitis B virus[49]. Further support for the notion that liver inflammation (irrespective of its aetiology) is important in mediating a link between NAFLD and CKD is found in other studies that have investigated the known links between hepatitis C virus infection and atherosclerosis and kidney disease[50-52].

## ***Atherogenic dyslipidemia, hypercoagulation, endothelial activation and increased oxidative stress***

With NAFLD there is often also an atherogenic dyslipidemia (typically characterized by increased small dense low-density lipoprotein cholesterol particles, low levels of high-density lipoprotein cholesterol and increased plasma triglyceride concentrations) that potentially increases the risk of reno-vascular damage. Increased procoagulant factors and profibrogenic growth factors also occur with NAFLD (**Figure 1**). Moreover, the release of key components of the renin-angiotensin system that may contribute to the pathophysiology of hypertension, may also be involved in liver disease progression in NASH[53,54]. Often with chronic inflammation, enhanced reactive oxygen species and increased activity of coagulation pathways, a common factor is endothelial cell activation, and all of these factors may play a role in CKD development and progression[55-57]. Patients with NAFLD often also have hypoadiponectinemia and plasma adiponectin levels are inversely associated with the severity of NAFLD histology, independently of other important confounding factors. An interesting hypothesis supports a role for fetuin A and adiponectin in the pathogenesis of CKD. Fetuin-A is a liver-secreted protein that regulates

adiponectin levels, whereas adiponectin is an adipose tissue-secreted protein with anti-inflammatory and anti-atherogenic effects. Recent studies have suggested that decreased plasma adiponectin levels may reduce activation of the energy sensor 5' adenosine monophosphate-activated protein kinase (AMPK), which is important in stimulating proinflammatory and profibrogenic mechanisms in both hepatocytes and podocytes, the unwarranted side effect of which may be to produce end-organ damage (i.e., end-stage liver and kidney diseases)[58].

### 5.3 Intestinal dysbiosis: a potential mediator involved in linking NAFLD, CKD, T2DM and CVD?

Dysbiosis is a perturbation of the normal intestinal microbiota and with dysbiosis, both qualitative and quantitative changes in the gut microbiota occur. Dysbiosis may potentially influence NAFLD, CKD and obesity via multiple and complex mechanisms. Several of the potential mechanisms linking NAFLD and CKD to dysbiosis are illustrated in **Figure 4**. Dysbiosis that often occurs with obesity[59] has recently been described in patients with T2DM[60-63], NAFLD[64, 65] or CKD[66, 67]. For example, in NAFLD, *Bacteroides* species are independently associated with NASH and *Ruminococcus* species with significant liver fibrosis[65]. In T2DM and NAFLD there is often a "functional" dysbiosis, with changes in microbial species affecting the metabolic and proinflammatory pathways (such as *Akkermantia muciniphila* and *Faecalibacterium prausnitzii*) that affect gut oxidative stress and butyrate production[68-71]. Increased amounts of *Akkermantia muciniphila* are also associated with higher L-cell activity (i.e., the neuroendocrine cells in the small intestine) with resulting increased glucagon-like peptide-1 (GLP-1) production that may improve glucose tolerance and increase satiety[72]. There is also now some data suggesting that dysbiosis occurs in CKD and the most often reported changes in gut microbiome in CKD which are related to lower levels of *Bifidobacteriaceae* and *Lactobacillaceae* and to higher levels of *Enterobacteriaceae*[66].

Microbial fermentation of dietary fibre in the intestine by anaerobic bacteria, such as *Lactobacilli* and *Bifidobacteria,* forms short chain fatty acids (SCFA). SCFAs include acetate, propionate and butyrate that may influence lipogenesis and gluconeogenesis. Bacteria, such as those from the *Clostridium*, *Eubacterium*, and *Butyrivibrio* genera, are able to produce butyrate in the gut lumen at mM levels[73, 74] and these bacteria are also able to produce intermediates such as formate, lactate and succinate, which facilitate

further bacterial growth. Dysbiosis is also frequently associated with an increased production of endotoxins from Gram-negative bacteria that can damage the intestinal barrier, affect vitamin absorption, and increase gut permeability with the potential for lipopolysaccharide (LPS) to enter the portal and systemic circulation. LPS causes disruption of the gut intracellular tight junctions, favouring the release of cytokines and gut microbiota DNA into the circulation and, consequently, into the liver. LPS promotes also inflammation within the liver and the resulting systemic inflammation may contribute to an increased risk of CKD.

Primary bile acids, such as chenodeoxycholic acid and cholic acid, are influenced in three ways by gut microbiota to produce potentially harmful secondary bile acids, such as urodeoxycholic acid, deoxycholic acid and lithocholic acid: (1) deconjugation of primary bile acids to form unconjugated bile acids that are passively or actively absorbed and returned directly to the liver for reconjugation; (2) chenodeoxycholic acid is modified through epimerization to produce urodeoxycholic acid; and (3) bacterial 7α-dehydroxylase converts cholic acid to deoxycholic acid, and chenodeoxycholic acid to lithocholic acid in the colon. Secondary bile acids are highly hydrophobic and toxic, and increased concentrations in the liver have been linked to inflammation, cholestasis and carcinogenesis[75]. The influence of bile acid metabolism on the kidneys is uncertain but secondary bile acids may exert the following toxic effects that have the potential to influence the development and progression of NAFLD: (1) increased intestinal permeability with decreased expression of tight junctions; this allows transfer of endotoxin products directly to the liver; and (2) the hydrophobicity of secondary bile acids allows their interaction with the phospholipids in cell membranes of hepatocytes, inducing perturbations of mitochondrial membranes. Thus, there is evidence suggesting that subtle alteration of bile acid metabolism by intestinal microbiota could influence the development, progression and complications of NAFLD. Furthermore, evidence supporting the notion that modifications to bile acids can affect the liver, is also provided by the recent results of the Farnesoid X nuclear receptor (FXR) ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT) trial[76]. The FLINT trial has tested the effects of obeticholic acid, an FXR-agonist created by adding an ethyl group to chenodeoxycholic acid. In this clinical trial, the treatment with obeticholic acid produced an improvement in liver histology in approximately 45% of the patients with biopsy-proven NASH[76].

The intestinal microbiota also produces molecules such as trimethylamine (TMA), pCresoyl and indole from dietary nutrients such as choline, phenylalanine/tyrosine and tryptophan, respectively. After further metabolism in the liver by oxidation or sulphation, ionically charged water soluble molecules, such as trimethylamine-N-oxide (TMAO), pCresoyl sulphate and indole sulphate, are produced that can be excreted in the urine. Indole sulphate is cleared by the proximal tubules and is proinflammatory (associated with NF-κB activation) and, therefore, potentially toxic to the kidneys by increasing the risk of tubule-interstitial fibrosis[67]. Other examples of molecules produced by the intestinal microbiota that are potentially toxic and excreted in the urine, are phenyl acetic acid and hippuric acid. TMAO has been shown to induce liver oxidative damage and atherosclerosis. The potential influence of TMAO on the vasculature[77] may also result in decreased kidney function and it possible that increased systemic TMAO levels could also have an adverse effect on the kidneys to increase risk of CKD. Plasma levels of TMAO are increased in patients with CKD, are associated with poorer long-term survival outcomes; and in animal studies diets that increase the circulating levels of TMAO, may contribute to progressive renal fibrosis and dysfunction[78].

**N.B.: *Note to Editors* – Table 1 insertion here.**

## 6. MANAGEMENT AND TREATMENT OPTIONS FOR NAFLD

Currently, there are no approved pharmacological agents for the treatment of NAFLD. However, based on the complex, biological mechanisms discussed above, several pharmacological agents for the treatment of NAFLD are currently under investigation[118-120.] Promising novel agents with anti-inflammatory, anti-fibrotic or insulin-sensitizing properties (e.g., dual peroxisome-proliferator-activated receptor [PPAR]-alpha/delta agonists, dual chemokine receptor [CCR]2/CCR5 antagonists and fatty acid/bile acid conjugates), inhibitors of *de novo* lipogenesis (aramchol), fibroblast growth factor [FGF]-19 or FGF-21 analogues, and anti-fibrotic agents (anti-lysyl oxidase-like [anti-LOXL2] monoclonal antibodies) are also being tested in late-phase randomized clinical trials in NASH[120].

The therapeutic approach to patients with NAFLD is multifactorial[1,3,118,119], as summarized in **Figure 5**. The first approach is the treatment of overweight and obesity (especially through appropriate changes in lifestyles or bariatric surgery for severe obesity), the optimization of glycemic control in patients with established diabetes and the treatment of all co-existing cardiometabolic risk factors, possibly with the use of therapies that may

have potential beneficial liver effects. The main goals of treatment are: (1) to improve insulin resistance; (2) to reduce intra-hepatic fat infiltration; and (3) to avoid the progression of NAFLD/NASH to more severe histological forms (cirrhosis, liver failure and hepatocellular carcinoma).

To date, there are no large studies examining the use of medications and lifestyle modification in both NAFLD and CKD. However, because NAFLD and CKD share multiple cardiometabolic risk factors and common pathogenetic pathways, it is reasonable to assume that prevention and treatment strategies for NAFLD and CKD are similar, sharing the specific aims of improving insulin resistance and modifying all the coexisting cardiometabolic risk factors.

The mainstay of management for NAFLD is lifestyle intervention, which includes a hypocaloric diet and regular physical exercise with a 5-10% weight reduction associated with improvement in hepatic steatosis and necroinflammation[1,3,118,119]. Resistance exercise may be more feasible than aerobic exercise for NAFLD patients with poor cardiorespiratory fitness or for those who cannot tolerate or participate in aerobic exercise. Notably, in a cohort of 261 patients with histologically-proven NASH who were treated with lifestyle modification for 52 weeks, Vilar-Gomez *et al*. recently found that patients with NASH resolution or improved/stabilized hepatic fibrosis were more likely also to improve or stabilize their kidney function, compared to those without NASH resolution or with impaired fibrosis, when adjusted by weight loss categories[121]. Bariatric surgery, as a non-pharmaceutical effective treatment to decrease body weight in patients with severe obesity, markedly improves all histological lesions of NASH, including hepatic fibrosis[1,3,118,119]. Whilst bariatric surgery is undoubtedly effective, there are limitations including complications, patient acceptability, service availability and costs.

To date, pharmacotherapy for NAFLD should probably be reserved for patients with NASH (particularly for those with significant fibrosis or with the presence of metabolic risk factors), who are at the highest risk for disease progression[1,3,118,119]. However, no drug has currently been tested in phase III trials and is approved for NASH by regulatory agencies. Therefore, no specific therapy can be firmly recommended and any drug treatment would be off-label.

The most available evidence for the treatment of NAFLD is the use of pioglitazone (i.e., a highly selective PPAR-gamma agonist) in patients with biopsy-proven NASH. Randomized clinical trials have documented that pioglitazone treatment improves hepatic steatosis and necroinflammation, but not hepatic fibrosis, in patients with NASH and that its interruption may frequently determine the re-appearance of the liver damage[1,3,118,119]. Recently, a randomized, double-blind, placebo-controlled trial (including 101 patients with T2DM or pre-diabetes and biopsy-proven NASH randomly treated with pioglitazone, 45 mg/day, or placebo for 18 months, followed by an 18-month open-label phase with pioglitazone treatment) reported that 51% of patients treated with pioglitazone had resolution of NASH[122]. Pioglitazone treatment was also associated with reduced intra-hepatic triglyceride content and improved systemic and hepatic insulin sensitivity. All 18-month metabolic and histologic improvements persisted over 36 months of therapy[122]. However, despite these encouraging data, pioglitazone is not licensed for the treatment of NASH, and concerns regarding fluid retention, weight gain, risk of bone fracture, and to a lesser extent bladder cancer have meant that the chronic use of pioglitazone in patients with NAFLD/NASH remains limited.

As previously mentioned, an interesting novel agent is the insulin-sensitizer FXR ligand obeticholic acid. In a multicentre, randomised, placebo-controlled trial of 283 individuals with non-cirrhotic NASH, treatment with obeticholic acid (25 mg daily) for 72 weeks significantly improved the biochemical and histological features of NASH (including hepatic steatosis, necroinflammation and fibrosis) compared with placebo[73]. However, this drug is not well tolerated and may produce side effects, such as cholestasis with itching (in about one-quarter of treated patients), and a substantial increase in total and low-density lipoprotein cholesterol concentrations[73].

Preliminary evidence derived from some retrospective, observational studies and a randomized phase 2 clinical trial also suggests some beneficial effects of GLP-1 agonists (liraglutide and exenatide) in improving serum liver enzyme levels and histological features of NASH, although it is uncertain whether this benefit results from concurrent weight loss[3,100,118,119,123]. The most common adverse events leading to the discontinuation of GLP-1 agonists are gastrointestinal events. Further evidence is required to support the efficacy of these hypoglycemic drugs in NASH.

Studies using metformin for the treatment of NAFLD have produced conflicting results. Collectively, these studies suggested that metformin treatment has beneficial effects on serum liver enzymes and insulin resistance, but has no beneficial effect on liver histology. Thus, metformin is not currently recommended as a specific treatment for liver disease in patients with NAFLD/NASH[1,3,118,119].

A number of large-scale clinical trials have demonstrated that statins substantially reduce CVD morbidity and mortality in both primary and secondary prevention[124]. In the past, statin use in patients with NAFLD may possibly have been hampered owing to concerns of liver toxicity, although such concerns are not justified based on the currently available data[1,3]. Statins can be safely used for dyslipidemia in patients with NAFLD/NASH. Recent post-hoc analyses of randomized controlled trials have also suggested that the cardio-protective effect of statins is more pronounced among CVD patients with mild-to-moderate baseline elevations in serum aminotransferase levels[125,126]. Statins are safe and may also reduce CVD events and mortality in patients with NAFLD. Although there are few and controversial data on the effects of statins on liver histology in patients with NAFLD, a recent large case-control study has shown that statin use was associated with protection towards the full spectrum of liver damage in individuals at risk of NASH; however, the presence of the I148M PNPLA3 risk variant limited this beneficial effect[127]. Consistent with this view, increasing evidence suggests that statins are also associated with a reduced risk of hepatocellular carcinoma[128]. On the other hand, post-hoc analyses of randomized clinical trials have shown that atorvastatin may be nephroprotective[129,130]. To date, however, there are no large randomized clinical trials testing the long-term effects of statins on histological liver endpoints in patients with NAFLD. Ongoing and future studies will clarify whether statins might also have a direct beneficial role in NAFLD treatment[131]. Similarly, it would be also extremely interesting to examine the potential beneficial effects of the newer inhibitors of the proprotein convertase subtilisin kexin type 9 (PCSK9) on liver histology in NAFLD.

No randomized clinical trials have specifically examined the effects of different anti-hypertensive agents on liver histology in hypertensive patients with NAFLD/NASH. However, renin-angiotensin system inhibitors should be the first-line choice in the treatment of hypertensive patients with NAFLD. To date, the potential anti-fibrogenic effect of these drugs is increasingly recognized in both animal and human studies[50]. Some preliminary clinical trials demonstrated that losartan or valsartan improved insulin

resistance, serum liver enzymes and other surrogate markers of NASH, whereas telmisartan improved hepatic necroinflammation and fibrosis[132,133]. Moreover, in a cross-sectional study of 290 hypertensive patients with biopsy-proven NAFLD, the use of renin-angiotensin system blockers was associated with less advanced hepatic fibrosis, providing further evidence that the renin-angiotensin system may be involved in NAFLD pathogenesis[134]. Similarly, in another recent cross-sectional study of 191 CKD patients with and without NAFLD the use of renin-angiotensin system blockers was associated with a lower degree of liver stiffness as measured by ultrasonography-based transient elastography[135]. At present, however, there are no robust data with histological end-points, as a primary outcome, to formally comment on the effectiveness of renin-angiotensin system blockers as a specific treatment for NAFLD/NASH[1,3,118,119].

Given that increased oxidative stress occurs in NAFLD, another therapeutic option for NAFLD treatment may be to decrease oxidative stress by administration of an antioxidant, such as vitamin E[1,3,118,119]. In the PIVENS (Pioglitazone versus Vitamin E versus Placebo for the Treatment of Nondiabetic Patients with Nonalcoholic Steatohepatitis) trial, involving 247 non-diabetic patients with histologically confirmed NASH, the treatment with vitamin E (at a dose of 800 U/day for 96 weeks), as compared with placebo, was associated with a significant improvement in serum liver enzyme levels and some histological features of NASH[136]. However, before vitamin E can be recommended for the treatment of NASH, further evidence is required to support efficacy and, importantly, the safety of this fat-soluble agent.

Pentoxifylline has been also shown to decrease oxidative stress and inhibit lipid oxidation. Some small clinical trials have examined the use of pentoxifylline in NAFLD, documenting a decrease in serum liver enzymes and an improvement in hepatic steatosis, necro-inflammation and fibrosis[1,3,119]. These studies suggest that this drug may have some benefit in NASH and has a very good safety profile. However, until more definitive data are available, its impact on histologic features of NASH remains elusive.

Treatments with long-chain polyunsaturated omega-3 fatty acids (n-3 PUFA) are safe but it is still uncertain whether treatment with these agents in NAFLD confers a benefit. Specifically, it remains uncertain whether treatment with certain types of n-3 PUFA may be more beneficial than others. Additionally, the timing of the treatment in NAFLD may also be important, as it is conceivable that these agents could benefit liver fat only, and have

limited, or no, effects on hepatic necroinflammation or fibrosis in NASH. In a phase 2 double-blind, randomized, placebo-controlled trial, treatment with low-dosage (1800 mg/day) or high-dosage (2700 mg/day) ethyl-eicosapentanoic acid for 12 months had no significant effects on the histologic features of NASH[137]. In contrast, others have shown a benefit of n-3 PUFA treatment on liver fat assessed by magnetic resonance imaging (and have suggested that a greater benefit in NAFLD was associated with docosahexanoic acid treatment)[138-140]. Additionally, it has been suggested that the PNPLA3 148MM may attenuate any beneficial effect conferred by n-3 PUFA treatment in NAFLD[141], emphasizing that future clinical trials testing new potential treatments for NAFLD should also perhaps consider the influence of different genotypes to modify any treatment effect.

Interestingly, a recent Bayesian network meta-analysis combining direct and indirect treatment comparisons has assessed the comparative effectiveness of pharmacological agents for the treatment of NASH[142]. Collectively, nine randomized, controlled trials including 964 patients with biopsy-proven NASH, comparing vitamin E, glitazones, pentoxifylline, or obeticholic acid to one another or placebo, were identified. This meta-analysis revealed only moderate-quality evidence for glitazones, pentoxifylline and obeticholic acid to decrease hepatic necroinflammation and for pentoxifylline and obeticholic acid to improve hepatic fibrosis[141]. Taken together, these data do not allow for straightforward recommendations for drug treatment of this disease.

## 7. CONCLUSIONS

From a pathophysiological perspective, the liver and kidneys share a number of pathways that are intrinsically linked to each other. Mounting data now indicate that the prevalence of CKD is markedly increased among patients with NAFLD, and that the presence and severity of NAFLD is associated with an increased incidence of CKD, independently of multiple cardio-renal risk factors (including the features of metabolic syndrome).

Taken together, these findings suggest that patients with NAFLD should be screened for CKD even in the absence of other risk factors for the disease, and that better treatment of NAFLD might also help to prevent or slow the development and progression of CKD. CKD occurs also in patients with T2DM. To date, however, there are no specific criteria or characteristics that can be used to distinguish CKD in patients with T2DM, from CKD in patients with NAFLD who do not have T2DM.

We suggest that patients with NAFLD and renal dysfunction should be treated early by a multidisciplinary team, involving specialists in hepatology, diabetology and nephrology. However, in order to assess definitively the existence of a causal relationship between NAFLD and the development and progression of CKD, we emphasise that large randomized, double-blind, placebo-controlled trials with incident CKD outcomes that focus on treatments for liver disease in NAFLD are needed.

## 8. KEY POINTS

- Accumulating evidence indicates that the presence and severity of NAFLD is strongly associated with an increased prevalence of CKD.
- The presence and severity of NAFLD predicts the development of incident CKD, independently of traditional cardio-renal risk factors.
- Experimental evidence suggests that NAFLD exacerbates hepatic and peripheral insulin resistance, confers a predisposition to atherogenic dyslipidemia, and causes the release of several proinflammatory, procoagulant, prooxidant and profibrogenic mediators that play important roles in the pathophysiology of CKD.
- However, despite the growing evidence linking NAFLD to CKD, it has not been definitively established whether a causal association exists.
- These findings call for a more active and systematic search for CKD in patients with NAFLD.

## 9. GLOSSARY TERMS

NAFLD = a clinico-pathological spectrum of liver diseases that encompasses simple fatty infiltration in more than 5% of hepatocytes (simple steatosis), fatty infiltration plus inflammation (steatohepatitis, NASH), fibrosis and, ultimately, cirrhosis.

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## Competing interests statement

The authors declare no competing financial interests.

## Author Contributions

Both authors have contributed equally to write this article.

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## FIGURE LEGENDS

**Figure 1. Proposed pragmatic algorithm for the assessment and disease severity monitoring in the presence of suspected NAFLD and metabolic risk factors or CKD.** The algorithm has been developed by the authors using both available evidence and guidelines, as well as personal opinion where uncertainty exists and evidence was not available.

**Figure 2. Potential factors linking diet, adipose tissue accumulation, and intestinal dysbiosis to NAFLD and CKD, and links between NAFLD and CKD and T2DM and cardiovascular disease.**

*Figure 2. Abbreviations:*
AGEs, advanced glycation end-products; CETP, cholesterol ester transfer protein; FGF-23, fibroblast growth factor-23; IL-6, interleukin 6; LPS, lipopolysaccharide; NEFAs, non esterified fatty acids; PAI-1, plasminogen activator inhibitor-1; ROS, reactive oxygen species; SCFAs, short chain fatty acids; TNF-α, tumour necrosis factor-α; TGF-β, transforming growth factor-β; TMAO, trimethylamine oxide.

**Figure 3. Cellular pathways, signalling molecules and factors influencing NAFLD and CKD.**

*Figure 3. Abbreviations:*
AMPK, 5' adenosine monophosphate-activated protein kinase; Ask-1, apoptosis signal-regulating kinase 1 (Ask-1 is also known as mitogen-activated protein kinase kinase kinase 5 (MAP3K5)); CCL2 chemokine (C-C motif) ligand 2 (CCL2 is also referred to as monocyte chemoattractant protein 1 (MCP1)); CCR, chemokine receptor; FGF-21, fibroblast growth factor-21; JNK, C-Jun-N-terminal kinase; mTOR, mechanistic target of rapamycin/ mammalian target of rapamycin; PPARs, peroxisome activated proliferated receptors α, γ and δ; NF-kB, nuclear factor-kB; Nrf 2, nuclear factor (erythroid-derived 2)-like 2; FXR, farnesoid X receptor; SREBPs, sterol regulatory element binding protein.

**Figure 4. Dysbiosis: potential molecules and pathways linking perturbations of gut microbiota with NAFLD, CKD and obesity.**
L cells are enteric endocrine cells that secrete peptides capable of stimulating insulin secretion and modulating satiety. Short chain fatty acids (SCFAs) are produced from the fermentation of carbohydrate and are able to modify gluconeogenesis (propionate), lipogenesis (acetate) and autophagy of the colonic epithelium (butyrate). Butyrate provides an energy source for colonic cells protecting against autophagy and is a critical modulator of the colonic inflammatory response.

*Figure 4. Abbreviations:*
SCFAs, short chain fatty acids; LPS, lipopolysaccharide; TMA, trimethylamine; TMAO, trimethylamine oxide; PYY; peptide YY (PYY is also known as peptide tyrosine or pancreatic peptide YY 3-36; GLP-1, glucagon like peptide 1.

**Figure 5. Management strategies of NAFLD.**

**Table 1. Summary of the effects of changes in putative molecules/pathways relevant to the pathogenesis of NAFLD and CKD.**

| | | Properties/potential functions of molecules/pathways relevant to liver and kidneys | Key potential effects in NAFLD and effects of modification | Key potential effects in CKD and effects of modification |
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| **Molecules/pathways and direction of changes relevant to NAFLD and CKD** | | | | |
| **Nutrients and related molecules** | **Increased Fructose Intake & Uric acid** | Increased dietary fructose may decrease cellular ATP, increase IMP and increase purine metabolism resulting in increased serum uric acid concentrations. | Increased lipogenesis (fructose). | Renal toxicity/calculi. Inflammasome activation/macrophage accumulation (uric acid). |
| | **Decreased Vitamin $D_3$** | Insulin resistance (increased hepatic lipogenesis and gluconeogenesis. Kupffer cells: toll-like receptor activation (TLR 2, 4 and 9). Stellate cells: down-regulation of vitamin D receptor. | Hepatic inflammation and fibrosis[79, 80]. | Glomerular and tubular effects. Podocyte injury. Proteinuria[81]. |
| **Nuclear transcription factors and related molecules** | **Decreased PPAR-alpha, gamma and delta activity (**nuclear transcription factors) | PPAR-alpha is the master regulator of hepatic beta-oxidation (mitochondrial and peroxisomal) and microsomal omega-oxidation. PPAR-delta is crucial to the regulation of forkhead box-containing protein O (FOXO) subfamily-1 expression and, hence, the modulation of enzymes that trigger hepatic gluconeogenesis. In addition, PPAR-delta activates hepatic stellate cells aiming to the hepatic recovery from chronic insults. Decreased fatty acid oxidation (PPAR-alpha) Kupffer cell and stellate cell activation (PPAR-delta) Increased triacylglycerol in adipose tissue (PPAR-gamma)[82]. | Decreased free fatty flux to liver (decreased hepatic di-acyl glycerol and tri-acylglycerol) (mainly PPAR-gamma). | Increased matrix (PPARs alpha, gamma and delta). Profibrogenic (PPAR-alpha[83] and delta[84]. |
| | **Nuclear erythroid 2–related factor 2 (Nrf-2)** | Nuclear transcription factor ubiquitously expressed in human tissues, especially the liver. Regulates the basal and stress-inducible expression of a battery of genes encoding key components of the glutathione-based and thioredoxin-based antioxidant | Regulates the expression of several antioxidant and detoxifying enzymes and has direct, metabolic, anti-inflammatory and pro-autophagic actions. Activation of Nrf-2 may attenuate fibrosis progression[85]. | Modifying redox state may benefit CKD[86]. |

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| | | systems, as well as aldo-keto reductase, glutathione S-transferase, and NADPH drug metabolising isoenzymes[85]. | | |
| | **Decreased FXR activity** (nuclear transcription factor) | Increased bile acid and cholesterol synthesis. | Proinflammatory and profibrotic activities. FXR agonist activity may be beneficial in some with NASH[76]. | Proximal tubule inflammation. Profibrogenic. |
| | **Increased free cholesterol and SREBP-1c, and SREBP-2 activity** (nuclear transcription factors) **Increased CETP activity (CETP transport of neutral lipids) Increased Syndecan-1** (transmembrane heparan sulfate proteoglycan bound to hepatocyte membranes). | Increased fatty acid synthesis and cholesterol synthesis (SREBP-1c and SREBP 2, respectively). CETP secretion (Kupffer cells). Increased CETP activity and Syndecan-1 cause atherogenic dyslipidemia. Syndecan-1 is a regulator of triglyceride-rich lipoprotein clearance[87]. | Cholesterol retention in liver and kidney cells. CETP activity associated with NASH[88]. Defective syndecan-1 sulphation increases shedding and impaired triglyceride-rich lipoprotein clearance [89]. | Cholesterol retention in liver and kidney cells. Defective syndecan-1 sulphation increases shedding and atherogenic dyslipidemia in CKD[89]. |
| **Energy sensors and related molecules** | **Decreased 5' AMPK activity** (ubiquitous kinase and energy sensor responds to increase in the AMP/ATP ratio). **Decreased adiponectin** (Adiponectin binds to these Adipo-R1 and R2 receptors and signals via stimulation of 5′-AMP-activated protein kinase (AMPK) and potentially other intracellular pathways)[90]. | Increased hepatic gluconeogenesis. Decreased inflammation (Kupffer cells)[91]. Profibrogenic stellate cells. | Increased hepatic glucose production and inflammation[91]. | Effects on podocytes, endothelium, proximal tubular cells to decrease glomerular membrane integrity and endothelial activation. The adiponectin–AMPK pathway may play a crucial role in both the maintenance of podocyte function and the inhibition of reactive oxigen species[90]. |
| | **Increased mTOR (mTORC1 and 2) activity** mTOR is a serine/threonine kinase that responds to changes in cellular nutrient levels. There are two distinct signaling molecular complexes, mTOR complex 1 (mTORC1) and mTORC2. **Decreased adiponectin and increased fetuin A** | Cellular nutrient sensor signalling molecules. Hepatic secretion of fetuin A regulates adiponectin secretion by adipose tissue [92]. Adiponectin has many anti-inflammatory activities and suppresses tumour necrosis factor-alpha (TNFα), a cytokine of key importance in NAFLD. The anti-inflammatory effects of adiponectin are also exerted by induction of the anti-inflammatory cytokines interleukin-10 (IL-10) or IL-1 receptor | mTORC1 promotes anabolism by stimulating synthesis of proteins, lipids, and nucleotides and blocking catabolism. mTORC1 activation in NAFLD and CKD inhibits autophagy and promotes insulin resistance, ectopic lipid accumulation, lipotoxicity, and proinflammatory monocyte recruitment in the liver and kidney. mTORC1 inhibition may decrease lipid, inflammation and fibrosis in | See NAFLD. Activation of proinflammatory pathways, up-regulation of adhesion molecules, endothelial dysfunction (adiponectin). Pro-fibrogenic liver and kidney (podocytes) (low adiponectin). Adiponectin plays a protective role to reduce albuminuria by directly affecting podocyte function via the AMPK-Nox4 pathway[90]. |

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| | | antagonist and up-regulation of heme-oxygenase-1[92]. | NAFLD and CKD.<br><br>Adiponectin is able to regulate steatosis, insulin resistance, inflammation and fibrosis. NAFLD is also associated with decreased liver expression of the two adiponectin receptors (Adipo-R1 and R2) thereby contributing to a state of hepatic adiponectin resistance[92]. | |
| **Regulator of growth factors, cytokines and cell death** | **Apoptosis signal regulating kinase-1 (ASK-1)**<br>Serine/threonine kinase belonging to the mitogen-activated protein kinase (MAPK) family. | | Inflammation and fibrogenesis (activated in response to stresses, like reactive oxygen species (ROS), tumour necrosis factor-alpha (TNF-alpha), lipopolysaccharide (LPS), and endoplasmic reticulum (ER) stress).<br>Activates downstream terminal MAPK kinases p38 and c-Jun N-terminal kinase (JNK), which promote insulin resistance, cell death, proinflammatory cytokine/chemokine production, and fibrogenesis. | Impact on the kidneys is less clear. |
| **Chemokines and receptors** | **Increased chemokines and receptors (CCR 2/5 Receptors)**<br>The control of cell migration by chemokines involves interactions with two types of receptors: seven trans-membrane chemokine-type G protein-coupled receptors and cell surface or extracellular matrix-associated glycosaminoglycans[93]. | Modify leukocyte migration into tissues and consequent inflammation, tissue remodelling and fibrosis[94, 95]. | Hepatic secretion of CCL2 attracts proinflammatory cells to liver. Inhibition of CCL2/CCR2 decreases inflammation and fibrosis in liver. Pharmacological inhibition of monocyte recruitment using a CCL2-inhibitor, accelerated regression of liver fibrosis in two independent experimental models[94]. | In the kidneys, tubular cells and podocytes secrete chemokines CCL2 and CCL5 in response to diverse proinflammatory stimuli to promote tubulo-interstitial inflammation and fibrosis, which are reversed by chemokine antagonists[95]. |
| **Lectins** | **Decreased Galectin 3** | Lectin (carbohydrate binding protein) expressed immune and epithelial cells and regulates cell proliferation, apoptosis, and cell adhesion[96]. | Galectin-3 is up-regulated in the liver and kidney of patients with NASH and CKD. Inhibitor ameliorates diet-induced NASH[97]. | Receptor function for advanced glycation end-products (AGEs) and advanced lipoxidation end-products (ALEs) to potentially |

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| | | | | damage end organs[96]. Galectin-3 may aid resolution of inflammation and fibrogenesis. Decreased galectin-3 may impair removal of AGEs and ALEs[96].<br><br>Galectin 3 is positively associated with impaired renal function. |
| **Gastrointestinal-mediated effects** | **Altered intestinal microbiota** | Alteration of gut hormone production affecting glucose control.<br>Alteration of short chain fatty acid production, influencing glucose and lipid metabolism.<br>Translocation of bacterial lipopolysaccharide (LPS) influencing gut permeability, vitamin absorption and hepatic mitochondrial function contributing to liver inflammation; and perturbation of bile acid and trimethyl-amine (TMA) metabolism, increasing liver toxicity and cardiovascular risk. | Decreased Bacteroidetes, Lactobacillaceae, and Prevotellaceae families and increased intestinal permeability[98, 99].<br>*Bacteroides* species are independently associated with NASH and *Ruminococcus* species with significant liver fibrosis [65]. In NAFLD there is often a "functional" dysbiosis, with changes in microbial species that affect metabolic and inflammatory pathways such as *Akkermantia muciniphila*, and *Faecalibacterium prausnitzii that* affect gut oxidative stress and butyrate production) [68-71]. Increased amounts of *Akkermantia muciniphila* is also associated with higher L-cell activity and resulting increased production of glucagon-like peptide -1 (GLP-1) that improves glucose tolerance and increases satiety [72]. | Indoxylsulfate, p-cresyl sulfate, and trimethylamine-N-oxide (TMAO) are associated with CKD[78, 100-102]. |
| | **Decreased Incretins, e.g. GLP-1 agonists** | Peptide secreted by L cells (neuroendocrine cells) small intestine. Increased insulin secretion, improved glucose tolerance and decreased appetite. | Weight loss, improvement in NASH in 45% of treated patients. GLP-1 agonist activity may be beneficial in NASH[103]. | Treatment with GLP-1 agonists decreased activation of the renin–angiotensin system [104] and renal anti-inflammatory, antifibrotic and antioxidative effects [105 106]. |
| **Signalling molecules regulating tissue** | **Decreased FGF-21** | FGFs are signalling proteins that regulate embryonic development, | FGF-21 administration ameliorates adipose and hepatic | FGF-21 administration has improved experimental |

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| **regeneration and metabolism** | | tissue regeneration, and diverse metabolic functions by binding extracellularly to four cell surface tyrosine kinase FGF receptors (FGFRs 1–4)[107]. | insulin sensitivity, suppresses hepatic gluconeogenesis and lipogenesis, and enhances free fatty acid (FFA) oxidation and mitochondrial function. FGF-21 has anti-inflammatory and anti-fibrogenic activity by inhibiting the key nuclear factor kB (NF-kB) and transforming growth factor-beta (TGF-beta)[108]. | NASH[109] and CKD [107, 108]. |
| **Glucose transporters** | **Sodium-glucose cotransporter-2 (SGLT2)** | SGLT2 expressed in the S1 segment of the renal proximal tubule and regulates glucose reabsorption from tubular fluid. | SGLT2 inhibitors prevented diet-induced hepatic steatosis[110], necroinflammation and fibrosis, independently of anti-hyperglycemic action[110, 111]. | SGLT2 inhibitors block the activity of the SGLT2 protein, leading to glycosuria and decreased plasma glucose levels.<br>SGLT2 inhibitors have decreased inflammatory and fibrogenic responses, oxidative stress, and cell apoptosis in diverse experimental models of CKD[112]. |
| **Regulators of expression and translation of genes** | **miRNAs**<br>(miRNAs regulate translation (increased) with poor binding to mRNAs or repress gene expression with tight binding to mRNAs)[113]. | Ubiquitous modifiers of gene function. | Possible role for hepatic miRNAs in the pathogenesis of NAFLD-related fibrosis[114, 115].<br><br>Anti-miRNA21 antisense oligonucleotides induced weight loss, normalized metabolic dysregulation, and improved hepatic and renal inflammation and fibrosis, effects at least partly mediated by PPAR-alpha up-regulation[116, 117]. | Anti-miRNA21 antisense oligonucleotides induced weight loss, normalized metabolic dysregulation, and improved renal inflammation and fibrosis[113]. |