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Managing the Burden of Non-NASH NAFLD

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Abstract

Purpose of Review—The purpose of this review article is to raise awareness of the significance of steatosis that exist within the spectrum of nonalcoholic fatty liver disease (NAFLD). While the impact of nonalcoholic steatohepatitis (NASH), and its potential for histologic progression to cirrhosis and hepatocellular carcinoma is widely appreciated, the impact of non-NASH NAFLD (steatosis) on morbidity and mortality is less well recognized.

Recent Findings—NAFLD is a spectrum of hepatic pathology with a rising prevalence worldwide. Steatosis without fibrosis carries a low risk of progression to cirrhosis but likely confers an increased risk of diabetes mellitus and cardiovascular disease.

Summary—About a quarter of the world population is affected by NAFLD. NAFLD represents a burden to affected individuals, economics of the health care system and contributes significantly to morbidity and mortality worldwide. An increased level of awareness and knowledge about risk factors and diagnostic strategies is needed to identify patients affected with disease.

Keywords

nonalcoholic fatty liver disease; nonalcoholic steatohepatitis; insulin resistance; cardiovascular disease; weight loss

Introduction

Nonalcoholic fatty liver disease (NAFLD) is now the most common form of liver disease worldwide. It represents a spectrum of disorders affecting the liver that is rapidly becoming not only a significant indication for liver transplantation and cause of liver-related morbidity

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Compliance with Ethics Guidelines

Conflicts of Interest

Christopher J. Danford, Jorge E. Sanchez, and Kathleen E. Corey each declare that they have no conflicts of interest.

Human and Animal Rights Informed Consent

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and mortality, but also a significant cause of extrahepatic morbidity and mortality. NAFLD is defined as evidence of hepatic steatosis (>5%) by imaging or histology and the absence of other causes of steatosis such as significant alcohol consumption, medications or hereditary disorders [1]. It exists along a spectrum of disease from a state of steatosis to advanced disease states: steatosis with fibrosis or non-alcoholic steatohepatitis (NASH) with or without fibrosis and finally, cirrhosis. Steatosis, in the absence of fibrosis, represents the most benign state within the spectrum of disease and carries little to no risk of progression. However, steatosis confers an increased risk of diabetes mellitus and cardiovascular disease and recent data suggest that steatosis with fibrosis, in the absence of NASH, is associated with risk of progressive liver disease.

Epidemiology

The prevalence of NAFLD is estimated to be 25% worldwide and 24% in the North America [2]. Populations with higher prevalence of NAFLD include those with obesity, the metabolic syndrome (central obesity, insulin resistance, dyslipidemia, hypertension) and diabetes mellitus. In addition, NAFLD prevalence varies by ethnicity with the highest rates seen in Hispanics [3]. In contrast to other causes of chronic liver disease such as viral hepatitis infections and alcoholic liver disease, which have remained stable in the last decades, the prevalence of NAFLD has steadily increased [4]. Given that the prevalence of many of these risk factors are expected to increase, especially obesity, it is likely that the prevalence of NAFLD will continue to rise and contribute greatly to the burden of chronic liver disease worldwide.

NAFLD is associated with a significant burden to the affected individual and the healthcare system. Affected individuals often complain of fatigue and abdominal pain which can limit physical function, affect job stability and reduce quality of life [5]. The number of patients utilizing healthcare resources for NAFLD and the many associated comorbidities continue to rise. Younossi et al studied the economic burden of NAFLD in the United States and estimated the annual burden associated with all incident and prevalent NAFLD cases to be $103 billion with a 10-year economic burden of up to about $1 trillion after adjusting for annual growth in prevalence of obesity [6].

Pathophysiology

Nonalcoholic fatty liver disease is often thought of as the hepatic manifestation of the metabolic syndrome. The pathophysiology of NAFLD is a complex interaction between genetic, environmental, and gut microbial modulators resulting in the excessive accumulation of triglycerides in hepatocytes. Accumulation of hepatic triglycerides results from an imbalance between uptake from dietary intake, plasma free fatty acids (FFA) flux from adipose tissue, and hepatic *de novo lipogenesis* (DNL) and export in the form of very low density lipoprotein (VLDL), FFA oxidation, and phospholipid synthesis [7].

Dietary intake, plasma FFA flux from adipose tissue, and DNL contribute 15, 59, and 26% respectively to hepatic lipid uptake in NAFLD [8]. Multiple studies in mice have shown increased hepatic steatosis in mice fed high-fructose and high-trans fat diets [9][10]. In

addition, when this diet is withdrawn, steatosis reverses [11]. High saturated fat and cholesterol diets also lead to and steatosis [12][13]. A similar diet high in unsaturated fat and cholesterol and low in polyunsaturated fat has been demonstrated in human subjects with NASH and is associated with increased insulin resistance and other features of the metabolic syndrome [14].

Circulating plasma FFAs are largely derived from lipolysis from adipose tissue. Hepatocyte FFA uptake is largely a function of plasma FFA concentration and transport proteins. NAFLD patients are often obese with hyperlipidemia and have upregulation of fatty acid transport proteins, resulting in a significant contribution of plasma FFA flux from adipose tissue to hepatic steatosis [8][15].

De novo lipogenesis (DNL), regulated by insulin and glucose levels, is also increased in NAFLD. Hepatic insulin resistance and hyperglycemia result in increased DNL. Hyperglycemia activates the transcription factor, carbohydrate responsive element binding protein (ChREBP), which in turn activates liver pyruvate kinase resulting in increased fatty acid production [16]. Hyperinsulinemia upregulates another transcription factor, sterol regulatory element-bind protein-1c (SREBP-1c), which induces expression of lipogenesis genes resulting in increased DNL [17][18].

Changes in the gut microbiome have also been associated with the metabolic syndrome and NAFLD. When fed a high-fat diet, most C57BL/6J mice develop steatosis and other features of the metabolic syndrome (responders), while some mice did not (non-responders). When the fecal microbiota of those responder mice were transplanted into non-responders, they developed similar features of the metabolic syndrome and upregulation of transcription factors ChREBP and SREBP-1c [19]. The exact mechanism through which the gut microbiome exerts its effects on hepatic steatosis is not clear. Increased intestinal permeability resulting in liver inflammation and impaired insulin signaling is suggested by some studies associating small intestinal bacterial overgrowth (SIBO) in NAFLD, but results have been inconsistent [20][21][22]. Gut bacteria may also play a role in increasing free fatty acid absorption and efflux to the liver. Germ-free mice transplanted with the microbiome from conventional mice have increased monosaccharide absorption, increased DNL, and suppress Fasting-induced adipocyte factor (Fiaf), an intestinally-produced circulating lipoprotein lipase inhibitor, resulting in increased triglyceride storage [23][24]. Colonic bacteria are also involved in directly increased fatty acid production through the fermentation of non-digestible carbohydrates into short-chain fatty acids (SCFA) [25]. In a human study, subjects with metabolic syndrome who received fecal transplant from lean donors had a non-significant lowering of SCFA levels compared to those who received autologous transplants as well as a significant increase in butyrate-producing bacteria [26].

Increasingly, genetic variations that affect lipid metabolism are being increasingly recognized as playing a crucial role in the development of NAFLD and the discrepancy seen between rates of the metabolic syndrome and prevalence of NAFLD among different ethnicities [27]. Several genome-wide association analyses have shown that a missense mutation in the *PNPLA3* gene, with highest prevalence in Hispanics, is associated with insulin resistance and increased risk of NAFLD [28][29]. Another genome-wide association

analysis showed a variant in the gene *TM6SF2* confers increased risk of NAFLD and is associated with increased hepatic triglycerides and decreased VLDL production [30]. Variants in the apolipoprotein C gene are also associated with NAFLD in a population of Asian Indian men resulting in increased fasting and postprandial hypertriglyceridemia and resultant hepatic steatosis [31].

Identification of Patients with NAFLD

The majority of patients with NAFLD are asymptomatic or have nonspecific symptoms (fatigue, nausea, abdominal pain) and have normal and mild abnormalities on physical exam (hepatomegaly, obese). Most patients are diagnosed with NAFLD via incidental finding of steatosis on imaging or incidentally noted elevated liver enzymes which prompt imaging. In addition, patient with NAFLD risk factors including obesity, the metabolic syndrome and diabetes are sometimes screened by clinicians for NAFLD although data for the efficacy of this is lacking [32].

In the care of patients with NAFLD, it is imperative to identify those patients with advanced disease from those patients with steatosis. Ultrasound has a sensitivity of 93% when there is more than 33% of steatosis, but sensitivity is less than 30% in those with <33% steatosis [33]. CT has a sensitivity of about 82% in detecting moderate to severe steatosis and is based on attenuation (Hounsfield units). Given that many patients with NAFLD may have a mild degree of steatosis, the sensitivity of CT for mild steatosis is lower than that for moderate to severe [34]. The low sensitivity coupled with exposure to radiation makes CT a less desirable method for evaluation of NAFLD. MRI (including MR spectroscopy) is able to detect steatosis with high accuracy with sensitivity for mild hepatic steatosis of >5% of about 80–90% but it is expensive and therefore not cost-effective [34]. Both CT and MRI do not differentiate steatosis from steatohepatitis. Despite these limitations, ultrasound is a reasonable first test for a patient with a high likelihood of NAFLD. If an ultrasound is negative and a high pre-test probability remains CT or MRI should be performed.

Serum liver chemistry tests also do not reliably predict the presence of steatosis or any other entity in the NAFLD spectrum. Serum ALT has been looked at in many studies and it is concluded that steatosis (or any other form of NAFLD) can exist with normal ALT levels and that the histology in patients with normal ALT level does not differ from those with higher ALT levels [35].

The gold standard for the diagnosis of NAFLD, differentiating between steatosis and NASH, and staging fibrosis is liver biopsy. Since fibrosis is associated with worse outcomes and overall mortality, selection of patients with liver biopsy is based on a prediction of likelihood of significant fibrosis. The NAFLD fibrosis score (NFS), calculated by using body mass index, presence of diabetes/impaired fasting glucose, platelet count, albumin, ALT and AST level, is a model that helps identify patients with more advanced disease who may benefit from a liver biopsy [36]. More recently, imaging modalities such as vibration-controlled transient elastography (fibroscan) or elastography with acoustic radiation and magnetic resonance elastography (MRE) have been used to quantify the degree of liver fibrosis. These modalities are not widely available and can be costly. The controlled attenuation parameter

(evaluated with transient elastography) is a new non-invasive technique that is being used in select centers to measure the degree of steatosis but its use remains to be validated. There modalities can accurately determine the extremes of fibrosis (stage 0–1 vs 2–4) but lack the ability to differentiate reliably between stages.

Morbidity and Mortality

Mortality

While NAFLD is an important cause of morbidity and mortality worldwide, the implications of steatosis alone are less clear. Several studies have found increased mortality in NASH subjects compared to the general population, but steatosis, itself, has not been associated with increased mortality [37][38]. In a retrospective Swedish study, mortality over a 28-year period of biopsy-proven NAFLD patients was compared to the general Swedish population. All-comer NAFLD patients had 69% increased risk of mortality compared to the general population, however, when divided into NASH and steatosis subgroups, mortality among steatosis patients was no longer statistically significant [37]. Kim et al. used data from the National Health and Nutrition Examination Survey (NHANES) to extract non-invasive markers of fibrosis among ultrasonographical-identified NAFLD patients. They found no increased mortality among all-comer NAFLD identified by ultrasonography compared to NHANES controls, but statistically significant increased mortality among the subgroup identified by non-invasive fibrosis markers to have advanced fibrosis [38].

In contrast with the above studies, a recent longitudinal study in patients with NAFLD indicated increased mortality even in non-NASH patients in the presence of fibrosis. In this study, fibrosis stage was independently associated with long term overall mortality, liver transplantation and liver-related events. In the same study, patients that had steatosis with fibrosis had similar outcomes to patients with NASH with fibrosis [39].

Morbidity

Increasing evidence suggests that NAFLD independently contributes to cardiovascular disease, type 2 diabetes mellitus and renal disease [40]. Several mechanisms have been proposed including hepatic inflammation, hepatic dysfunction or steatosis. This suggest that steatosis is not as benign as currently known to be and that perhaps steatosis has metabolic effects leading to increased morbidity.

Liver disease

Steatosis and steatosis with nonspecific inflammation in the absence of fibrosis carry a low risk of progression to end stage liver disease. Liver-related mortality is not elevated among those with steatosis compared to the general population. In a cohort of 40 patients with steatosis followed over a median 11 years, none developed clinical signs of fibrosis or cirrhosis [41]. Similarly, in a Danish study of 417 patients with biopsy-proven steatosis (170 NAFLD, 247 alcoholic), only 2 (1.2%) NAFL patients clinically developed cirrhosis over a median of 20.4 years of follow-up compared to 54 (22%) alcoholic patients (median 21.0 years of follow-up) [42]. Histologic data, however, suggests progression of liver disease even among steatosis or steatosis with nonspecific inflammation patients. A retrospective review

of 70 patients with biopsy-proven NAFLD showed, of 25 patients with steatosis, 16 (64%) developed NASH and 6 (24%) bridging fibrosis over a median 3.7 years of follow-up. Disease progression was associated with increased weight and incidence of diabetes [43]. Wong et al. showed in a prospective paired-biopsy study including 12 with steatosis, 3 (23%) developed NASH on repeat biopsy after 3 years [44]. A systematic review of paired-biopsy studies found an annual fibrosis progression rate of 0.07 for patients with steatosis compared with 0.14 for NASH indicating progression of 1 stage of fibrosis every 14.3 years for NAFL patients and every 7.1 years for NASH patients [45]. This discrepancy between clinical and histologic studies may indicate that progression of liver disease in steatosis patients is reduced and individuals may succumb to comorbid conditions such as cardiovascular disease prior to developing advanced liver disease.

Angulo et al., however, showed that in the presence of fibrosis even steatosis carries an increased risk of mortality, liver transplantation, and liver-related events [39]. Patients with advanced disease are at higher risk for progression to cirrhosis than those with just steatosis [46] and often times identification of these patients goes unnoticed because of a lack of diagnostic tests for advanced disease.

Hepatocellular Carcinoma

The incidence of hepatocellular carcinoma (HCC) is significantly increased in patients with obesity and diabetes [47]. In addition, the incidence of HCC is increased among patients with NAFLD, especially NASH. Studies of populations with cryptogenic cirrhosis and HCC revealed that HCC likely developed in the setting of unrecognized NASH [48][49]. HCC can develop in NASH in the presence or absence of fibrosis, but data on HCC development in steatosis is lacking. While the incidence of hepatocellular carcinoma (HCC) is significantly increased in patients with obesity and diabetes [47] the role of steatosis in this risk is unknown.

Cardiovascular Disease

NAFLD is an independent risk factor for cardiovascular disease [50][51] and has been associated with increased risk of ischemic heart disease, heart failure, valvular disease, and arrhythmias [52]. Evidence for increased cardiovascular risk in steatosis patients independent of NASH is limited. The degree to which steatosis increases one’s cardiovascular risk is not as well defined as the risk across the NAFLD spectrum and needs further research.

Some studies note an association between hepatic steatosis and coronary artery defects. Mellinger et al. noted a significant association between hepatic steatosis, as identified by CT, and coronary calcifications using CT [53] with an odds ratio of 1.2. Another study by Osawa et al. noted that a significantly higher percentage of subjects with hepatic steatosis (measured by liver to spleen fat ratio) have a higher number of CT-detected coronary plaques than subjects without steatosis [54]. These studies were performed independent of CVD risk factors and suggest that steatosis itself is associated with coronary artery defects, though they cannot fully isolate the contribution of non-NASH NAFLD.

A case-control study examined 150 biopsy-proven NAFLD patients and quantified liver fat using MRI-proton density fat fraction (MRI-PDFF), dividing patients into above the median (15.4% fat fraction) and below the median groups. They found an association with between fat quantity and cardiovascular risk factors (abdominal obesity, low HDL, high triglycerides, and high fasting glucose) independent of biopsy-proven NASH, but did not specifically look at cardiovascular outcomes [55]. Another study looked at echocardiographic speckle-tracking findings of left ventricular global longitudinal strain (LV-GLS) in NAFLD patients compared to healthy controls. This study found significantly lower LV-GLS in steatosis, borderline NASH, and NASH patients compared to healthy controls, but no difference between the NAFLD subgroups [56].

Musso et al. looked at 40 non-obese, normolipidemic, non-diabetic patients with biopsy-proven NAFLD compared to 40 healthy controls. Patients were administered oral fat load and glucose tolerance tests and lipid profiles, adipose insulin resistance, and adipokines were measured postprandially. Steatosis and NASH patients demonstrated similar fasting values, however, NASH patients demonstrated more atherogenic lipoprotein profiles compared to simple steatosis patients suggesting a dysfunctional response to fat ingestion in NASH predisposing to increased cardiovascular risk compared to steatosis [57].

Type 2 Diabetes

There have been multiple studies showing that intrahepatic lipid content is independently associated with decreased insulin sensitivity, and that the presence of steatosis in the liver increases the risk of developing type 2 diabetes mellitus. Also important is the fact that a decrease in the hepatic fatty content leads to a reduction in the risk of developing type 2 diabetes mellitus [58–60]. This suggests that steatosis and diabetes mellitus are closely interrelated in terms of cause and effect. However, these have been mostly small studies in specific obese adult populations and need further validation and the majority of these studies have not differentiated between NASH and non-NASH NAFLD.

One systematic review found 6 studies using ultrasound-diagnosed NAFLD and 12 studies using LFT abnormalities to diagnose NAFLD. Among the ultrasound studies, 4/6 found NAFLD predicted type 2 diabetes mellitus (T2DM) independent of age, obesity, family history, and BMI (all studies were independent of age). All studies using LFT abnormalities to diagnose NAFLD predicted T2DM independent of age and BMI [61]. Steatosis and diabetes share similar risk factors and pathogenesis and it is not clear from current studies whether increased risk of T2DM in NAFLD stems from something intrinsic to NAFLD itself or is related to NASH and associated inflammation.

The study by Musso et al. in which 40 biopsy-proven NAFLD patients were administered oral fat load and glucose tolerance tests also looked at postprandial adipose and hepatic insulin resistance. Similar to their cardiovascular findings, NASH was associated with impaired B-cell function and increased adipose and hepatic insulin resistance compared to steatosis [57].

Colon Cancer

The second-leading cause of death in NAFLD patients after cardiovascular disease is extrahepatic malignancy [37]. NAFLD has been implicated in increased risk of gastric, esophageal, pancreatic, renal, melanoma, breast, and prostate cancer [62] though the evidence for a role in these cancers is not well-proven and often conflicting. The association between colorectal cancer and NAFLD, however, is much more established though it does not appear that this risk extends to steatosis. In one cross-sectional study of 199 biopsy-proven NAFLD patients, NAFLD patients had an increased risk of colorectal adenomas and advanced neoplasms compared to healthy controls (34.7% vs. 21.5% and 18.6% vs. 5.5% respectively). NASH patients had higher risk of adenomas and advanced neoplasms compared to steatosis patients (51.0% vs. 25.6% and 34.7% vs. 14.0% respectively) and the difference between steatosis patients and healthy controls was not statistically significant [63]. While data is limited, it does not appear steatosis in and of itself confers additional risk of colorectal cancer compared to the general population.

Chronic Kidney Disease

Numerous studies have noted a correlation between chronic kidney disease (CKD) and NAFLD [64]. A small number of these studies have sought to break down the influence of steatosis compared to NASH. In one study of 87 biopsy-proven NAFLD patients, 14 were found to have microalbuminuria. While there was no statistically significant difference in the prevalence of microalbuminuria between NASH and non-NASH patients, there were a small number of steatosis patients in the study (1 with microalbuminuria and 4 without). In addition, fibrosis scores were significantly higher in NAFLD patients with microalbuminuria than those without [65]. A cross-sectional study of 174 biopsy-proven NAFLD patients showed significantly increased rates of CKD in NASH patients compared to non-NASH patients. Out of 92 NASH and 87 non-NASH patients, 24 (14%) were found to have CKD with 19 (21%) having NASH and 5 (6%) non-NASH [66].

Management

Lifestyle Interventions

The most effective treatment for NAFLD is lifestyle interventions aimed at weight loss. Weight loss (either through dietary or exercise intervention) of about 3–5% has been associated with a reduction in the amount of steatosis [1]. In a meta-analysis by Musso et al. where four randomized studies were evaluated, more than 7% weight loss was needed to show statistically significant improvement in the NAFLD Activity score for patients with NASH but there were no improvements in fibrosis [57]. In other recent studies, ≥10% weight loss was needed to make improvements in biopsy-proven NASH fibrosis [67][68] with one study citing steatosis improvement in 100% of patients, steatohepatitis resolution in 90% of patients and fibrosis regression in 45% of patients with at least 10% total body weight loss. In the same study, a weight loss of at least 5% had some benefit with steatosis improvement in about 35%of patients. Although the optimal diet has not been agreed upon, for improvement of steatosis data supports a diet low in sugars, carbohydrates, saturated fats and high in fiber, healthy fats (monounsaturated, omega 3) with the goal of gradual weight loss of about 2lbs per week improves disease [69]. In a study by Kistler et al, 54% of 813

adults patients with NAFLD reported an activity level of inactive [70]. In the same study, NAFLD patients who achieved a MET of 6 or more for 75 minutes a week had lower odds of having NASH than those who did not. Another study showed that moderate to vigorous physical activity (>250 min weekly) significantly reduced NAFLD pathophysiology parameters such as levels of hepatic steatosis by fibroscan and abdominal visceral adiposity by MRI [71]. Current recommendations for treatment of all types of NAFLD (steatosis, steatosis with fibrosis, NASH and NASH with fibrosis) advocate for lifestyle interventions in the form of both diet and exercise as they are thought to have a greater effect together than either one alone [72].

Pharmacologic Interventions

Pharmacologic treatment of NAFLD is targeted to those patients with advanced disease at risk for progression such as steatosis with fibrosis, NASH and NASH with fibrosis. Pharmacological treatment such as vitamin E and pioglitazone have shown improvement in steatosis and inflammation in patients with NASH without DM and in patients with NASH with DM, respectively [73]. Omega-3 fatty acids have shown improvement in radiographic steatosis and pentoxifylline has shown improvement in steatosis and ballooning in patients with NASH but these treatments need further validation [74][75]. Metformin, ursodiol and orlistat have shown no benefits [32]. Ursodeoxycholic acid (UDCA), HMG Co-A-reductase inhibitors and ezetemibe have also been studied and so far have not achieved beneficial results or have yielded conflicting results [76].

Surgical Interventions

Bariatric surgery improves all stages of NAFLD (and might even cure NAFLD by reducing steatosis significantly to less than <5%) not only through its effect on weight, but through an improvement in insulin resistance and in the pro-inflammatory state caused by obesity [77].

In summary, therapy for all NAFLD should focus on weight reduction through diet and exercise. Pharmacological and surgical therapy do not apply for patients with steatosis or non-NASH without fibrosis.

Conclusion

The available literature on NAFLD clearly demonstrate that it is a worldwide health problem. The significance of non-NASH NAFLD (steatosis) within the spectrum of NAFLD remains to be defined. The pathophysiology of steatosis and its progression to NASH is not currently well known, though research into the microbiome and genetic underpinnings of the disease is promising. The available data suggests a relatively benign course of steatosis compared to NASH in terms of overall mortality and increased risk extrahepatic complications of NAFLD. However, it does appear that steatosis progresses to NASH at least histologically, but the clinical significance of this remains unclear and how and whom to follow remains ill-defined. Finally, treatment options for NAFLD across the spectrum remains limited, but more so for steatosis. Given the relatively benign course of steatosis, treatment of comorbidities and lifestyle interventions aimed at diet and exercise are currently

recommended. Further research is needed to identify which patients with steatosis may progress so that they can be safely and effectively be chosen for therapy.

References

1. Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, et al. The diagnosis and management of non-alcoholic fatty liver disease: Practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. Hepatology. 2012; 55:2005–23. [PubMed: 22488764]

2•. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease—Meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology. 2016; 64:73–84. A recent study decribing NAFLD epidemiology and the global epidemic. [PubMed: 26707365]

3. Sherif, ZA., Saeed, A., Ghavimi, S., Nouraie, S-M., Laiyemo, AO., Brim, H., et al. Dig Dis Sci [Internet]. Vol. 61. Springer; US: 2016. Global Epidemiology of Nonalcoholic Fatty Liver Disease and Perspectives on US Minority Populations; p. 1214-25.Available from: http://link.springer.com/10.1007/s10620-016-4143-0

4. Younossi ZM, Stepanova M, Afendy M, Fang Y, Younossi Y, Mir H, et al. Changes in the Prevalence of the Most Common Causes of Chronic Liver Diseases in the United States From 1988 to 2008. Clin Gastroenterol Hepatol. 2011; 9:524–30. [PubMed: 21440669]

5. Younossi, Z., Henry, L. Gastroenterology [Internet]. Vol. 150. Elsevier, Inc; 2016. Contribution of Alcoholic and Nonalcoholic Fatty Liver Disease to the Burden of Liver-Related Morbidity and Mortality; p. 1778-85.Available from: http://dx.doi.org/10.1053/j.gastro.2016.03.005

6. Younossi ZM, Blissett D, Blissett R, Henry L, Stepanova M, Younossi Y, et al. The economic and clinical burden of nonalcoholic fatty liver disease in the United States and Europe. Hepatology. 2016; 64:1577–86. [PubMed: 27543837]

7. Liu W, Robert Baker BD, Tavleen Bhatia B, Lixin Zhu B, Susan Baker BS. Pathogenesis of nonalcoholic steatohepatitis. Cell Mol Life Sci. 2016; 73:1969–87. [PubMed: 26894897]

8. Donnelly KL, Smith CI, Schwarzenberg SJ, Jessurun J, Boldt MD, Parks EJ. Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. J Clin Invest. 2005; 115:1343–51. [PubMed: 15864352]

9. Tetri LH, Basaranoglu M, Brunt EM, Yerian LM, Neuschwander-Tetri BA. Severe NAFLD with hepatic necroinflammatory changes in mice fed trans fats and a high-fructose corn syrup equivalent. AJP Gastrointest Liver Physiol. 2008; 295:G987–95.

10. Kohli R, Kirby M, Xanthakos SA, Softic S, Feldstein AE, Saxena V, et al. High-fructose, medium chain trans fat diet induces liver fibrosis and elevates plasma coenzyme Q9 in a novel murine model of obesity and nonalcoholic steatohepatitis. Hepatology. 2010; 52:934–44. [PubMed: 20607689]

11. Neuschwander-Tetri BA, Ford DA, Acharya S, Gilkey G, Basaranoglu M, Tetri LH, et al. Dietary trans-fatty acid induced NASH is normalized following loss of trans-fatty acids from hepatic lipid pools. Lipids. 2012; 47:941–50. [PubMed: 22923371]

12. Charlton M, Krishnan A, Viker K, Sanderson S, Cazanave S, McConico A, et al. Fast food diet mouse: novel small animal model of NASH with ballooning, progressive fibrosis, and high physiological fidelity to the human condition. AJP Gastrointest Liver Physiol. 2011; 301:G825–34.

13. Mells JE, Fu PP, Kumar P, Smith T, Karpen SJ, Anania FA. Saturated fat and cholesterol are critical to inducing murine metabolic syndrome with robust nonalcoholic steatohepatitis. J Nutr Biochem. 2015; 26:285–92. [PubMed: 25577467]

14. Musso G, Gambino R, De Michieli F, Cassader M, Rizzetto M, Durazzo M, et al. Dietary habits and their relations to insulin resistance and postprandial lipemia in nonalcoholic steatohepatitis. Hepatology. 2003; 37:909–16. [PubMed: 12668986]

15. Berk PD, Verna EC. Nonalcoholic Fatty Liver Disease. Lipids and Insulin Resistance. Clin Liver Dis. 2016; 20:245–62. [PubMed: 27063267]

16. Kawaguchi T, Takenoshita M, Kabashima T, Uyeda K. Glucose and cAMP regulate the L-type pyruvate kinase gene by phosphorylation/dephosphorylation of the carbohydrate response element

binding protein. Proc Natl Acad Sci U S A [Internet]. 2001; 98:13710–5. Available from: http://www.pnas.org/cgi/content/long/98/24/13710.

17. Horton JD, Shah Na, Warrington Ja, Anderson NN, Park SW, Brown MS, et al. Combined analysis of oligonucleotide microarray data from transgenic and knockout mice identifies direct SREBP target genes. Proc Natl Acad Sci U S A. 2003; 100:12027–32. [PubMed: 14512514]
18. Horton JD, Shimomura I, Ikemoto S, Bashmakov Y, Hammer RE. Overexpression of sterol regulatory element-binding protein-1a in mouse adipose tissue produces adipocyte hypertrophy, increased fatty acid secretion, and fatty liver. J Biol Chem. 2003; 278:36652–60. [PubMed: 12855691]
19. Le Roy T, Llopis M, Lepage P, Bruneau A, Rabot S, Bevilacqua C, et al. Intestinal microbiota determines development of non-alcoholic fatty liver disease in mice. Gut. 2013; 62:1787–94. [PubMed: 23197411]
20. Wigg AJ, Roberts-Thomson IC, Dymock RB, McCarthy PJ, Grose RH, Cummins AG. The role of small intestinal bacterial overgrowth, intestinal permeability, endotoxaemia, and tumour necrosis factor α in the pathogenesis of non-alcoholic steatohepatitis. Gut. 2001; 48:206–11. [PubMed: 11156641]
21. Sabaté JM, Jouët P, Harnois F, Mechler C, Msika S, Grossin M, et al. High prevalence of small intestinal bacterial overgrowth in patients with morbid obesity: A contributor to severe hepatic steatosis. Obes Surg. 2008; 18:371–7. [PubMed: 18286348]
22. Miele L, Valenza V, La Torre G, Montalto M, Cammarota G, Ricci R, et al. Increased intestinal permeability and tight junction alterations in nonalcoholic fatty liver disease. Hepatology [Internet]. 2009; 49:1877–87. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19291785.
23. Bäckhed F, Ding H, Wang T, Hooper LV, Koh GY, Nagy A, et al. The gut microbiota as an environmental factor that regulates fat storage. Proc Natl Acad Sci U S A [Internet]. 2004; 101:15718–23. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=524219&tool=pmcentrez&rendertype=abstract.
24. Bäckhed F, Manchester JK, Semenkovich CF, Gordon JI. Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. Proc Natl Acad Sci [Internet]. 2007; 104:979–84. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1764762&tool=pmcentrez&rendertype=abstract.
25. den Besten G, van Eunen K, Groen AK, Venema K, Reijngoud D-J, Bakker BM. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. J Lipid Res [Internet]. 2013; 54:2325–40. Available from: http://www.jlr.org/content/54/9/2325.abstract%5Cnhttps://www.ncbi.nlm.nih.gov/pmc/articles/PMC3735932/pdf/2325.pdf.
26. Vrieze A, van Nood E, Holleman F, Salojarvi J, Kootte RS, Bartelsman JF, et al. Transfer of Intestinal Microbiota from Lean Donors Increases Insulin Sensitivity in Subjects with Metabolic Syndrome. Gastroenterology [Internet]. 2012; 143:913–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22728514%5Cnhttp://ac.els-cdn.com/S001650851200892X/1-s2.0-S001650851200892X-main.pdf?_tid=a54e2204413c2bb7b689a0274be0d7a7&acdnat=1342205913_c73a01b843c214454b64498ffd4ef5a5.
27. Kalia, HS., Gaglio, PJ. Clin Liver Dis [Internet]. Vol. 20. Elsevier Inc; 2016. The Prevalence and Pathobiology of Nonalcoholic Fatty Liver Disease in Patients of Different Races or Ethnicities; p. 215-24.Available from: http://dx.doi.org/10.1016/j.cld.2015.10.005
28•. Romeo S, Kozlitina J, Xing C, Pertsemlidis A, Cox D, Pennacchio LA, et al. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. Nat Genet. 2008; 40:1461–5. A paper describing the discovery of PNPLA3, one of the first and most important genes conferring risk of fatty liver disease. [PubMed: 18820647]
29. Wagenknecht LE, Palmer ND, Bowden DW, Rotter JI, Norris JM, Ziegler J, et al. Association of PNPLA3 with non-alcoholic fatty liver disease in a minority cohort: The Insulin Resistance Atherosclerosis Family Study. Liver Int. 2011; 31:412–6. [PubMed: 21281435]
30•. Kozlitina J, Smagris E, Stender S, Nordestgaard BG, Zhou HH, Tybjærg-Hansen A, et al. Exome-wide association study identifies a TM6SF2 variant that confers susceptibility to nonalcoholic fatty liver disease. Nat Genet. 2014; 46:352–6. A paper describing the discovery of a second important gene conferring risk of NAFLD. [PubMed: 24531328]

31. Petersen KF, Dufour S, Hariri A, Nelson-Williams C, Foo JN, Zhang X-M, et al. Apolipoprotein C3 Gene Variants in Nonalcoholic Fatty Liver Disease. N Engl J Med. 2010; 362:1082–9. [PubMed: 20335584]
32. Corey KE, Rinella ME. Medical and Surgical Treatment Options for Nonalcoholic Steatohepatitis. Dig Dis Sci. 2016; 61:1387–97. [PubMed: 26942734]
33. Dasarathy, S., Dasarathy, J., Khiyami, A., Joseph, R., Lopez, R., McCullough, AJ. J Hepatol [Internet]. Vol. 51. European Association for the Study of the Liver; 2009. Validity of real time ultrasound in the diagnosis of hepatic steatosis: A prospective study; p. 1061-7.Available from: http://dx.doi.org/10.1016/j.jhep.2009.09.001
34. Lee SS, Park SH. Radiologic evaluation of nonalcoholic fatty liver disease. World J Gastroenterol. 2014; 20:7392–402. [PubMed: 24966609]
35. Mofrad P, Contos MJ, Haque M, Sargeant C, Fisher RA, Luketic VA, et al. Clinical and histologic spectrum of nonalcoholic fatty liver disease associated with normal ALT values. Hepatology. 2003; 37:1286–92. [PubMed: 12774006]
36•. Angulo P, Hui JM, Marchesini G, Bugianesi E, George J, Farrell GC, et al. The NAFLD Fibrosis Score: A Noninvasive System That Identifies Liver Fibrosis in Patients with NAFLD. Hepatology. 2007; 45:846–54. A paper describing the development of the NAFLD fibrosis score, a non-invasive way predictive tool of fibrosis in NAFLD. [PubMed: 17393509]
37. Söderberg C, Stål P, Askling J, Glaumann H, Lindberg G, Marmur J, et al. Decreased survival of subjects with elevated liver function tests during a 28-year follow-up. Hepatology. 2010; 51:595–602. [PubMed: 20014114]
38. Kim D, Kim WR, Kim HJ, Therneau TM. Association between noninvasive fibrosis markers and mortality among adults with nonalcoholic fatty liver disease in the United States. Hepatology. 2013; 57:1357–65. [PubMed: 23175136]
39••. Angulo P, Kleiner DE, Dam-Larsen S, Adams LA, Bjornsson ES, Charatcharoenwitthaya P, et al. Liver fibrosis, but no other histologic features, is associated with long-term outcomes of patients with nonalcoholic fatty liver disease. Gastroenterology. 2015; 149:389–397.e10. Important paper indicating non-NASH may not be as benign as previously thought. It showed even non-NASH in the presence of fibrosis predicts higher mortality and risk of liver transplantation. [PubMed: 25935633]
40. Byrne, CD., Targher, G. J Hepatol [Internet]. Vol. 62. European Association for the Study of the Liver; 2015. NAFLD: A multisystem disease; p. S47-64.Available from: http://dx.doi.org/10.1016/j.jhep.2014.12.012
41. Teli MR, James OFW, Burt AD, Bennett MK, Day' P. The Natural History of Nonalcoholic Fatty Liver: A Follow-up Study. Hepatology. 1995; 22:1714–9. [PubMed: 7489979]
42. Dam-Larsen S. Long term prognosis of fatty liver: risk of chronic liver disease and death. Gut. 2004; 53:750–5. [PubMed: 15082596]
43. Pais R, Charlotte F, Fedchuk L, Bedossa P, Lebray P, Poynard T, et al. A systematic review of follow-up biopsies reveals disease progression in patients with non-alcoholic fatty liver. J Hepatol. 2013; 59:550–6. [PubMed: 23665288]
44. Wong VW-S, Wong GL-H, Choi PC-L, Chan AW-H, Li MK-P, Chan H-Y, et al. Disease progression of non-alcoholic fatty liver disease: a prospective study with paired liver biopsies at 3 years. Gut [Internet]. 2010; 59:969–74. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20581244.
45. Singh, S., Allen, AM., Wang, Z., Prokop, LJ., Murad, MH., Loomba, R. Clin Gastroenterol Hepatol [Internet]. Vol. 13. Elsevier, Inc; 2015. Fibrosis Progression in Nonalcoholic Fatty Liver vs Nonalcoholic Steatohepatitis: A Systematic Review and Meta-analysis of Paired-Biopsy Studies; p. 643-54.Available from: http://dx.doi.org/10.1016/j.cgh.2014.04.014
46. Rinella ME. Nonalcoholic Fatty Liver Disease. JAMA. 2015; 313:2263–73. [PubMed: 26057287]
47. Polesel J, Zucchetto A, Montella M, Dal Maso L, Crispo A, La Vecchia C, et al. The impact of obesity and diabetes mellitus on the risk of hepatocellular carcinoma. Ann Oncol [Internet]. 2009; 20:353–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18723550.

48. Sørensen HT, Mellemkjaer L, Jepsen P, Thulstrup AM, Baron J, Olsen JH, et al. Risk of cancer in patients hospitalized with fatty liver: a Danish cohort study. J Clin Gastroenterol. 2003; 36:356–9. [PubMed: 12642745]
49. Marrero JA, Fontana RJ, Su GL, Conjeevaram HS, Emick DM, Lok AS. NAFLD may be a common underlying liver disease in patients with hepatocellular carcinoma in the United States. Hepatology [Internet]. 2002; 36:1349–54. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12447858.
50. Lin YC, Lo HM, Chen JD. Sonographic fatty liver, overweight and ischemic heart disease. World J Gastroenterol. 2005; 11:4838–42. [PubMed: 16097054]
51. Targher G, Bertolini L, Padovani R, Rodella S, Tessari R, Zenari L, et al. Prevalence of Nonalcoholic Fatty Liver Disease and Its Association With Cardiovascular Disease Among Type 2. Diabetes Care. 2007; 30:1212–8. [PubMed: 17277038]
52. Mantovani A, Ballestri S, Lonardo A, Targher G. Cardiovascular Disease and Myocardial Abnormalities in Nonalcoholic Fatty Liver Disease. Dig Dis Sci. 2016; 61:1246–67. [PubMed: 26809873]
53. Mellinger, JL., Pencina, KM., Massaro, JM., Hoffmann, U., Seshadri, S., Fox, CS., et al. J Hepatol [Internet]. Vol. 63. European Association for the Study of the Liver; 2015. Hepatic steatosis and cardiovascular disease outcomes: An analysis of the Framingham Heart Study; p. 470-6.Available from: http://dx.doi.org/10.1016/j.jhep.2015.02.045
54. Osawa K, Miyoshi T, Yamauchi K, Koyama Y, Nakamura K, Sato S, et al. Nonalcoholic hepatic steatosis is a strong predictor of high-risk coronary-artery plaques as determined by multidetector CT. PLoS One. 2015; 10:1–14.
55. Arulanandan A, Ang B, Bettencourt R, Hooker J, Behling C, Lin GY, et al. Association Between Quantity of Liver Fat and Cardiovascular Risk in Patients With Nonalcoholic Fatty Liver Disease Independent of Nonalcoholic Steatohepatitis. Clin Gastroenterol Hepatol. 2015; 13:1513–20. [PubMed: 25661453]
56. Karabay CY, Kocabay G, Kalayci A, Colak Y, Oduncu V, Akgun T, et al. Impaired left ventricular mechanics in nonalcoholic fatty liver disease: a speckle-tracking echocardiography study. Eur J Gastroenterol Hepatol March [Internet]. 2014; 26:325–31. Available from: http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=yrovftp&AN=00042737-201403000-00012.
57. Musso G, Cassader M, Rosina F, Gambino R. Impact of current treatments on liver disease, glucose metabolism and cardiovascular risk in non-alcoholic fatty liver disease (NAFLD): A systematic review and meta-analysis of randomised trials. Diabetologia. 2012; 55:885–904. [PubMed: 22278337]
58. Sada, Y., Katabami, T., Asai, S., Sato, T., Furukawa, K., Ishii, S., et al. Obes Res Clin Pract [Internet]. Vol. 5. Asia Oceania Assoc for the Study of Obesity; 2011. Intrahepatic lipid content is linked to insulin resistance in obese subjects; p. e79-156.Available from: http://www.sciencedirect.com/science/article/pii/S1871403X10002978
59. Lalia AZ, Dasari S, Johnson ML, Robinson MM, Konopka AR, DIstelmaier K, et al. Predictors of whole-body insulin sensitivity across ages and adiposity in adult humans. J Clin Endocrinol Metab. 2016; 101:626–34. [PubMed: 26709968]
60. Koska J, Stefan N, Permana PA, Weyer C, Sonoda M, Bogardus C, et al. Increased fat accumulation in liver may link insulin resistance with subcutaneous abdominal adipocyte enlargement, visceral adiposity, and hypoadiponectinemia in obese individuals. Am J Clin Nutr. 2008; 87:295–302. [PubMed: 18258617]
61. Lallukka S, Yki-Jarvinen H. Non-alcoholic fatty liver disease and risk of type 2 diabetes. Best Pract Res Clin Endocrinol Metab. 2016; 39:385–95.
62. Sanna C, Rosso C, Marietti M, Bugianesi E. Non-alcoholic fatty liver disease and extra-hepatic cancers. Int J Mol Sci. 2016; 17:717.
63. Wong VW-S, Wong GL-H, Tsang SW-C, Fan T, Chu WC-W, Woo J, et al. High prevalence of colorectal neoplasm in patients with non-alcoholic steatohepatitis. Gut [Internet]. 2011; 60:829–36. Available from: http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=21339204&retmode=ref&cmd=prlinks%5Cnpapers2://publication/doi/10.1136/gut.2011.237974.

64. Marcuccilli M, Chonchol M. NAFLD and chronic kidney disease. Int J Mol Sci. 2016; 17:562. [PubMed: 27089331]

65. Yilmaz Y, Alahdab YO, Yonal O, Kurt R, Kedrah AE, Celikel CA, et al. Microalbuminuria in nondiabetic patients with nonalcoholic fatty liver disease: Association with liver fibrosis. Metabolism. 2010; 59:1327–30. [PubMed: 20096896]

66. Yasui, K., Sumida, Y., Mori, Y., Mitsuyoshi, H., Minami, M., Itoh, Y., et al. Metabolism [Internet]. Vol. 60. Elsevier Inc; 2011. Nonalcoholic steatohepatitis and increased risk of chronic kidney disease; p. 735-9.Available from: http://dx.doi.org/10.1016/j.metabol.2010.07.022

67. Vilar-Gomez E, Martinez-Perez Y, Calzadilla-Bertot L, Torres-Gonzalez A, Gra-Oramas B, Gonzalez-Fabian L, et al. Weight loss through lifestyle modification significantly reduces features of nonalcoholic steatohepatitis. Gastroenterology. 2015; 149:367–378.e5. [PubMed: 25865049]

68. Glass, LM., Dickson, RC., Anderson, JC., Suriawinata, AA., Putra, J., Berk, BS., et al. Dig Dis Sci [Internet]. Vol. 60. Springer; US: 2015. Total Body Weight Loss of ≥10 % Is Associated with Improved Hepatic Fibrosis in Patients with Nonalcoholic Steatohepatitis; p. 1024-30.Available from: http://dx.doi.org/10.1007/s10620-014-3380-3

69. Pimentel CFMG, Lai M. Nutrition Interventions for Chronic Liver Diseases and Nonalcoholic Fatty Liver Disease. Med Clin North Am. 2016; 100:1303–27. [PubMed: 27745596]

70. Kistler KD, Brunt EM, Clark JM, Mae A, Sallis JF, Schwimmer JB, et al. Physical Activity Recommendations, Exercise Intensity, and Histological Severity of Nonalcoholic Fatty Liver Disease. Am J Gastroenterol. 2011; 2010:460–8.

71. Oh S, Shida T, Yamagishi K, Tanaka K, So R, Tsujimoto T, et al. Moderate to Vigorous Physical Activity Volume Is an Important Factor for Managing Nonalcoholic Fatty Liver Disease: A Retrospective Study. Hepatology. 2015; 61:1205–15. [PubMed: 25271091]

72. Hannah WN, Harrison SA. Lifestyle and Dietary Interventions in the Management of Nonalcoholic Fatty Liver Disease. Dig Dis Sci. 2016; 61:1365–74. [PubMed: 27052013]

73. Sanyal AJ, Chalasani N, Kowdley KV, McCullough A, Diehl AM, Bass NM, et al. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. N Engl J Med [Internet]. 2010; 362:1675–85. Available from: http://www.nejm.org/doi/abs/10.1056/NEJMoa0907929%5Cnhttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2928471&tool=pmcentrez&rendertype=abstract.

74. Spadaro L, Magliocco O, Spampinato D, Piro S, Oliveri C, Alagona C, et al. Effects of n-3 polyunsaturated fatty acids in subjects with nonalcoholic fatty liver disease. Dig Liver Dis. 2008; 40:194–9. [PubMed: 18054848]

75. Zein CO, Yerian LM, Gogate P, Lopez R, Kirwan JP, Feldstein AE, et al. Pentoxifylline improves nonalcoholic steatohepatitis: A randomized placebo-controlled trial. Hepatology. 2011; 54:1610–9. [PubMed: 21748765]

76. Ganesh S, Rustgi VK. Current Pharmacologic Therapy for Nonalcoholic Fatty Liver Disease. Clin Liver Dis. 2016; 20:351–64. [PubMed: 27063274]

77. Clanton J, Subichin M. The Effects of Metabolic Surgery on Fatty Liver Disease and Nonalcoholic Steatohepatitis. Surg Clin North Am. 2016; 96:703–15. [PubMed: 27473796]