


The Diagnosis and Management of Nonalcoholic Fatty Liver Disease: Practice Guidance from the American Association for the Study of Liver Diseases

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This is the author's manuscript of the article published in final edited form as:

Chalasani, N., Younossi, Z., Lavine, J. E., Charlton, M., Cusi, K., Rinella, M., Harrison, S. A., Brunt, E. M. and Sanyal, A. J. (), The Diagnosis and Management of Nonalcoholic Fatty Liver Disease: Practice Guidance from the American Association for the Study of Liver Diseases. Hepatology. Accepted Author Manuscript. <http://dx.doi.org/10.1002/hep.29367>

Abbreviations

NAFLD:	Nonalcoholic Fatty Liver Disease
NAFL:	Nonalcoholic Fatty Liver
NASH:	Nonalcoholic Steatohepatitis
T2DM:	Type 2 Diabetes Mellitus
AST:	Aspartate Aminotransferase
ALT:	Alanine Aminotransferase
RCT:	Randomized Controlled Trial
TONIC:	Treatment of Nonalcoholic Fatty Liver Disease in Children
NAS:	NAFLD Activity Score
MR:	Magnetic Resonance
MRE:	MR Elastography
VCTE:	Vibration Controlled Transient Elastography

FUNDING

The funding for the development of this Practice Guidance was provided by the American Association for the Study of Liver Diseases.

AASLD APPROVAL

This practice guidance was approved by the American Association for the Study of Liver Diseases on June 15, 2017.

Acknowledgments

This Practice Guidance was developed under the direction of the AASLD Practice Guidelines Committee, which approved the scope of the guidance and provided the peer review. Members of the Committee include Tram T. Tran, MD, FAASLD (Chair), Michael W. Fried, MD, FAASLD (Board Liaison), Jawad Ahmad, MD, FAASLD, Joseph Ahn, MD, Alfred Sidney Barritt IV, MD, MSCR, James R Burton, Jr., MD, Udeme Ekong, MD, Fredric Gordon, MD, FAASLD, Simon P. Horslen, MD, George Ioannou, MD, FAASLD, Whitney E. Jackson, MD, Patrick S Kamath, MD, Fasiha Kanwal, MD, MSHS, David G Koch, MD, Michael D. Leise, MD, Jacqueline G. O'Leary, MD, Raphael B. Merriman, MD, FACP, FRCPI, (Immediate Past Chair) Michael L. Schilsky, MD, FAASLD, Amit G. Singal, MD, (Vice-Chair), James R. Spivey, MD, Helen S. Te, MD, FAASLD, and Michael Volk, MD. Dr. Merriman served as primary reviewer for the AASLD Practice Guidelines Committee and declared no conflicts of interest. As this guidance document is an update of the previous AASLD Practice Guideline published in 2012, there will be inevitable similarities in the text between the two documents. Authors thank Ms. Megan Comerford from Indiana University for her editorial assistance.

Preamble

This guidance provides a data-supported approach to the diagnostic, therapeutic, and preventive aspects of NAFLD care. A “Guidance” document is different from a “Guideline.” Guidelines are developed by a multidisciplinary panel of experts and rate the quality (level) of the evidence and the strength of each recommendation using the Grading of Recommendations, Assessment Development, and Evaluation (GRADE) system. A guidance document is developed by a panel of experts in the topic, and guidance statements, not recommendations, are put forward to help clinicians understand and implement the most recent evidence.

This Practice Guidance was commissioned by the American Association for the Study of Liver Diseases (AASLD) and is an update to the Practice Guideline published in 2012 in conjunction with the American Gastroenterology Association and the American College of Gastroenterology (1). Sections where there have been no notable newer publications are not modified, so some paragraphs remain unchanged. This narrative review and guidance statements are based on the following: (1) a formal review and analysis of the recently published world literature on the topic (Medline search up to August 2016); (2) the American College of Physicians’ *Manual for Assessing Health Practices and Designing Practice Guidelines* (2); (3) guideline policies of the AASLD; and (4) the experience of the authors and independent reviewers with regards to NAFLD.

This practice guidance is intended for use by physicians and other health professionals. As clinically appropriate, guidance statements should be tailored for individual patients. Specific guidance statements are evidence-based whenever possible, and, when such evidence is not

AASLD approved version submitted to HEPATOLOGY on June 28, 2017

available or is inconsistent, guidance statements are made based on the consensus opinion of the authors (3). This is a practice guidance for clinicians rather than a review article, and interested readers can refer to several recent comprehensive reviews (4-9). As this guidance document is lengthy, to make it easier for the reader a list of all guidance statements and recommendations are provided in a tabular form as **Supplemental Table 1**.

Definitions

For defining nonalcoholic fatty liver disease (NAFLD), there must be (a) evidence of hepatic steatosis, either by imaging or histology, and (b) lack of secondary causes of hepatic fat accumulation such as significant alcohol consumption, long-term use of a steatogenic medication, or monogenic hereditary disorders (**Table 1**). In the majority of patients, NAFLD is commonly associated with metabolic comorbidities such as obesity, diabetes mellitus, and dyslipidemia. NAFLD can be categorized histologically into nonalcoholic fatty liver (NAFL) or nonalcoholic steatohepatitis (NASH) (**Table 2**). NAFL is defined as the presence of $\geq 5\%$ hepatic steatosis without evidence of hepatocellular injury in the form of hepatocyte ballooning. NASH is defined as the presence of $\geq 5\%$ hepatic steatosis and inflammation with hepatocyte injury (eg, ballooning), with or without any fibrosis. For defining “advanced” fibrosis, this guidance document will be referring specifically to stages 3 or 4, ie, bridging fibrosis or cirrhosis.

Incidence and Prevalence of NAFLD in the General Population

Incidence of NAFLD:

There is a paucity of data regarding the incidence of NAFLD in the general population. A few studies have reported incidence of NAFLD from Asian countries, which are briefly summarized below:

- In a study that followed 11,448 subjects for 5 years, the incidence of NAFLD documented by ultrasound was 12% ($n = 1,418$) (10).
- In a study of 635 Nagasaki atomic bomb survivors who were followed for 11.6 years, the incidence of NAFLD documented by ultrasound was 19.9 per 1,000 person-years (11).
- In 565 subjects, the incidence of NAFLD at 3 to 5 years, diagnosed using magnetic resonance imaging and transient elastography, was estimated to be 13.5% (34 per 1,000 person-years) (12).
- In a cohort study, 77,425 subjects free of NAFLD at baseline were followed for an average of 4.5 years. During 348,193.5 person-years of follow-up, 10,340 participants developed NAFLD documented by ultrasound, translating to an incidence rate of 29.7 per 1,000 person-years (13).

The incidence rates for NAFLD in the general population of Western countries are even less commonly reported:

- A study from England using ICD-10 codes reported an incidence rate for NAFLD of 29 per 100,000 person-years. Given the inaccuracy of administrative coding such as ICD-10, this study most likely underestimates the true incidence of NAFLD (14).
- A study from Israel reported an incidence rate of 28 per 1,000 person-years (15).
- A recent meta-analysis estimated that the pooled regional incidence of NAFLD from Asia to be 52.34 per 1,000 person-years (95% confidence interval [CI]: 28.31-96.77) while the

incidence rate from the West is estimated to be around 28 per 1,000 person-years (95% CI: 19.34-40.57) (16).

Prevalence of NAFLD:

In contrast to the incidence data, there is a significantly higher number of publications describing the prevalence of NAFLD in the general population. These studies are summarized in a recent meta-analysis of the epidemiology of NAFLD:

- The meta-analysis estimated that the overall global prevalence of NAFLD diagnosed by imaging is around 25.24% (95% CI: 22.10-28.65%) (16).
- The highest prevalence of NAFLD is reported from the Middle East (31.79% [95% CI:13.48-58.23]) and South America (30.45% [95% CI: 22.74-39.440]) while the lowest prevalence rate is reported from Africa (13.48% [5.69-28.69]).(16)

As described elsewhere, the gold standard for diagnosing NASH remains a liver biopsy. Given that liver biopsy is not feasible in studies of the general population, there is no direct assessment of the incidence or prevalence of NASH. Nevertheless, there have been some attempts to estimate the prevalence of NASH by indirect means (16,17). The data regarding the prevalence of NASH in the general population are summarized in the following paragraphs:

- The prevalence of NASH among NAFLD patients who had liver biopsy for a “clinical indication” is estimated to be 59.10% (95% CI: 47.55-69.73) (16).
- The prevalence of NASH among NAFLD patients who had liver biopsy without a specific “clinical indication” (random biopsy for living-related donors etc.) is estimated from 6.67% (95% CI:2.17-18.73) to 29.85% (95% CI: 22.72-38.12) (16).

- Given these estimates, one estimates that the prevalence of NASH in the general population ranges between 1.5% and 6.45% (16).

Prevalence of NAFLD in High-Risk Groups (Table 3)

Features of metabolic syndrome are not only highly prevalent in patients with NAFLD, but components of metabolic syndrome also increase the risk of developing NAFLD (16,18-20). This bidirectional association between NAFLD and components of metabolic syndrome has been strongly established. In this context, **Table 3** provides a list of the established conditions (obesity, type 2 diabetes, hypertension, dyslipidemia) and emerging conditions (sleep apnea, colorectal cancer, osteoporosis, psoriasis, endocrinopathies, and polycystic ovary syndrome independent of obesity) that are associated with NAFLD (21,22).

- Obesity (excessive body mass index [BMI] and visceral obesity) is the most common and well documented risk factor for NAFLD. In fact, the entire spectrum of obesity, ranging from overweight to obese and severely obese, is associated with NAFLD. In this context, the majority (>95%) of patients with severe obesity undergoing bariatric surgery will have NAFLD (23,24).
- Type 2 diabetes mellitus (T2DM): There is a very high prevalence of NAFLD in individuals with T2DM. In fact, some studies have suggested that about a third to two-thirds of diabetic patients have NAFLD (18,25-27). It is also important to remember the importance of bidirectional association between NAFLD and T2DM. In this context, T2DM and NAFLD can develop almost simultaneously in a patient, which confounds the prevalence of NAFLD in patients with T2DM or the prevalence of T2DM in patients with

NAFLD. Nevertheless, this association and its bidirectional causal relationship require additional investigation.(28)

- **Dyslipidemia:** High serum triglyceride levels and low serum high-density lipoprotein (HDL) levels are also common in patients with NAFLD. The prevalence of NAFLD in individuals with dyslipidemia attending lipid clinics has been estimated to be 50% (29,30). In a large, cross-sectional study conducted among 44,767 Taiwanese patients who attended a single clinic, the enrollees were stratified into 4 subgroups based on their total cholesterol to HDL-cholesterol and triglyceride to HDL-cholesterol ratios. The overall prevalence rate of NAFLD was 53.76%; however, the NAFLD prevalence rate for those with the lowest total cholesterol to HDL-cholesterol and triglyceride to HDL-cholesterol ratios was 33.41%, whereas the prevalence rate in the group with the highest ratios was 78.04%.
- **Age, gender, and ethnicity:** The prevalence of NAFLD may vary according to age, gender, and ethnicity (31-39). In fact, both the prevalence of NAFLD and the stage of liver disease appear to increase with age (34-37).

Although controversial, male gender has been considered a risk factor for NAFLD.

Furthermore, the prevalence of NAFLD in men is 2 times higher than in women (33,34,38).

The issues of ethnicity and its impact on NAFLD have evolved over the years. In fact, initial reports suggested that compared to non-Hispanic whites, Hispanic individuals have a

significantly higher prevalence of NAFLD, whereas non-Hispanic blacks have a significantly lower prevalence of NAFLD (39). Although the prevalence of NAFLD among American-

Indian and Alaskan-Native populations seem to be lower (0.6% to 2.2%), these rates need to be confirmed (31,32). It is intriguing that most of the recent data suggest the ethnic differences reported for NAFLD may be explained by the genetic variation related to the PNPLA-3 gene (40).

In summary, the incidence of NAFLD varies across the world, ranging from 28.01 per 1,000 person-year (95% CI: 19.34-40.57) to 52.34 per 1,000 person-years (95% CI: 28.31-96.77).

Natural History and Outcomes of Nonalcoholic Fatty Liver Disease

Over the past 2 decades, studies have reported the natural history of patients with NAFLD (1,19,41-52). There is growing evidence that patients with histologic NASH, especially those with some degree of fibrosis, are at higher risk for adverse outcomes such as cirrhosis and liver-related mortality (1,18,19,41-52). These studies have also shown the following:

- Patients with NAFLD have increased overall mortality compared to matched control populations without NAFLD (53,54).
- The most common cause of death in patients with NAFLD is cardiovascular disease, independent of other metabolic comorbidities.
- Although liver-related mortality is the 12th leading cause of death in the general population, it is the second or third cause of death among patients with NAFLD (55).
- Cancer-related mortality is among the top 3 causes of death in subjects with NAFLD (55).
- Patients with histologic NASH have an increased liver-related mortality rate (56,57).
- In a recent meta-analysis, liver-specific and overall mortality rates among NAFLD and NASH were determined to be 0.77 per 1,000 (range, 0.33-1.77) and 11.77 per 1,000 person-

years (range, 7.10-19.53) and 15.44 per 1,000 (range, 11.72-20.34) and 25.56 per 1,000 person-years (range, 6.29-103.80), respectively (16).

- The incidence risk ratios for liver-specific and overall mortality for NAFLD were also determined to be 1.94 (range, 1.28-2.92) and 1.05 (range, 0.70-1.56), respectively (16).
- The most important histologic feature of NAFLD associated with long term mortality is fibrosis; specifically, zone 3 sinusoidal fibrosis plus periportal fibrosis (stage 2) to advanced (bridging fibrosis [stage 3] or cirrhosis [stage 4]). These are independently predictive of liver-related mortality (44,58,59).
- NAFLD is now considered the third most common cause of hepatocellular carcinoma (HCC) in the United States, likely due to the enormous number of patients with the condition (60).

Given the growing epidemic of obesity, the incidence of NAFLD-related HCC has been shown to increase at a 9% annual rate (61).

- Patients with NAFLD-related HCC are older, have a shorter survival time, more often have heart disease, and are more likely to die from their primary liver cancer than other HCC patients (60).
- About 13% of HCC reported from a study of patients from Veteran Administration did not have cirrhosis. Among other factors, having NAFLD was independently associated with HCC in the absence of cirrhosis. This study confirms prior small reports of HCC in NAFLD patients without cirrhosis (62).
- It is important to recognize that most patients with cryptogenic cirrhosis may have what is considered “burned out” NAFLD (63). This particular group of patients with cryptogenic cirrhosis have a disproportionately high prevalence of metabolic risk factors (T2DM, obesity, metabolic syndrome) that resemble patients with NAFLD, but the pathologic assessment

seldom reports histological features consistent with NASH or even steatosis in the presence of cirrhosis (63,64).

Important Outcomes in Patients with NAFLD

One of the important surrogates for advanced liver disease is documentation of progressive hepatic fibrosis. In the recent meta-analysis, hepatic fibrosis progression in patients with histological NASH at baseline showed a mean annual fibrosis progression rate of 0.09 (95% CI: 0.06-0.12) (16). Several studies investigated the natural history of NASH cirrhosis in comparison to patients with hepatitis C cirrhosis (9,65,66). One large, prospective, US-based study observed a lower rate of decompensation and mortality in patients with NASH cirrhosis as compared to patients with hepatitis C cirrhosis (65). However, a more recent international study of 247 NAFLD patients with advanced fibrosis (bridging fibrosis and cirrhosis) followed over a mean duration of 85.6 ± 54.5 months showed an overall 10-year survival of 81.5%—a survival rate not different from matched patients with hepatitis C cirrhosis (1). This is confirmed with increasing numbers of patients with NAFLD presenting with HCC or requiring liver transplantation. In fact, NASH is now ranked as the second most common cause of liver transplantation and will likely overtake hepatitis as the number one cause of liver transplantation in the future, as more hepatitis C virus patients are treated with highly curative antiviral regimens (9,67).

As noted previously, another important, long-term outcome of liver disease is the development of HCC. The current HCC incidence rate among NAFLD patients was determined to be 0.44 (range: 0.29-0.66) per 1,000 person-years (16). In another study of patients with HCC, 54.9% of

the HCC cases were related to hepatitis C virus, 16.4% to alcoholic liver disease, 14.1% were related to NAFLD, and 9.5% to hepatitis B virus. However, it is estimated that the risk for developing HCC in noncirrhotic NAFLD patients is very small given the extremely large number of patients with noncirrhotic NAFLD within the general population (61).

Alcohol Consumption and Definition of NAFLD

By definition, NAFLD indicates the lack of evidence for ongoing or recent consumption of significant amounts of alcohol. However, the precise definition of significant alcohol consumption in patients with suspected NAFLD is uncertain. A consensus meeting recommended that, for NASH clinical trials candidate eligibility purposes, significant alcohol consumption be defined as >21 standard drinks per week in men and >14 standard drinks per week in women over a 2-year period prior to baseline liver histology (68). According to the National Institute on Alcohol Abuse and Alcoholism, a standard alcoholic drink is any drink that contains about 14 g of pure alcohol (69). Unfortunately, the definition of significant alcohol consumption in published NAFLD literature has been inconsistent (70).

Guidance Statement

- 1. Ongoing or recent alcohol consumption > 21 standard drinks on average per week in men and > 14 standard drinks on average per week in women is a reasonable threshold for significant alcohol consumption when evaluating patients with suspected NAFLD.*

Evaluation of Incidentally Discovered Hepatic Steatosis

Some patients undergoing thoracic and abdominal imaging for reasons other than liver symptoms, signs, or abnormal biochemistry may demonstrate unsuspected hepatic steatosis. A

recent study showed that 11% of patients with incidentally discovered hepatic steatosis may be at high risk for advanced hepatic fibrosis based on the calculated NAFLD Fibrosis Score (71).

However, the natural history and optimal diagnostic and management strategies for this patient population have not been investigated.

Guidance Statements

2. *Patients with unsuspected hepatic steatosis detected on imaging who have symptoms or signs attributable to liver disease or have abnormal liver chemistries should be evaluated as though they have suspected NAFLD and worked up accordingly.*
3. *Patients with incidental hepatic steatosis detected on imaging who lack any liver-related symptoms or signs and have normal liver biochemistries should be assessed for metabolic risk factors (eg, obesity, diabetes mellitus, dyslipidemia) and alternate causes for hepatic steatosis such as significant alcohol consumption or medications.*

Screening for NAFLD in Primary Care, Diabetes, and Obesity Clinics

It can be argued that there should be systematic screening for NAFLD, at least among higher-risk individuals with diabetes or obesity. For example, not only do patients with type 2 diabetes have higher prevalence of NAFLD, but the available evidence suggests higher prevalence of NASH and advanced stages of fibrosis among type 2 diabetes patients (72-74). However, there are significant gaps in our knowledge regarding the diagnosis, natural history, and treatment of NAFLD. A recent, cost-effective analysis using a Markov model suggested that screening for NASH in individuals with diabetes is not cost-effective at present, due to disutility associated with available treatment (75). Since liver biochemistries can be normal in patients with NAFLD, they may not be sufficiently sensitive to serve as screening tests, whereas liver ultrasound or

transient elastography are potentially more sensitive, but their utility as screening tools is unproven. Some experts recently have called for “vigilance” for chronic liver disease in patients with type 2 diabetes, but not routine screening (76).

Guidance Statements

4. *Routine Screening for NAFLD in high-risk groups attending primary care, diabetes, or obesity clinics is not advised at this time due to uncertainties surrounding diagnostic tests and treatment options, along with lack of knowledge related to long-term benefits and cost-effectiveness of screening.*
5. *There should be a high index of suspicion for NAFLD and NASH in patients with type 2 diabetes. Clinical decision aids such as NAFLD Fibrosis Score or FIB4 or vibration controlled transient elastography (VCTE) can be used to identify those at low or high risk for advanced fibrosis (bridging fibrosis or cirrhosis).*

Screening of Family Members

Several studies suggest familial clustering of NAFLD (77-80). In a retrospective cohort study, Willner et al observed that 18% of patients with NASH have a similarly affected first-degree relative (80). In a familial aggregation study of overweight children with and without NAFLD, after adjusting for age, gender, race, and BMI, the heritability of magnetic resonance (MR)–measured liver fat fraction was 0.386, and fatty liver was present in 18% of family members of children with NAFLD in the absence of elevated alanine aminotransferase (ALT) and obesity (81). Data reporting the heritability of NAFLD have been highly variable, ranging from no detectable heritability, in a large Hungarian twin cohort, to nearly universal heritability, in a

study of obese adolescents (77,82,83). In an ongoing, well-characterized cohort of community-dwelling twins in California, using MRI to quantify steatosis and fibrosis, both steatosis and fibrosis correlated between monozygotic, but not dizygotic, twin pairs, and, after multivariable adjustment, the heritability of hepatic steatosis and fibrosis was 0.52 (95% CI, 0.31-0.73; $p < 1.1 \times 10^{-11}$) and 0.50 (95% CI, 0.28-0.72; $p < 6.1 \times 10^{-1}$), respectively (84).

Guidance Statement

6. *Systematic screening of family members for NAFLD is not recommended currently.*

Initial Evaluation of the Patient with Suspected NAFLD

The diagnosis of NAFLD requires that (a) there is hepatic steatosis by imaging or histology, (b) there is no significant alcohol consumption, (c) there are no competing etiologies for hepatic steatosis, and (d) there are no coexisting causes of chronic liver disease.

Common alternative causes of hepatic steatosis are significant alcohol consumption, hepatitis C, medications, parenteral nutrition, Wilson disease, and severe malnutrition (**Table 1**). When evaluating a patient with newly suspected NAFLD, it is important to exclude coexisting etiologies for chronic liver disease including hemochromatosis, autoimmune liver disease, chronic viral hepatitis, alpha-1 antitrypsin deficiency, Wilson disease, and drug induced liver injury.

Serologic evaluation can uncover laboratory abnormalities in patients with NAFLD that do not always reflect the presence of another liver disease. Two examples of this are elevated serum

ferritin and autoimmune antibodies. Mildly elevated serum ferritin is a common feature of NAFLD that does not necessarily indicate hepatic iron overload, though it can impact disease progression. While the data are somewhat conflicting, serum ferritin >1.5 upper limit of normal was associated with more advanced fibrosis in a retrospective cohort of 628 adults (85). If serum ferritin and transferrin saturation are elevated in a patient with suspected NAFLD, genetic hemochromatosis should be excluded. Mutations in the HFE gene occur with variable frequency in patients with NAFLD, and the clinical significance is unclear (86). Liver biopsy should be considered in the setting of high ferritin and a high iron saturation to determine the presence or extent of hepatic iron accumulation and to exclude significant hepatic injury in a patient with suspected NAFLD. Low titers of serum autoantibodies, particularly antismooth muscle and antinuclear antibodies, are common in patients with NAFLD and are generally considered to be an epiphenomenon of no clinical consequence, though they often require liver biopsy to exclude autoimmune disease. In a study of 864 well-characterized NAFLD subjects from the NASH Clinical Research Network, significant elevations in serum autoantibodies (anti-nuclear antibodies >1:160 or anti-smooth muscle antibodies >1:40) were present in 21% and were not associated with more advanced disease or atypical histologic features (87).

While other diseases are being excluded, history should be carefully taken for the presence of commonly associated comorbidities including central obesity, hypertension, dyslipidemia, diabetes or insulin resistance, hypothyroidism, polycystic ovary syndrome, and obstructive sleep apnea.

Guidance Statements

7. *When evaluating a patient with suspected NAFLD, it is essential to exclude competing*

etiologies for steatosis and coexisting common chronic liver disease.

- 8. In patients with suspected NAFLD, persistently high serum ferritin, and increased iron saturation, especially in the context of homozygote or heterozygote C282Y HFE mutation, a liver biopsy should be considered.*
- 9. High serum titers of autoantibodies in association with other features suggestive of autoimmune liver disease (> 5 upper limit of normal aminotransferases, high globulins, or high total protein to albumin ratio) should prompt a work-up for autoimmune liver disease.*
- 10. Initial evaluation of patients with suspected NAFLD should carefully consider the presence of commonly associated comorbidities such as obesity, dyslipidemia, insulin resistance or diabetes, hypothyroidism, polycystic ovary syndrome, and sleep apnea.*

Noninvasive Assessment of Steatohepatitis and Advanced Fibrosis in NAFLD

The natural history of NAFLD is fairly dichotomous—NAFL is generally benign, whereas NASH can progress to cirrhosis, liver failure, and liver cancer. Liver biopsy is currently the most reliable approach for identifying the presence of steatohepatitis and fibrosis in patients with NAFLD, but it is generally acknowledged that biopsy is limited by cost, sampling error, and procedure-related morbidity and mortality. Serum aminotransferase levels and imaging tests such as ultrasound, computerized tomography, and MR do not reliably reflect the spectrum of liver histology in patients with NAFLD. Therefore, there has been significant interest in developing clinical prediction rules and noninvasive biomarkers for identifying steatohepatitis in patients with NAFLD, but their detailed discussion is beyond the scope of this practice guidance (47).

Noninvasive Quantification of Hepatic Steatosis in NAFLD

AASLD approved version submitted to HEPATOLOGY on June 28, 2017

Some studies suggest that degree of steatosis may predict the severity of histological features (eg, ballooning and steatohepatitis) (88) and the incidence and prevalence of diabetes in patients with NAFLD (89-91). MR imaging, either by spectroscopy (92) or by proton density fat fraction (93,94), is an excellent noninvasive modality for quantifying hepatic steatosis and is being widely used in NAFLD clinical trials (95). The use of transient elastography to obtain continuous attenuation parameters is a promising tool for quantifying hepatic fat in an ambulatory setting (74,96). However, the utility of noninvasively quantifying hepatic steatosis in patients with NAFLD in routine clinical care is limited.

Noninvasive Prediction of Steatohepatitis in Patients with NAFLD

The presence of metabolic syndrome is a strong predictor for the presence of steatohepatitis in patients with NAFLD (47,97-100). Although NAFLD is highly associated with components of metabolic syndrome, the presence of increasing an number of metabolic diseases such as insulin resistance, type 2 diabetes, hypertension dyslipidemia, and visceral obesity seems to increase the risk of progressive liver disease (16,18,41). Therefore, patients with NAFLD and multiple risk factors such as type 2 diabetes mellitus and hypertension are at the highest risk for adverse outcomes (20,101). Circulating levels of cytokeratin-18 fragments have been investigated extensively as novel biomarkers for the presence of steatohepatitis in patients with NAFLD (47,102,103). This test is currently not available in a clinical care setting.

Noninvasive Assessment of Advanced Fibrosis in Patients with NAFLD

The commonly investigated noninvasive tools for the presence of advanced fibrosis in NAFLD include clinical decision aids (eg, NAFLD fibrosis score, FIB-4 index, APRI), serum biomarkers

(Enhanced liver fibrosis panel, Fibrometer, FibroTest, Hepascore), or imaging (eg, transient elastography, MR elastography, acoustic radiation force impulse imaging, supersonic shear wave elastography) (104).

The NAFLD Fibrosis Score is based on 6 readily available variables (age, BMI, hyperglycemia, platelet count, albumin, aspartate aminotransferase to ALT ratio) and is calculated using the published formula (<http://gihep.com/calculators/hepatology/nafld-fibrosis-score/>). In a meta-analysis of 13 studies consisting of 3,064 patients, (47) the NAFLD Fibrosis Score had an area under the receiver operating curve (AUROC) of 0.85 for predicting advanced fibrosis (ie, bridging fibrosis with nodularity or cirrhosis). A score <-1.455 had 90% sensitivity and 60% specificity to exclude advanced fibrosis, whereas a score >0.676 had 67% sensitivity and 97% specificity to identify the presence of advanced fibrosis. FIB-4 index (<http://gihep.com/calculators/hepatology/fibrosis-4-score/>) is an algorithm based on platelet count, age, AST, and ALT that offers dual cutoff values (patients with score < 1.45 are unlikely, whereas patients with score >3.25 are likely to have advanced fibrosis) (104). A recent study that compared various risk scores and elastography (MR as well as transient elastography) against liver histology showed that NAFLD Fibrosis Score and FIB-4 were (a) better than other indices such as BARD, APRI and AST/ALT ratio and (b) as good as MR elastography for predicting advanced fibrosis in patients with biopsy-proven NAFLD (105).

The Enhanced Liver Fibrosis (ELF) panel consists of plasma levels of 3 matrix turnover proteins (hyaluronic acid, TIMP-1, and PIIINP) had an AUROC of 0.90 with 80% sensitivity and 90% specificity for detecting advanced fibrosis (bridging fibrosis or cirrhosis) (106). This panel has

been recently approved for commercial use in Europe, but is not available for clinical use in the United States.

VCTE (Fibroscan ®), which measures liver stiffness noninvasively, was recently approved by the United States Food and Drug Administration for use in both adults and children with liver diseases. Two recent studies investigated the performance of VCTE in patients with suspected NAFLD using an M probe. Tapper et al reported the performance of VCTE in 164 patients with biopsy-proven NAFLD (median BMI 32.2 kg/m²) from the United States (107,108). The optimal liver stiffness measurement cutoff for advanced fibrosis was 9.9 kPa with 95% sensitivity and 77% specificity. The AUROC for detecting advanced fibrosis was 0.93 (95% CI: 0.86-0.96).

Interestingly, in 27% of the participants the VCTE yielded unreliable results (107). Imajo et al reported the performance of VCTE an with M probe in 142 Japanese patients with biopsy-proven NAFLD (mean BMI 28.1 kg/m²) (108). The failure rate for VCTE in this cohort was 10.5%. The AUROC for VCTE for identifying advanced fibrosis (bridging fibrosis and cirrhosis) was 0.88 (95% CI: 0.79-0.97). The NASH Clinical Research Network (NASH CRN) recently reported its experience with VCTE in 511 patients with biopsy-proven NAFLD (mean BMI 33.6 kg/m²) across 8 clinical centers in the United States, using a machine guided protocol with either an M + or XL + probe (109). Failure rate for obtaining a reliable liver stiffness measurement was 2.6%. MR elastography (MRE) is excellent for identifying varying degrees of fibrosis in patients with NAFLD (110,111). In the study by Imajo et al, MRE performed better than VCTE for identifying fibrosis stage 2 or above, but they both performed equally well in identifying fibrosis stage 3 or above (ie, bridging fibrosis). AUROCs for TE and MRE were 0.88 and 0.89, respectively.

Recent genome-wide association studies have associated several genetic polymorphisms, notably PNPLA3 variants, with steatohepatitis and advanced fibrosis in patients with NAFLD (112-121). However, testing for these genetic variants in routine clinical care is currently not advocated.

Guidance Statements

- 11. In patients with NAFLD, the metabolic syndrome predicts the presence of steatohepatitis, and its presence can be used to target patients for a liver biopsy.*
- 12. NAFLD Fibrosis Score or FIB-4 index are clinically useful tools for identifying NAFLD patients with higher likelihood of having bridging fibrosis (stage 3) or cirrhosis (stage 4).*
- 13. VCTE or MRE are clinically useful tools for identifying advanced fibrosis in patients with NAFLD.*

When to Obtain a Liver Biopsy in Patients with NAFLD

Liver biopsy remains the gold standard for characterizing liver histologic alterations in patients with NAFLD. However, biopsy is expensive, requires expertise for interpretation, and carries some morbidity and very rare mortality risk. Thus, it should be performed in those who would benefit the most from diagnosis, therapeutic guidance, and prognostic information.

Guidance Statements

- 14. Liver biopsy should be considered in patients with NAFLD who are at increased risk of having steatohepatitis and/or advanced fibrosis.*
- 15. The presence of metabolic syndrome, NAFLD Fibrosis Score or FIB-4, or liver stiffness*

measured by VCTE or MRE may be used for identifying patients who are at risk for steatohepatitis and/or advanced fibrosis.

- 16. Liver biopsy should be considered in patients with suspected NAFLD in whom competing etiologies for hepatic steatosis and the presence and/or severity of coexisting chronic liver diseases cannot be excluded without a liver biopsy.*

Histopathology of Adult NAFLD

The histopathologic features of adult NAFLD are prototypic, regardless of underlying pathogenesis, with the exception of severe alcoholic hepatitis, which has lesions not shared by severe NASH (122). The goals for histopathologic evaluation of liver biopsy in a subject with suspected NAFLD include confirming or excluding the diagnosis and providing commentary on severity of the disease. It is currently the standard to report grade (necroinflammatory “activity”) separately from stage, which comments on location of abnormal collagen deposition and architectural remodeling, ie, “fibrosis.” The following diagnostic categories for NAFLD have been utilized by the NASH CRN: Not NAFLD (< 5% steatosis, by definition); NAFL, not NASH ($\geq 5\%$ steatosis, with or without lobular and portal inflammation); Borderline steatohepatitis, zone 3 or Borderline steatohepatitis, zone 1 (most, but not all criteria for steatohepatitis present, with accentuation of steatosis or injury in zone 3 or zone 1, respectively), and Definite steatohepatitis (all criteria present, including steatosis, hepatocellular ballooning, and lobular inflammation) (123). Any of these diagnostic categories, including Not NAFLD, may have no fibrosis or any amount of fibrosis up to cirrhosis. Specifically, stage 1 is zone 3 (perivenular),

perisinusoidal, or periportal fibrosis; stage 2 is both zone 3 and periportal fibrosis; stage 3 is bridging fibrosis with nodularity; stage 4 is cirrhosis.

Histopathologic features of NAFLD in children may differ from those in adult NAFLD, particularly in younger years: Steatosis may be more abundant, or accentuated in zone 1 hepatocytes, and inflammation and fibrosis may be concentrated in portal tracts initially. Ballooning is less frequent (124-126). Interested readers may refer to other recent publications for detailed description of pathological features of fatty liver disease in adults and children (126,127).

There are 2 systems for semiquantitative assessment of necroinflammatory lesions in NAFLD: NAFLD Activity Score (NAS) from the NASH CRN (128), and Steatosis Activity Fibrosis (SAF) from the European Fatty Liver Inhibition of Progression Consortium (129,130). Both utilize the lesions stated above, but exact criteria and stated goals for utilization differ. The former was developed as a method of comparing biopsies in clinical trials, but stands separately from a pattern-based diagnosis; the latter utilizes the score for diagnosis as well as for use in clinical trials. Clinicians and pathologists benefit from familiarity with understanding the details of these systems prior to implementation. Even though NAFLD and NASH result in diffuse parenchymal involvement, as with other forms of chronic liver injury, there is well-recognized regional variability. Sampling “error,” however, remains a concern for diagnosis (131) and for clinical trials with histologically-based entry criteria and outcomes. Approaches to lessen the effects of sampling error include large needle size (eg, 2-3 cm in length and 16 gauge) (132-134) and at least 1 core biopsy (133). The study by Vuppalanchi et al (133) noted that a diagnosis of definite NASH was more common with 2 cores, in biopsies ≥ 25 mm, and when a single expert pathologist read a biopsy twice.

Guidance Statements

- 17. Clinically useful pathology reporting should include a distinction between NAFL (steatosis), NAFL with inflammation, and NASH (steatosis with lobular and portal inflammation and hepatocellular ballooning). A comment on severity (mild, moderate, severe) may be useful. Specific scoring systems such as NAS (128) and/or Steatosis Activity Fibrosis (SAF) (128,129) may be used as deemed appropriate.*
- 18. The presence or absence of fibrosis should be described. If present, a further statement related to location, amount, and parenchymal remodeling is warranted.*

MANAGEMENT OF PATIENTS WITH NAFLD

WHOM TO TREAT: The management of NAFLD should consist of treating liver disease as well as the associated metabolic comorbidities such as obesity, hyperlipidemia, insulin resistance, and T2DM. As patients with NAFLD without steatohepatitis or any fibrosis have excellent prognosis from a liver standpoint, pharmacological treatments aimed primarily at improving liver disease should generally be limited to those with biopsy-proven NASH and fibrosis.

- 19. Pharmacological treatments aimed primarily at improving liver disease should generally be limited to those with biopsy-proven NASH and fibrosis.*

Lifestyle Intervention

Lifestyle modification consisting of diet, exercise, and weight loss has been advocated to treat patients with NAFLD. The best data generated to date demonstrates that overall weight loss is the key to improvement in the histopathologic features of NASH. In a meta-analysis of 8

randomized controlled trials, 4 with post-treatment histology, those adults who were able to lose at least 5% of body weight had improvement in hepatic steatosis, while $\geq 7\%$ body weight reduction was associated with NAS improvement (135). These data have been supported by a more recent 12-month prospective trial with paired liver biopsies in 261 patients (136). In this trial, a dose response curve was demonstrated wherein the greater the degree of weight loss, the more significant the improvement in histopathology such that $\geq 10\%$ weight loss was associated with improvement in all features of NASH, including portal inflammation and fibrosis. However, it is important to note that those patients losing $\geq 5\%$ body weight stabilized or improved fibrosis in 94% of the cases. Unfortunately, only 50% of patients were able to achieve at least a 7% weight loss at 12 months in this trial.

Compliance with a calorie-restricted diet over the long term is associated with mobilization of liver fat and improvement in cardiovascular risk (137). The specific macronutrient composition of the diet, over months to years, appears to be less relevant than the end result of sustained weight loss. Prospective trials comparing various macronutrient diets in NAFLD patients are limited by a lack of sufficient power as well as pre- and post-treatment histopathology. Data suggests, however, that decreasing caloric intake by at least 30% or by approximately 750-1000 kcal/day results in improvement in insulin resistance and hepatic steatosis (138,139). The Mediterranean diet (higher in monounsaturated fatty acids) has also been studied in comparison to a high-fat, low-carbohydrate diet for 6 weeks and, while there was no change in weight loss, MRI results showed significant improvement in steatosis in the Mediterranean diet group. Ultimately, rigorous, prospective, longer-term trials with histopathologic endpoints are required before recommendations related to specific macronutrient diets can be made.

The majority of NAFLD patients are engaged in minimal physical activity (140), and this has been associated with an increased risk of metabolic syndrome and NAFLD (141). Large, randomized controlled trials assessing the effect of exercise on histopathology in NASH are lacking; however, a recent meta-analysis showed an improvement in hepatic steatosis with exercise, but no improvement in alanine aminotransferase levels. The optimal duration and intensity of exercise remains undetermined. However, data suggest that patients who maintain physical activity more than 150 minutes/week or increase their activity level by more than 60 minutes/week have more pronounced decrement in serum aminotransferases, independent of weight loss (142). This is supported by a large Korean population study demonstrating that exercise frequency of ≥ 5 times/week, consisting of moderate exercise (carrying light loads, riding a bike at a steady pace, or playing tennis for at least 10 minutes), was associated with the greatest benefit in prevention of NAFLD development or improvement in patients that previously had NAFLD, independent of BMI over the 5-year follow-up (143). The effects of exercise on underlying NASH are less clear, but from a large, retrospective assessment of biopsy-proven NAFLD patients, moderate-intensity exercise (metabolic equivalents of 3-5.9) or total exercise per week was not associated with improvement in NASH severity or fibrosis. However, patients meeting vigorous (≥ 6 metabolic equivalents) activity recommendations did have improvement in NASH, although doubling of the vigorous activity recommendations was required to have a benefit on fibrosis (140).

Both diet and exercise counseling are often recommended for patients with NAFLD to achieve weight loss goals. Unfortunately, data evaluating the efficacy of combination diet and exercise on NAFLD are limited. When focusing on weight loss alone in a pooled analysis of 18 trials, combination diet plus exercise resulted in a 1.14 kg greater weight loss than diet alone (144).

Focusing on NAFLD, a systematic review of combined diet and aerobic exercise programs showed improvement in liver fat assessment and/or liver enzymes with 3 to 6 months of follow-up (145). In the largest paired biopsy study to date, 1 year of a calorically restricted diet (750 kcal/day) plus recommendations to walk 200 minutes/week resulted in a dose response relationship of weight loss to histopathologic improvement in inflammation, ballooning, and fibrosis (136).

Guidance Statements

20. *Weight loss generally reduces hepatic steatosis, achieved either by hypocaloric diet alone or in conjunction with increased physical activity. A combination of a hypocaloric diet (daily reduction by 500-1000 kcal) and moderate intensity exercise is likely to provide the best likelihood of sustaining weight loss over time.*
21. *Weight loss of at least 3 to 5% of body weight appears necessary to improve steatosis, but a greater weight loss (7 to 10%) is needed to improve the majority of the histopathologic features of NASH, including fibrosis.*
22. *Exercise alone in adults with NAFLD may prevent or reduce hepatic steatosis, but its ability to improve other aspects of liver histology remains unknown.*

Insulin Sensitizers

Metformin

Several studies investigated the effect of metformin on aminotransferases and/or liver histology in patients with NASH (146-156). Although several studies have shown an improvement in serum aminotransferases and insulin resistance, metformin does not significantly improve liver

histology. Two published meta-analyses conclude that metformin therapy did not improve liver histology in patients with NAFLD and NASH (157,158).

Guidance Statement

23. Metformin is not recommended for treating NASH in adult patients.

Thiazolidinediones

Thiazolidinediones are ligands for the nuclear transcription factor PPAR- γ with broad effects on glucose and lipid metabolism, as well as on vascular biology and inflammation (159). The ability of thiazolidinediones to reverse adipose tissue dysfunction and insulin resistance in obesity and T2DM have led to randomized controlled trials (RCTs) exploring their role in NASH (160).

Studies with rosiglitazone reported an improvement in hepatic steatosis, but not of necroinflammation or fibrosis (161,162). Rosiglitazone is no longer available in most countries, and its prescribing remains severely restricted in the United States due to controversial findings of an increase in coronary events, although no firm association was found after an extensive review of all evidence by the Food and Drug Administration (163).

In an early proof-of-concept study, Belfort et al conducted an RCT of pioglitazone (45 mg/day) in 55 patients with NASH and prediabetes or T2DM (164). Treatment improved insulin sensitivity and aminotransferases, steatosis, inflammation, and ballooning. The NAS improved with pioglitazone in 73% compared to 24% of placebo-treated patients ($P < 0.001$) and there was a trend towards improvement in fibrosis among patients randomized to pioglitazone ($P = 0.08$). In a recent study, Cusi et al treated 101 patients with biopsy-

proven NASH having either prediabetes ($n = 49$) or T2DM ($n = 52$) with a hypocaloric diet (a 500 kcal/day deficit from weight-maintaining caloric intake) and pioglitazone, 45 mg/day, or placebo for 18 months, followed by an 18-month open-label phase with pioglitazone treatment (165). The primary outcome was a reduction of at least 2 points in the NAS (in 2 different histologic categories) without worsening of fibrosis. In patients treated with pioglitazone, 58% achieved the primary outcome and 51% had resolution of NASH (both $P < 0.001$). Pioglitazone treatment also improved fibrosis ($P = 0.039$). Metabolic and histologic improvements continued over 36 months of therapy (165). Adverse events were overall no different between groups, but weight gain was greater with pioglitazone (2.5 kg vs placebo at 18 months; and a total of 3.0 kg over 36 months).

Pioglitazone is also of benefit in patients with NASH without diabetes. Aithal et al performed an RCT with either pioglitazone 30 mg/day or placebo for 12 months in 74 patients with NASH (166). While steatosis did not improve significantly compared to placebo, treatment did significantly ameliorate hepatocellular injury and fibrosis. In the Pioglitazone versus Vitamin E versus Placebo for the Treatment of Nondiabetic Patients with Nonalcoholic Steatohepatitis (PIVENS) trial, a large multicenter RCT in nondiabetic patients with NASH, 247 patients were randomized to pioglitazone (30 mg/day), vitamin E (800 IU/day), or placebo for 24 months (167). The primary endpoint was an improvement in NAS by ≥ 2 points with at least 1-point improvement in hepatocellular ballooning and 1-point improvement in either the lobular inflammation or steatosis score, and no increase in the fibrosis score. This was achieved in 19% in the placebo group compared to 34% in the pioglitazone group ($P = 0.04$ vs placebo) and 43% in the vitamin E group ($P = 0.001$ vs placebo) (168). Because this study consisted of 2 primary

comparisons (pioglitazone vs placebo and vitamin E vs placebo), a P-value of 0.025 was considered to be significant *a priori*. Therefore, although there were histological benefits associated with pioglitazone, this study concluded that pioglitazone did not meet the primary end point. However, resolution of NASH, a key secondary end point, was achieved in a significantly higher number of patients receiving pioglitazone than receiving placebo (47% vs 21%, $P < 0.001$) (167). Vitamin E and pioglitazone were well tolerated and there were no differences in other adverse events.

Weight gain is the most common side effect associated with pioglitazone treatment, likely from improved adipose tissue insulin action and increased adipocyte triglyceride synthesis. It ranges from 2.5 kg to 4.7 kg in RCTs of 12- to 36-month duration (165-167). Bladder cancer has been a concern, with population-based studies reporting either positive or negative associations (169-171). However, Lewis et al followed 193,099 persons aged ≥ 40 years for up to 16 years and found no statistically significant association between bladder cancer risk and use of pioglitazone or increasing duration of therapy (172). Finally, bone loss may occur in women treated with thiazolidinediones (169).

Guidance Statements

24. *Pioglitazone improves liver histology in patients with and without T2DM with biopsy-proven NASH. Therefore, it may be used to treat these patients. Risks and benefits should be discussed with each patient prior to starting therapy.*
25. *Until further data supports its safety and efficacy, pioglitazone should not be used to treat NAFLD without biopsy-proven NASH.*

Glucagon-Like Peptide-1 Analogues

There has been an interest in investigating the role of glucagon-like peptide-1 as therapeutic agents in patients with NAFLD and NASH (173-177). In a recently published randomized placebo-controlled trial consisting of 52 patients with biopsy-proven NASH, liraglutide administered subcutaneously once daily for 48 weeks was associated with greater resolution of steatohepatitis and less progression of fibrosis (174). As expected, liraglutide was associated with greater weight loss but also gastrointestinal side effects.

Guidance Statement

26. It is premature to consider glucagon-like peptide-1 agonists to specifically treat liver disease in patients with NAFLD or NASH.

Vitamin E

Oxidative stress is considered a key mechanism of hepatocellular injury and disease progression in subjects with NASH. Vitamin E is an antioxidant and has been investigated as a treatment for NASH (178-182). Comparison between these trials is difficult due to varying criteria for entry into the study, different doses of vitamin E and unclear formulations of vitamin E used that could affect its bioavailability, the additional use of other antioxidants or other drugs, and limited histologic data to assess outcomes. Also, most studies were relatively under-powered and did not meet or publish CONSORT criteria for clinical trials. Despite these limitations, it can be summarized that (1) the use of vitamin E is associated with a decrease in aminotransferases in subjects with NASH, (2) studies in which histologic endpoints were evaluated indicate that vitamin E results in improvement in steatosis, inflammation, and ballooning and resolution of steatohepatitis in a proportion of nondiabetic adults with NASH, and (3) vitamin E did not have

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an effect on hepatic fibrosis. In the PIVENS clinical trial (167), the pure form of rrr α -tocopherol was orally administered at a dose of 800 IU/day for 96 weeks. The primary endpoint was achieved in a significantly greater number of participants receiving vitamin E compared to placebo (42% vs 19%, $P < 0.001$, number needed to treat = 4.4). In the Treatment of Nonalcoholic Fatty Liver Disease in Children trial (TONIC), which tested vitamin E (800 IU/day) or metformin (500 mg twice daily) against placebo in children with biopsy-proven NAFLD, resolution of NASH was significantly greater in children treated with vitamin E than in children treated with placebo (58% vs 28%, $P = 0.006$) (183). Two recent meta-analyses reported significant histological benefits with vitamin E in patients with NASH (184,185).

There are also lingering concerns about the long-term safety of vitamin E. One meta-analysis suggested that doses of >800 IU/day were associated with increased all-cause mortality (186). However, this meta-analysis has been criticized because several studies with low mortality were excluded and concomitant vitamin A and other drug administration as well as common factors such as smoking were not considered. A subsequent analysis of these trials with the addition of more studies suggested the differences were driven by imbalance in males in the trials in question (187). A large meta-analysis that included 57 studies and 246,371 subjects followed from 1 to 10 years did not demonstrate a relationship between vitamin E supplementation and all-cause mortality (188). In a large RCT published in 2011, vitamin E administered at a dose of 400 IU/day was unexpectedly and unexplainably associated with a modest increase in the risk of prostate cancer (absolute increase of 1.6 per 1,000 person-years of vitamin E use) (189), and this risk may be modified by baseline selenium concentration (190) or genetic variants associated with vitamin metabolism (191).

Guidance Statements

27. *Vitamin E (rrr α -tocopherol) administered at a daily dose of 800 IU/day improves liver histology in nondiabetic adults with biopsy-proven NASH and therefore may be considered for this patient population. Risks and benefits should be discussed with each patient prior to starting therapy.*

28. *Until further data supporting its effectiveness become available, vitamin E is not recommended to treat NASH in diabetic patients, NAFLD without liver biopsy, NASH cirrhosis, or cryptogenic cirrhosis.*

Bariatric Surgery

NAFLD at all stages is more common in those who meet criteria for bariatric surgery.

Nonsurgical weight loss is effective in improving all histologic features of NAFLD including fibrosis, though most patients had early stage fibrosis (136). However, sustained weight loss is difficult to achieve and harder yet to sustain. Bariatric surgery improves or eliminates comorbid disease in most patients and improves long-term survival and death from cardiovascular disease (CVD) and malignancy, the 2 most common causes of death in NAFLD(192-195). While there are no RCTs of bariatric surgery in NASH (and unlikely to be in the future), there are several retrospective and prospective cohort studies and 2 large single-center studies with follow-up liver biopsies. Mathurin et al prospectively correlated clinical and metabolic data with liver histology at time of surgery and 1 and 5 years after bariatric surgery in 381 adult patients with severe obesity (196). Gastric band, bilio-intestinal bypass, and gastric bypass were done in 56%, 23%, and 21%, respectively. Compared to baseline, there was a significant improvement in the prevalence and severity of steatosis and ballooning at 1 and 5 years following bariatric surgery.

In patients with probable or definite NASH at baseline ($n = 99$), there was a significant improvement in steatosis, ballooning, and NAS and resolution of probable or definite NASH at 1 and 5 years following bariatric surgery. Most histological benefits were evident at 1 year with no differences in liver histology between 1 and 5 years following bariatric surgery. Intriguingly, a minor but statistically significant increase in mean fibrosis score was noted at 5 years after the bariatric surgery (from 0.27 ± 0.55 at baseline to 0.36 ± 0.59 , $P = 0.001$). Despite this increase, at 5 years, 96% of patients exhibited fibrosis score ≤ 1 and 0.5% had bridging fibrosis, indicating that there is no clinically significant worsening in fibrosis that can be attributed directly to the procedure. In a follow-up study focused on those with NASH at baseline undergoing bariatric surgery, Lassailly et al prospectively examined 109 patients with NASH at the time of bariatric surgery and performed follow-up biopsies 1 year later. Eighty-five percent of patients had NASH resolution (95% CI: 75.8%-92.2%). Importantly, in contrast to prior data, fibrosis improved at 1 year after surgery in 33% of patients (197). Furthermore, a meta-analysis of available data in 2015 also showed that the majority of patients undergoing bariatric surgery appear to improve or completely resolve the histopathologic features of steatosis, inflammation, and ballooning. Fibrosis also improved by a weighted mean decrease by 11.9% in the incidence of fibrosis (198).

The safety and efficacy of bariatric surgery in patients with NASH cirrhosis is not well established. An analysis performed from the Nationwide Inpatient Sample (1998-2007) estimated perioperative mortality and inpatient hospital stays for patients undergoing bariatric surgery with and without cirrhosis. Compared to those without cirrhosis (0.3%, $n = 670,095$), mortality was higher in those with compensated cirrhosis (0.9%, $n = 3,888$) and much higher in those with decompensated cirrhosis (16.3%, $n = 62$) (199). A recent systematic review of bariatric surgery in 122 patients with cirrhosis (97% Child's A cirrhosis) described 1.6% early

and 2.45% late, surgery-related mortality (200). Noteworthy is 0% mortality associated with surgery among 41 cirrhotic patients who had sleeve gastrectomy.

Guidance Statements

29. *Foregut bariatric surgery can be considered in otherwise eligible obese individuals with NAFLD or NASH.*

30. *It is premature to consider foregut bariatric surgery as an established option to specifically treat NASH.*

31. *The type, safety, and efficacy of foregut bariatric surgery in otherwise eligible obese individuals with established cirrhosis due to NAFLD are not established. In otherwise eligible patients with compensated NASH or cryptogenic cirrhosis, foregut bariatric surgery may be considered on a case by case basis by an experienced bariatric surgery program.*

Ursodeoxycholic Acid, Omega-3 Fatty Acids, and Miscellaneous Agents

Several studies (180,201-204) have investigated ursodeoxycholic acid (conventional and high doses) to improve aminotransferases and steatosis in patients with NAFLD and liver histology in patients with NASH. All but one study (203) have been proof-of-concept studies with small numbers of participants and/or surrogate endpoints. Notably, a single, large, multicenter RCT convincingly showed that ursodeoxycholic acid offers no histological benefit over placebo in patients with NASH (203). Omega-3 fatty acids, currently approved in the United States to treat hypertriglyceridemia, have been investigated to treat NAFLD both in animal models and in humans (205). In a review of the published literature in 2010, Masterton et al (206) found experimental evidence to support the use of omega-3 fatty acids in patients with NAFLD to improve liver disease, but the interpretation of human studies was limited by small sample size

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and methodological flaws. However, 2 recently reported studies failed to show convincing therapeutic benefit for omega-3 fatty acids in patients with NAFLD or NASH (207,208). More than a dozen other miscellaneous agents have been investigated in small, proof-of-concept studies, and their detailed evaluation is beyond the scope of this guidance.

Guidance Statements

32. Ursodeoxycholic acid *is not recommended for the treatment of NAFLD or NASH.*
33. Omega-3 fatty acids *should not be used as a specific treatment of NAFLD or NASH, but they may be considered to treat hypertriglyceridemia in patients with NAFLD.*

Alcohol Use in Patients with NAFLD and NASH

Heavy alcohol consumption is a risk factor for chronic liver disease and should be avoided by patients with NAFLD and NASH. The National Institute on Alcohol Abuse and Alcoholism defines heavy or at-risk drinking as more than 4 standard drinks on any day or more than 14 drinks per week in men or more than 3 drinks on any day or 7 drinks per week in women (209). Although several cross-sectional studies (210-216) have suggested a beneficial effect of light alcohol consumption (on average less than 1 drink per day) on the presence (defined either biochemically or by imaging) and severity of NAFLD, a recent meta-regression analysis of 42,059 participants combined from 6 studies raised the possibility of potential confounding caused by lower BMI among those who are moderate drinkers (217). There are no longitudinal studies reporting the effect of ongoing alcohol consumption on disease severity or natural history of NAFLD or NASH. The effects of light drinking on the cardiovascular system and cancer risks, if any, have not been investigated in individuals with NAFLD.

Guidance Statements

34. *Patients with NAFLD should not consume heavy amounts of alcohol.*

35. *There are insufficient data to make recommendations with regards to nonheavy consumption of alcohol by individuals with NAFLD.*

Management of Cardiovascular Disease and Dyslipidemia

There is a strong association between NAFLD and increased risk of CVD events and mortality that withstands correction for traditional CVD risk factors (218,219). Debate remains over the causal relationship between NAFLD and CVD; however, NAFLD at minimum represents a risk marker, and thus, attention to and control of CVD risk factors is critical. Furthermore, there are many mechanistic links between NAFLD and various stages of the atherosclerotic process and cardiac structure and function. Some of these include but are not limited to endothelial dysfunction, atherogenic dyslipidemia and impaired cardiac mechanics (220).

Patients with NAFLD have a pro-atherogenic lipid profile characterized by high triglyceride , increased very low density lipoprotein and high apolipoprotein B to apolipoprotein A-1 ratio, as well as a higher concentration of small dense LDL coupled with low HDL concentration (221).

These changes seem to be driven by hepatic lipid concentration and insulin resistance, predominately at the level of adipose tissue, rather than by the presence of NASH, *per se* (222,223). Although we have limited evidence of the long-term benefits of treating patients with NAFLD specifically, targeted treatment of atherogenic dyslipidemia in patients with diabetes or metabolic syndrome does reduce CVD and favorably impacts mortality. A recent *post hoc* analysis of the cardiovascular outcomes study, GREACE, observed that statins significantly

improved aminotransferases and cardiovascular outcomes in patients with elevated aminotransferases presumed due to NAFLD (224). Another post hoc analysis of the IDEAL trial suggested a benefit of high dose statins in those with baseline elevation in ALT compared to moderate intensity statins (225). Thus, it is reasonable to incorporate lipid-lowering therapy in patients with NAFLD who meet criteria based on current recommendations (226). While reluctance to use statins in patients with suspected or established chronic liver disease, including NAFLD and NASH, may persist, several studies have established the safety of statins in patients with liver disease regardless of baseline elevation in liver chemistries. Furthermore, the risk of statin induced hepatotoxicity is not higher in those with chronic liver disease (227,228). Although elevated aminotransferases are not uncommon in patients receiving statins, serious liver injury from statins is quite rare in clinical practice.

Clinical trials of statins as treatment for NASH are limited and have shown inconsistent results, with liver enzymes improving modestly or not at all and variable effects on histology when this was assessed (229-232). One small RCT did not demonstrate a benefit of simvastatin in reducing liver enzymes or liver histology (233).

Guidance Statements

36. Patients with NAFLD are at high risk for cardiovascular morbidity and mortality. Thus, aggressive modification of CVD risk factors should be considered in all patients with NAFLD.

37. Patients with NAFLD or NASH are not at higher risk for serious liver injury from statins. Thus, statins can be used to treat dyslipidemia in patients with NAFLD and NASH. While statins may be used in patients with NASH cirrhosis, they should be avoided in patients with

decompensated cirrhosis.

Agents in Registration Trials

Currently, obeticholic acid (NCT02548351) and elafibranor (NCT02704403) are 2 compounds that are being tested in phase 3 registration trials. Obeticholic acid, a potent farnesoid X receptor agonist, administered at 25 mg per day dose improved steatohepatitis and fibrosis over a 72 week period in a large, multicenter, phase 2b clinical trial (234). In this study, OCA was associated with dyslipidemia and itching. This compound was recently approved by the United States Food and Drug Administration for treating patients with primary biliary cirrhosis who are unresponsive to ursodeoxycholic acid therapy in a dose up to 10 mg/day. Elafibranor (a dual PPAR α/δ agonist) 120 mg/day, in a recently reported phase 2 study exhibited an efficacy signal for improving NASH without fibrosis worsening over a 12 month study period (235). While this treatment was associated with improved cardiometabolic profiles, there was a mild, reversible increase in serum creatinine.

Guidance Statement

38. Until further safety and efficacy data become available in patients with NASH, we recommend that obeticholic acid should not be used off-label to treat NASH.

NASH, Obesity and Liver Transplantation

Pre-Liver Transplant Considerations

NASH and cryptogenic cirrhosis are highly prevalent among patients awaiting liver transplantation, and, in fact, NASH is on a trajectory to become the most common indication for liver transplantation in the United States (45,67).

Higher BMI, common among patients with NASH, is associated with an increased risk of clinical decompensation while awaiting liver transplantation (236,237) and may present technical challenges to performing liver transplantation. Although an analysis of the United Network for Organ Sharing database reported a higher frequency of post-transplant complications and increased graft loss and mortality among patients with class III obesity ($\text{BMI} > 40 \text{ kg/m}^2$) at the time of transplant (238), when corrected for ascites, higher BMI does not appear to independently confer an increased risk of mortality or allograft failure (239,240). The effects of fluid retention on BMI and variability in the distribution of body fat reduce the utility of BMI as a sole factor in determining transplant candidacy. An upper limit of BMI that identifies candidates as technically inoperable or too high-risk for adverse post-transplant outcomes has not been identified for liver transplant recipients. In contrast, pre-transplant weight reduction and subsequently successful liver transplantation has been reported in a series of waitlisted patients with class III obesity (241). Substantial success has been reported in improving pre-transplant body habitus and weight through intensive diet and exercise in obese patients being considered for liver transplantation. The role of bariatric surgery as an adjunct to liver transplantation, particularly sleeve gastrectomy, which preserves absorptive dynamics of almost all medications as well as access to the lower esophagus, is under evaluation (241).

Obesity is strongly associated with sarcopenia, which has been consistently identified as an independent predictor of post-transplant mortality and graft loss (242-244). Because of the high prevalence of obesity and sarcopenia among patients with NASH and cryptogenic cirrhosis, a

multifaceted assessment of nutritional status is recommended. Preoperative nutritional status assessment with some combination of computed tomography (243,245-247), dual energy X-ray absorptiometry (248), hand-grip strength (248), and triceps skinfold thickness (249) have all been reported to be useful in this setting.

As described previously, NASH is associated with a high frequency of cardiovascular disease (218,250,251). Noninvasive functional cardiac testing (eg, with dobutamine stress echocardiography) is recommended in patients with NASH cirrhosis, with progression to coronary angiography when noninvasive testing is abnormal or inconclusive (252). NASH is also associated with an increased prevalence of chronic kidney disease, and is, in fact, the most rapidly growing indication for simultaneous liver kidney transplantation in the United States (253). Because of the high prevalence of sarcopenia among patients with NASH, serum creatinine may overestimate glomerular filtration rate. Direct measures of glomerular filtration rate or determination of cystatin C (eg, with the creatinine-cystatin C equation) is more accurate than estimates of renal function that are derived from serum creatinine alone (254).

Post-Liver Transplant Considerations

Post-transplant outcomes are generally good following liver transplantation for NASH, with 1- and 3-year patient and graft survival rates that are comparable to other indications (45). The excellent 5-year graft survival suggests that recurrence of NASH is an uncommon cause of mortality and graft loss, at least in midterm (45). Some histological evidence of NAFLD is common following liver transplantation. Steatosis at or above grade 2 (34-66% by biopsy), for example, is seen in ~60% of recipients by the end of the second postoperative year, a rate that is higher than seen among patients undergoing liver transplantation for indications other than

NASH (255). NASH with progressive fibrosis, eg, METAVIR stage ≥ 2 (more than septal formation, thus bridging fibrosis and cirrhosis), is uncommon, occurring in ~5% of recipients by the fifth postoperative year (255,256). A recent single-center experience suggested higher incidence of advanced fibrosis (up to 27%), but this study suffers from modest sample size and selection bias (257).

In general, management recommendations for liver transplant recipients are similar to those for other patients with NASH. Ongoing attention to and assistance with achieving and maintaining a healthy weight and diet are important in the management of post-transplant NASH as weight gain is common following liver transplantation, exacerbated by immunosuppression and debility (258). Metabolic syndrome is very common in liver transplant recipients, particularly those with a history of NASH (259). There are some important pharmacological considerations that relate to the high prevalence of the metabolic syndrome among patients. Calcineurin inhibitors and corticosteroids exacerbate diabetes and impair insulin secretion (260,261).

Guidance Statements

39. Patients with NASH cirrhosis have high prevalence of cardiovascular disease. Thus, careful attention should be paid to identifying cardiovascular disease, whether clinically apparent or occult, during the transplant evaluation process.

Miscellaneous Guidance Statements Pertinent to Clinical Practice

40. Patients with NASH cirrhosis should be screened for gastroesophageal varices according to the AASLD[and American College of Gastroenterology practice guidelines (262).

41. *Patients with cirrhosis suspected due to NAFLD should be considered for HCC screening according to the AASLD practice guidelines (263).*
42. *Current evidence does not support routine screening and surveillance for HCC in patients with noncirrhotic NASH.*
43. *Current evidence does not support routinely repeating a liver biopsy in patients with NAFL or NASH, but this may be considered on a case by case basis.*

Aspects of NAFLD Specific to Children and Adolescents

NAFLD in childhood may be due to more penetrant genetic risk or enhanced sensitivity to environmental provocation. Adults with onset of NAFLD in childhood may be most at risk for early or severe complications. The definitions of NAFLD and its subphenotypes in childhood are the same as in adults. Children with NAFLD are reported as early as 2 years and with NASH-related cirrhosis as early as age 8 years (124,264).

Prevalence and Risk Factors

Estimation of population prevalence of NAFLD in children presents difficulties for the same reasons detailed above in adults. Estimates vary based upon the type of test or imaging, the cut-points for detection, and the age, sex, race, and ethnicity of the geographic region sampled. A school-based study of obese children in Minnesota, California, Texas, and Louisiana, using abnormal serum ALT as a surrogate marker ($>40\text{U/L}$), found that 23% of 17-18 year olds had elevated unexplained ALT (264). An autopsy study using the “gold standard” of liver histology examined 742 children aged 2 to 19 years who died from unnatural causes. The estimated NAFLD prevalence was 9.6% when adjusted for age, gender, race, and ethnicity (124). A recent AASLD approved version submitted to HEPATOLOGY on June 28, 2017

meta-analysis demonstrated the pooled mean prevalence of NAFLD to be 7.6% in children from general population studies and 34.2% in studies based on pediatric obesity clinics (265). This meta-analysis highlights the higher prevalence of NAFLD in boys relative to girls, with prevalence increasing incrementally with BMI z-score.

In a study of children with obesity with NAFLD and obstructive sleep apnea with chronic intermittent hypoxemia, the severity of hypoxemia was found to be associated with histological measures of NAFLD severity, particularly related to fibrosis stage (266). Histologic abnormalities in children with NAFLD and normal or mildly elevated ALT levels may show significant histological abnormalities, including advanced fibrosis in children with mildly elevated ALT, so use of ALT alone may underestimate the extent of liver injury (267). In a screening program of children with overweight and obesity referred from primary care, evaluation of 347 children suspected of NAFLD on the basis of elevated ALT underwent evaluation. NAFLD was diagnosed in 55% of these children, with liver diseases other than NAFLD found in 18%; autoimmune hepatitis was the most common alternative diagnosis. Advanced fibrosis (bridging fibrosis and cirrhosis) was present in 11% of the referred children with NAFLD. Screening ALT with 2 times the upper limit of normal had a sensitivity of 57% and a specificity of 71% (268).

Penetrance of NAFLD has been demonstrated in family members of children with NAFLD (81). The likelihood of first, second, and third degree relatives who exhibited abnormally high fat fractions (by MRI estimation) relative to body mass index was much more highly correlated in

those related to a child with NAFLD than to those who were related to an age, gender, and BMI-matched child without NAFLD.

Natural History of NAFLD in Children

A retrospective single center report described the natural history of NAFLD in 66 children (269).

Only 5 had serial biopsies, obtained for unspecified reasons over varying intervals, averaging 41 months between biopsies. Of these 5 children, 4 had progression of fibrosis. Four of the 5 underwent liver transplantation and 2 died of cirrhosis. The NASH Clinical Research Network reported the shorter-duration follow-up data on patients with NAFLD who received placebo along with standard-of-care lifestyle advice as part of the TONIC clinical trial. Forty-seven participants 8 to 17 years old at enrollment underwent 2 liver biopsies over 96 weeks.

Remarkably, 5 developed type 2 diabetes during the study, which was related to baseline BMI z-score, HbA1c value, and ballooning score. Fibrosis stage remained the same or progressed in 60% of subjects, and those in whom fibrosis stage did not improve were more likely to be white, older, and with higher baseline NAS (270). More robust prospective data are needed on larger numbers of children to better detail the natural history of NAFLD in children.

Screening for NAFLD in Children

NAFLD is under-diagnosed in children due to lack of recognition, screening, or appreciation of associated complications by health care providers. One study showed that less than a third of children with obesity were screened for NAFLD with laboratory testing at clinic visits (271).

Children may not be recognized as having obesity at visits, and age-appropriate norms for BMI may go unacknowledged. Abdominal adiposity may mask detection of hepatomegaly by

palpation during physician examination. As in adults, children with features of metabolic syndrome such as obesity, hypertension, insulin resistance, and dyslipidemia (81) are at higher risk for NAFLD, and particular histopathological features of NAFLD correlate with components of metabolic syndrome. Thus, identification of children at risk for NAFLD could occur in general health provider settings as well as in specialty clinics for nutrition, gastroenterology, hepatology, endocrinology, dyslipidemia, pulmonology, and bariatric surgery. Children may also exhibit NAFLD incidentally discovered while undergoing imaging, but there are no studies evaluating how to proceed with children identified in this fashion. Recently, the summary report of an expert committee suggested biannual screening for liver disease with serum ALT and AST starting at age 10 years in children with obesity and those with BMI in the 85th to 94th percentile with other risk factors (272).

Diagnosis in Children

Given the relatively early onset, caregivers must give additional consideration to the possibility of monogenic disorders that present as fatty liver disease in very young children. Considerations include inborn errors of fatty acid or carnitine metabolism, peroxisomal disorders, lysosomal storage disorders, celiac disease, Wilson disease, and cystic fibrosis (273). However, as in adults, positive serum autoantibodies are present in a significant population of children with biopsy-proven NAFLD, and on some occasions liver biopsy is required to discriminate between autoimmune hepatitis and NAFLD (81). Obviously, the confounding factor of alcohol use or abuse is much less common in children and standard questionnaires for quantifying alcohol intake are usually unnecessary. At the current time, no predictive panel of proteomic, lipidomic,

genomic, metabolomic, or clinical markers can reliably discriminate between NAFLD and NASH in children.

Guidance Statements

44. *Children with fatty liver who are very young or not overweight should be tested for monogenic causes of chronic liver disease such as fatty acid oxidation defects, lysosomal storage diseases, and peroxisomal disorders, in addition to those causes considered for adults (Table 1).*
45. *Low serum titers of autoantibodies are often present in children with NAFLD, but higher titers, particularly in association with higher serum aminotransferases, high globulins, or high total protein to albumin ratio should prompt a liver biopsy to exclude autoimmune hepatitis and related autoimmune disorders.*
46. *Due to a paucity of evidence, a formal recommendation cannot be made with regards to screening for NAFLD in children with overweight and obesity.*

When to Obtain a Liver Biopsy for Suspected Pediatric NAFLD?

The decision to perform a liver biopsy in a child to confirm the diagnosis of NAFLD must be weighed against the risks associated with biopsy and the likelihood that the result will impact management. In children with an uncertain diagnosis, biopsy may rule out potential drug hepatotoxicity, presence of more than one diagnosis, or lack of clarity due to presence of serum autoantibodies. When there is an interest in grading or staging NAFLD, instead of submitting all children with NAFLD to a liver biopsy, it would be optimal to identify those children who are more likely to have NASH, or to identify children with advanced fibrosis. Current radiologic

imaging technologies serving as surrogates for liver fibrosis on biopsy include, as in adults, assessments of transient elastography, MRE, and acoustic radiation force impulse imaging. At present, none of these techniques have been sufficiently validated to serve as sufficient replacements for tissue sampling (274). The continued paucity of natural history data confounds the decision to biopsy, as alteration of long-term outcomes with treatment based on severity of histology at baseline remains unknown.

As in adults, development of noninvasive biomarkers or imaging to identify those at risk for more rapid progression or severe disease onset is desirable. Particularly, accurate markers of cellular injury and fibrosis are needed. Two studies suggested that Enhanced Liver Fibrosis score can be used to accurately predict fibrosis in children with NAFLD, but these studies assayed a relatively small number of children, and fewer with advanced fibrosis (275,276).

There is reported benefit in predicting fibrosis stage in pediatric patients with an AUROC of 0.92, though only 9 of the 76 subjects studied had bridging fibrosis or more (273). In one study consisting of 134 children with NAFLD, serum keratin 18 levels measured within 2 days of a liver biopsy showed a very strong correlation with steatosis, inflammation, hepatocellular ballooning, fibrosis, steatohepatitis, and the NAFLD activity score (277).

Guidance Statements

47. Liver biopsy in children with suspected NAFLD should be performed in those in whom the diagnosis is unclear or in whom there is possibility of multiple diagnoses, or prior to initiating potentially hepatotoxic medical therapy.

48. A liver biopsy to establish a diagnosis of NASH should be obtained prior to starting children

on pharmacologic therapy for NASH.

NAFLD Histology in Children

Histopathology of children with NAFLD can differ from that found in adults. In some instances, as in adults, children's biopsies may show pronounced features of hepatocellular injury, lobular inflammation, and peri-sinusoidal fibrosis, but there is a unique pattern also recognized in children. This pattern is typified by either diffuse, marked, macrovesicular, hepatocellular steatosis or zone 1, periportal steatosis, portal inflammation, and portal fibrosis in the absence of ballooning (264,278,279). The etiopathogenesis, prognosis and response to treatment may be different in children with these findings.

Guidance Statement

49. Pathologists interpreting pediatric liver biopsies should recognize the unique pattern frequently found in children with NAFLD to appropriately characterize pediatric NAFLD.

Treatment in Children

Recommendations for pediatric treatment options are limited by a small number of randomized clinical trials and insufficient information on natural history to assess risk-benefit. The overall goal is to improve a child's quality of life and reduce longer-term cardiovascular and liver morbidity and mortality. Given that early onset likely indicates higher likelihood of later complications, attempts should be made to identify children who will benefit from intervention.

Lifestyle modification

Because most pediatric NAFLD patients have obesity, addressing this is the first step. An open

label study (280) in 84 Italian children with biopsy-proven NAFLD showed that >20% body weight reduction over 12 months resulted in improvement in serum ALT and steatosis by ultrasonography in most children with NAFLD. Reportedly, 94% of the 70 enrolled subjects were able to achieve this weight loss goal using caloric restriction and exercise advice. Because liver biopsies were not performed at the end of the study, the effect of lifestyle intervention on liver histology could not be determined. In another study, Nobili et al (281) randomized 53 children with biopsy-proven NAFLD to lifestyle modification plus antioxidant therapy or lifestyle modification and placebo. Antioxidant therapy did not improve liver histology, but children in both groups who had already reduced their weight through designated lifestyle changes showed significant improvement in steatosis, inflammation, ballooning, and the NAS.

In one study consisting of 51 children with severe obesity (BMI z-score >3.5) and NAFLD, intensive life style modification (either in an inpatient or ambulatory setting) offered sustained biochemical benefits in comparison to usual care (282).

No information exists on recommending any particular type of diet or exercise. Similarly, the degree of weight loss needed to improve various histological aspects of NASH in children is unknown. Further studies are needed to assess the efficacy of specific diets. Recommendations for overweight pediatric NAFLD patients should include consultation with a registered dietitian to assess quality of diet and measurement of caloric intake, adoption of American Heart Association dietary strategies, and regular aerobic exercise, progressing in difficulty as fitness allows (283).

Pharmacotherapy

As in adults, clinical trials for pediatric NAFLD generally targeted insulin resistance or oxidative stress. Open-label, proof-of-concept studies have utilized changes in serum ALT or liver brightness on ultrasound as endpoints (273). Agents evaluated thus far include metformin, vitamin E, ursodeoxycholic acid, and delayed-release cysteamine. A large multicenter RCT using change in histology as a secondary endpoint, called TONIC, compared the efficacy of vitamin E or metformin to placebo in 8 to 17 year olds with NAFLD (183). Although the primary outcome of sustained reduction of ALT was not different among the 3 groups, there were statistically significant improvements in NAS and resolution of NASH ($P < 0.006$) with vitamin E treatment compared to placebo over 96 weeks (183). In this study, metformin administered at a 500 mg, twice daily dose had no effect on liver biochemistries or liver histology. The results from another large multicenter RCT comparing the effect of delayed-release cysteamine to placebo were just reported (284). In this trial, the primary outcome, requiring reduction in NAS of 2 or more without worsening of fibrosis, was not achieved over the 52-week treatment interval. Interestingly, a secondary outcome comparing reduction in serum ALT on treatment to placebo did achieve significance. There has been some interest to evaluate omega-3 fatty acids to treat NAFLD in children. While a combination of eicosapentaenoic acid and docosahexaenoic acid failed to show significant therapeutic benefit in one study (285), docosahexaenoic acid administered at 250 mg/day for 6 months showed significant improvement in hepatic fat as well as cardiometabolic risk factors in another study (286).

Guidance Statements

50. *Intensive lifestyle modification improves aminotransferases and liver histology in*
51. *Children with NAFLD and thus should be the first line of treatment.*
52. *Metformin at 500 mg twice daily offers no benefit to children with NAFLD and thus should*

not be prescribed to specifically treat NAFLD or NASH. The effect of metformin administered at a higher dose is not known.

53. Vitamin E (RRR α -tocopherol) 800 IU/day offers histological benefits to some children with biopsy-proven NASH. Long-term safety of high-dose vitamin E in children is unknown.

Vitamin E may be used to treat NASH in children, but risks and benefits should be discussed with each patient.

Table 1: Common Causes of Secondary Hepatic Steatosis

Macrovesicular steatosis <ul style="list-style-type: none">- Excessive alcohol consumption- Hepatitis C (genotype 3)- Wilson disease- Lipodystrophy- Starvation- Parenteral nutrition- Abetalipoproteinemia- Medications (eg, mipomersen, lomitapide, amiodarone, methotrexate, tamoxifen, corticosteroids)
Microvesicular steatosis <ul style="list-style-type: none">- Reye syndrome- Medications (valproate, anti-retroviral medicines)- Acute fatty liver of pregnancy- HELLP syndrome- Inborn errors of metabolism (eg, lecithin-cholesterol acyltransferase deficiency, cholesterol ester storage disease, Wolman disease)

Table 2: Nonalcoholic Fatty Liver Disease and Related Definitions

Nonalcoholic Fatty Liver Disease (NAFLD)	Encompasses the entire spectrum of fatty liver disease in individuals without significant alcohol consumption, ranging from fatty liver to steatohepatitis to cirrhosis.
Nonalcoholic Fatty Liver (NAFL)	Presence of $\geq 5\%$ hepatic steatosis without evidence of hepatocellular injury in the form of ballooning of the hepatocytes or evidence of fibrosis. The risk of progression to cirrhosis and liver failure is considered minimal.
Nonalcoholic Steatohepatitis (NASH)	Presence of $\geq 5\%$ hepatic steatosis with inflammation and hepatocyte injury (ballooning) with or without fibrosis. This can progress to cirrhosis, liver failure, and rarely liver cancer.
NASH Cirrhosis	Presence of cirrhosis with current or previous histological evidence of steatosis or steatohepatitis
Cryptogenic Cirrhosis	Presence of cirrhosis with no obvious etiology. Patients with cryptogenic cirrhosis are heavily enriched with metabolic risk factors such as obesity and metabolic syndrome.
NAFLD Activity Score (NAS)	An unweighted composite of steatosis, lobular inflammation, and ballooning scores. NAS is a useful tool to measure changes in liver histology in patients with NAFLD in clinical trials. Fibrosis is scored separately (126).
Steatosis Activity Fibrosis (SAF) Score	A semiquantitative score consisting of steatosis amount, activity (lobular inflammation plus ballooning), and fibrosis (130).

Table 3: Risk Factors Associated with NAFLD

Common conditions with established association	Other conditions associated with NAFLD
Obesity	Hypothyroidism
Type 2 diabetes mellitus	Obstructive sleep apnea
Dyslipidemia	Hypopituitarism
Metabolic syndrome**	Hypogonadism
Polycystic ovary syndrome	Pancreato-duodenal resection
	Psoriasis

** The Adult Treatment Panel III clinical definition of the metabolic syndrome requires the presence of 3 or more of the following features: (1) waist circumference greater than 102 cm in men or greater than 88 cm in women, (2) triglyceride level 150 mg/dL or greater, (3) HDL cholesterol level less than 40 mg/dL in men and less than 50 mg/dL in women, (4) systolic blood pressure 130 mm Hg or greater or diastolic pressure 85 mm Hg or greater, and (5) fasting plasma glucose level 110 mg/dL or greater (287).

Supplemental Table 1: Summary of Guidance Statements

Alcohol Consumption and Definition of NAFLD

1. *Ongoing or recent alcohol consumption > 21 standard drinks on average per week in men and > 14 standard drinks on average per week in women is a reasonable threshold for significant alcohol consumption when evaluating patients with suspected NAFLD.*

Incidentally Discovered Hepatic Steatosis

2. *Patients with unsuspected hepatic steatosis detected on imaging who have symptoms or signs attributable to liver disease or have abnormal liver chemistries should be evaluated as though they have suspected NAFLD and worked up accordingly.*
3. *Patients with incidental hepatic steatosis detected on imaging who lack any liver-related symptoms or signs and have normal liver biochemistries should be assessed for metabolic risk factors (eg, obesity, diabetes mellitus, dyslipidemia) and alternate causes for hepatic steatosis such as significant alcohol consumption or medications.*

Screening for NAFLD in Primary Care, Diabetes, and Obesity Clinics

4. *Routine Screening for NAFLD in high-risk groups attending primary care, diabetes, or obesity clinics is not advised at this time due to uncertainties surrounding diagnostic tests and treatment options, along with lack of knowledge related to long-term benefits and cost-effectiveness of screening.*
5. *There should be a high index of suspicion for NAFLD and NASH in patients with type 2 diabetes. Clinical decision aids such as NAFLD Fibrosis Score or FIB4 or vibration controlled transient elastography (VCTE) can be used to identify those at low or high risk for advanced fibrosis (bridging fibrosis or cirrhosis).*

Screening of Family Members

6. *Systematic screening of family members for NAFLD is not recommended currently.*

Initial Evaluation of the Patient with Suspected NAFLD

7. *When evaluating a patient with suspected NAFLD, it is essential to exclude competing etiologies for steatosis and coexisting common chronic liver disease.*
8. *In patients with suspected NAFLD, persistently high serum ferritin, and increased iron saturation, especially in the context of homozygote or heterozygote C282Y HFE mutation, a liver biopsy should be considered.*
9. *High serum titers of autoantibodies in association with other features suggestive of autoimmune liver disease (> 5 upper limit of normal aminotransferases, high globulins, or high total protein to albumin ratio) should prompt a work-up for autoimmune liver disease.*
10. *Initial evaluation of patients with suspected NAFLD should carefully consider the presence of commonly associated comorbidities such as obesity, dyslipidemia, insulin resistance or diabetes, hypothyroidism, polycystic ovary syndrome, and sleep apnea.*

Noninvasive Assessment of Steatohepatitis and Advanced Fibrosis in NAFLD

11. *In patients with NAFLD, the metabolic syndrome predicts the presence of steatohepatitis, and its presence can be used to target patients for a liver biopsy.*
12. *NAFLD Fibrosis Score or FIB-4 index are clinically useful tools for identifying NAFLD patients with higher likelihood of having bridging fibrosis (stage 3) or cirrhosis (stage 4).*
13. *VCTE or MRE are clinically useful tools for identifying advanced fibrosis in patients with NAFLD.*

When to Obtain a Liver Biopsy in Patients with NAFLD

- 14. Liver biopsy should be considered in patients with NAFLD who are at increased risk of having steatohepatitis and/or advanced fibrosis.*
- 15. The presence of metabolic syndrome, NAFLD Fibrosis Score or FIB-4, or liver stiffness measured by VCTE or MRE may be used for identifying patients who are at risk for steatohepatitis and/or advanced fibrosis.*
- 16. Liver biopsy should be considered in patients with suspected NAFLD in whom competing etiologies for hepatic steatosis and the presence and/or severity of coexisting chronic liver diseases cannot be excluded without a liver biopsy.*

Histopathology of Adult NAFLD

- 17. Clinically useful pathology reporting should include a distinction between NAFL (steatosis), NAFL with inflammation, and NASH (steatosis with lobular and portal inflammation and hepatocellular ballooning). A comment on severity (mild, moderate, severe) may be useful. Specific scoring systems such as NAS (128) and/or Steatosis Activity Fibrosis (SAF) (128,129) may be used as deemed appropriate.*
- 18. The presence or absence of fibrosis should be described. If present, a further statement related to location, amount, and parenchymal remodeling is warranted.*

Management Of Patients With NAFLD

- 19. Pharmacological treatments aimed primarily at improving liver disease should generally be limited to those with biopsy-proven NASH and fibrosis.*

Lifestyle Intervention

20. *Weight loss generally reduces hepatic steatosis, achieved either by hypocaloric diet alone or in conjunction with increased physical activity. A combination of a hypocaloric diet (daily reduction by 500-1000 kcal) and moderate intensity exercise is likely to provide the best likelihood of sustaining weight loss over time.*
21. *Weight loss of at least 3 to 5% of body weight appears necessary to improve steatosis, but a greater weight loss (7 to 10%) is needed to improve the majority of the histopathologic features of NASH, including fibrosis.*
22. *Exercise alone in adults with NAFLD may prevent or reduce hepatic steatosis, but its ability to improve other aspects of liver histology remains unknown.*

Insulin Sensitizers

23. *Metformin is not recommended for treating NASH in adult patients.*
24. *Pioglitazone improves liver histology in patients with and without T2DM with biopsy-proven NASH. Therefore, it may be used to treat these patients. Risks and benefits should be discussed with each patient prior to starting therapy.*
25. *Until further data supports its safety and efficacy, pioglitazone should not be used to treat NAFLD without biopsy-proven NASH.*
26. *It is premature to consider glucagon-like peptide-1 agonists to specifically treat liver disease in patients with NAFLD or NASH.*

Vitamin E

27. *Vitamin E (rrr α -tocopherol) administered at a daily dose of 800 IU/day improves liver histology in nondiabetic adults with biopsy-proven NASH and therefore may be considered for this patient population. Risks and benefits should be discussed with each*

patient prior to starting therapy.

28. Until further data supporting its effectiveness become available, vitamin E is not recommended to treat NASH in diabetic patients, NAFLD without liver biopsy, NASH cirrhosis, or cryptogenic cirrhosis.

Bariatric Surgery

29. Foregut bariatric surgery can be considered in otherwise eligible obese individuals with NAFLD or NASH.

30. It is premature to consider foregut bariatric surgery as an established option to specifically treat NASH.

31. The type, safety, and efficacy of foregut bariatric surgery in otherwise eligible obese individuals with established cirrhosis due to NAFLD are not established. In otherwise eligible patients with compensated NASH or cryptogenic cirrhosis, foregut bariatric surgery may be considered on a case by case basis by an experienced bariatric surgery program.

Ursodeoxycholic Acid, Omega-3 Fatty Acids, and Miscellaneous Agents

32. Ursodeoxycholic acid is not recommended for the treatment of NAFLD or NASH.

33. Omega-3 fatty acids should not be used as a specific treatment of NAFLD or NASH, but they may be considered to treat hypertriglyceridemia in patients with NAFLD.

Alcohol Use in Patients with NAFLD and NASH

34. Patients with NAFLD should not consume heavy amounts of alcohol.

35. There are insufficient data to make recommendations with regards to nonheavy consumption of alcohol by individuals with NAFLD.

Management of Cardiovascular Disease and Dyslipidemia

36. Patients with NAFLD are at high risk for cardiovascular morbidity and mortality. Thus,

aggressive modification of CVD risk factors should be considered in all patients with NAFLD.

- 37. Patients with NAFLD or NASH are not at higher risk for serious liver injury from statins. Thus, statins can be used to treat dyslipidemia in patients with NAFLD and NASH. While statins may be used in patients with NASH cirrhosis, they should be avoided in patients with decompensated cirrhosis.*

Agents in Registration Trials

- 38. Until further safety and efficacy data become available in patients with NASH, we recommend that obeticholic acid should not be used off-label to treat NASH.*

NASH, Obesity and Liver Transplantation

- 39. Patients with NASH cirrhosis have high prevalence of cardiovascular disease. Thus, careful attention should be paid to identifying cardiovascular disease, whether clinically apparent or occult, during the transplant evaluation process.*

Miscellaneous Guidance Statements Pertinent to Clinical Practice

- 40. Patients with NASH cirrhosis should be screened for gastroesophageal varices according to the AASLD[and American College of Gastroenterology practice guidelines (262).*
- 41. Patients with cirrhosis suspected due to NAFLD should be considered for HCC screening according to the AASLD practice guidelines (263).*
- 42. Current evidence does not support routine screening and surveillance for HCC in patients with noncirrhotic NASH.*
- 43. Current evidence does not support routinely repeating a liver biopsy in patients with NAFL or NASH, but this may be considered on a case by case basis.*

Aspects of NAFLD Specific to Children and Adolescents

44. *Children with fatty liver who are very young or not overweight should be tested for monogenic causes of chronic liver disease such as fatty acid oxidation defects, lysosomal storage diseases, and peroxisomal disorders, in addition to those causes considered for adults (Table 1).*

45. *Low serum titers of autoantibodies are often present in children with NAFLD, but higher titers, particularly in association with higher serum aminotransferases, high globulins, or high total protein to albumin ratio should prompt a liver biopsy to exclude autoimmune hepatitis and related autoimmune disorders.*

46. *Due to a paucity of evidence, a formal recommendation cannot be made with regards to screening for NAFLD in children with overweight and obesity.*

When to Obtain a Liver Biopsy for Suspected Pediatric NAFLD?

47. *Liver biopsy in children with suspected NAFLD should be performed in those in whom the diagnosis is unclear or in whom there is possibility of multiple diagnoses, or prior to initiating potentially hepatotoxic medical therapy.*

48. *A liver biopsy to establish a diagnosis of NASH should be obtained prior to starting children on pharmacologic therapy for NASH.*

NAFLD Histology in Children

49. *Pathologists interpreting pediatric liver biopsies should recognize the unique pattern frequently found in children with NAFLD to appropriately characterize pediatric NAFLD.*

Treatment in Children

50. *Intensive lifestyle modification improves aminotransferases and liver histology in*

51. *Children with NAFLD and thus should be the first line of treatment.*

52. *Metformin at 500 mg twice daily offers no benefit to children with NAFLD and thus should not be prescribed to specifically treat NAFLD or NASH. The effect of metformin administered at a higher dose is not known.*

53. *Vitamin E (RRR α -tocopherol) 800 IU/day offers histological benefits to some children with biopsy-proven NASH. Long-term safety of high-dose vitamin E in children is unknown. Vitamin E may be used to treat NASH in children, but risks and benefits should be discussed with each patient.*

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;55:2005–2023.
2. Eddy DM. *A Manual for Assessing Health Practices and Designing Practice Policies: The Explicit Approach*. Philadelphia: American College of Physicians; 1992.
3. Guyatt GH, Oxman AD, Vist GE, Kunz R, Falck-Ytter Y, Alonso-Coello P, et al. GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. *BMJ*. 2008;336:924–926.
4. Rinella ME, Sanyal AJ. Management of NAFLD: a stage-based approach. *Nat Rev Gastroenterol Hepatol*. 2016;13:196–205.
5. Hannah WN, Harrison SA. Noninvasive imaging methods to determine severity of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. *Hepatology*. 2016;
6. Gawrieh S, Chalasani N. Pharmacotherapy for Nonalcoholic Fatty Liver Disease. *Semin Liver Dis*. 2015;35:338–348.
7. Khan RS, Newsome PN. Non-alcoholic fatty liver disease and liver transplantation. *Metabolism*. 2016;65:1208–1223.
8. Bedossa P, Patel K. Biopsy and noninvasive methods to assess progression of nonalcoholic fatty liver disease. *Gastroenterology*. 2016;150:1811–1822.e4.
9. Ahmed A, Wong RJ, Harrison SA. Nonalcoholic fatty liver disease review: diagnosis, treatment, and outcomes. *Clin Gastroenterol Hepatol*. 2015;13:2062–2070.

AASLD approved version submitted to HEPATOLOGY on June 28, 2017

10. Sung K-C, Wild SH, Byrne CD. Development of new fatty liver, or resolution of existing fatty liver, over five years of follow-up, and risk of incident hypertension. *J Hepatol*. 2014;60:1040–1045.
11. Tsuneto A, Hida A, Sera N, Imaizumi M, Ichimaru S, Nakashima E, et al. Fatty liver incidence and predictive variables. *Hypertens Res*. 2010;33:638–643.
12. Wong VW-S, Wong GL-H, Yeung DK-W, Lau TK-T, Chan CK-M, Chim AM-L, et al. Incidence of non-alcoholic fatty liver disease in Hong Kong: a population study with paired proton-magnetic resonance spectroscopy. *J Hepatol*. 2015;62:182–189.
13. Chang Y, Jung H-S, Cho J, Zhang Y, Yun KE, Lazo M, et al. Metabolically healthy obesity and the development of nonalcoholic fatty liver disease. *Am J Gastroenterol*. 2016;111:1133–1140.
14. Whalley S, Puvanachandra P, Desai A, Kennedy H. Hepatology outpatient service provision in secondary care: a study of liver disease incidence and resource costs. *Clin Med*. 2007;7:119–124.
15. Zelber-Sagi S, Lotan R, Shlomai A, Webb M, Harrari G, Buch A, et al. Predictors for incidence and remission of NAFLD in the general population during a seven-year prospective follow-up. *J Hepatol*. 2012;56:1145–1151.
16. 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.
17. Golabi P, Sayiner M, Fazel Y, Koenig A, Henry L, Younossi ZM. Current complications and challenges in nonalcoholic steatohepatitis screening and diagnosis. *Expert Rev Gastroenterol Hepatol*. 2016;10:63–71.
18. Byrne CD, Targher G. NAFLD: a multisystem disease. *J Hepatol*. 2015;62:S47–64.
19. Argo CK, Caldwell SH. Epidemiology and natural history of non-alcoholic steatohepatitis. *Clin Liver Dis* 2009;13:511–531.
20. 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;9:524–530.e1; quiz e60.
21. Fujioka K. Current and emerging medications for overweight or obesity in people with comorbidities. *Diabetes Obes Metab*. 2015;17:1021–1032.
22. VanWagner LB, Rinella ME. Extrahepatic Manifestations of Nonalcoholic Fatty Liver Disease. *Curr Hepatol Rep*. 2016;15:75–85.

23. Sasaki A, Nitta H, Otsuka K, Umemura A, Baba S, Obuchi T, et al. Bariatric surgery and non-alcoholic Fatty liver disease: current and potential future treatments. *Front Endocrinol.* 2014;5:164.
24. Subichin M, Clanton J, Makuszewski M, Bohon A, Zografakis JG, Dan A. Liver disease in the morbidly obese: a review of 1000 consecutive patients undergoing weight loss surgery. *Surg Obes Relat Dis.* 2015;11:137–141.
25. Leite NC, Salles GF, Araujo ALE, Villela-Nogueira CA, Cardoso CRL. Prevalence and associated factors of non-alcoholic fatty liver disease in patients with type-2 diabetes mellitus. *Liver Int.* 2009;29:113–119.
26. Prashanth M, Ganesh HK, Vima MV, John M, Bandgar T, Joshi SR, et al. Prevalence of nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus. *J Assoc Physicians India.* 2009;57:205–210.
27. Fan N, Zhang L, Xia Z, Peng L, Wang Y, Peng Y. Sex-specific association between serum uric acid and nonalcoholic fatty liver disease in type 2 diabetic patients. *J Diabetes Res.* 2016;2016:3805372.
28. Fruci B, Giuliano S, Mazza A, Malaguarnera R, Belfiore A. Nonalcoholic Fatty liver: a possible new target for type 2 diabetes prevention and treatment. *Int J Mol Sci.* 2013;14:22933–22966.
29. Assy N, Kaita K, Mymin D, Levy C, Rosser B, Minuk G. Fatty infiltration of liver in hyperlipidemic patients. *Dig Dis Sci.* 2000;45:1929–1934.
30. Wu K-T, Kuo P-L, Su S-B, Chen Y-Y, Yeh M-L, Huang C-I, et al. Nonalcoholic fatty liver disease severity is associated with the ratios of total cholesterol and triglycerides to high-density lipoprotein cholesterol. *J Clin Lipidol.* 2016;10:420–425.e1.
31. Fischer GE, Bialek SP, Homan CE, Livingston SE, McMahon BJ. Chronic liver disease among Alaska-Native people, 2003-2004. *Am J Gastroenterol.* 2009;104:363–370.
32. Bialek SR, Redd JT, Lynch A, Vogt T, Lewis S, Wilson C, et al. Chronic liver disease among two American Indian patient populations in the southwestern United States, 2000-2003. *J Clin Gastroenterol.* 2008;42:949–954.
33. Fattahi MR, Niknam R, Safarpour A, Sepehrimanesh M, Lotfi M. The prevalence of metabolic syndrome in non-alcoholic fatty liver disease; a population-based study. *Middle East J. Dig. Dis.* 2016;8:131–137.
34. Park KS, Lee YS, Park HW, Seo SH, Jang BG, Hwang JY, et al. Factors associated or related to with pathological severity of nonalcoholic fatty liver disease. *Korean J Intern Med.* 2004;19:19–26.

35. Koehler EM, Schouten JNL, Hansen BE, van Rooij FJA, Hofman A, Stricker BH, et al. Prevalence and risk factors of non-alcoholic fatty liver disease in the elderly: results from the Rotterdam study. *J Hepatol*. 2012;57:1305–1311.
36. Kagansky N, Levy S, Keter D, Rimon E, Taiba Z, Fridman Z, et al. Non-alcoholic fatty liver disease—a common and benign finding in octogenarian patients. *Liver Int*. 2004;24:588–594.
37. Frith J, Day CP, Henderson E, Burt AD, Newton JL. Non-alcoholic fatty liver disease in older people. *Gerontology*. 2009;55:607–613.
38. Zelber-Sagi S, Nitzan-Kaluski D, Halpern Z, Oren R. Prevalence of primary non-alcoholic fatty liver disease in a population-based study and its association with biochemical and anthropometric measures. *Liver Int*. 2006;26:856–863.
39. Masuoka HC, Chalasani N. Nonalcoholic fatty liver disease: an emerging threat to obese and diabetic individuals. *Ann N Y Acad Sci*. 2013;1281:106–122.
40. Dongiovanni P, Anstee QM, Valenti L. Genetic predisposition in NAFLD and NASH: impact on severity of liver disease and response to treatment. *Curr Pharm Des*. 2013;19:5219–5238.
41. Caldwell S, Argo C. The natural history of non-alcoholic fatty liver disease. *Dig Dis*. 2010;28:162–168.
42. Angulo P, Keach JC, Batts KP, Lindor KD. Independent predictors of liver fibrosis in patients with nonalcoholic steatohepatitis. *Hepatology*. 1999;30:1356–1362.
43. Nouredin M, Yates KP, Vaughn IA, Neuschwander-Tetri BA, Sanyal AJ, McCullough A, et al. Clinical and histological determinants of nonalcoholic steatohepatitis and advanced fibrosis in elderly patients. *Hepatology*. 2013;58:1644–1654.
44. 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.
45. Charlton MR, Burns JM, Pedersen RA, Watt KD, Heimbach JK, Dierkhising RA. Frequency and outcomes of liver transplantation for nonalcoholic steatohepatitis in the United States. *Gastroenterology*. 2011;141:1249–1253.
46. Bedogni G, Miglioli L, Masutti F, Castiglione A, Crocè LS, Tiribelli C, et al. Incidence and natural course of fatty liver in the general population: the Dionysos study. *Hepatology*. 2007;46:1387–1391.
47. Musso G, Gambino R, Cassader M, Pagano G. Meta-analysis: natural history of non-alcoholic fatty liver disease (NAFLD) and diagnostic accuracy of non-invasive tests for liver disease severity. *Ann. Med*. 2011;43:617–649.

48. Vernon G, Baranova A, Younossi ZM. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. *Aliment Pharmacol Ther.* 2011;34:274–285.
49. Zezos P, Renner EL. Liver transplantation and non-alcoholic fatty liver disease. *World J Gastroenterol.* 2014;20:15532–15538.
50. 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.
51. Dam-Larsen S, Franzmann M, Andersen IB, Christoffersen P, Jensen LB, Sørensen TIA, et al. Long term prognosis of fatty liver: risk of chronic liver disease and death. *Gut.* 2004;53:750–755.
52. Rafiq N, Bai C, Fang Y, Srishord M, McCullough A, Gramlich T, et al. Long-term follow-up of patients with nonalcoholic fatty liver. *Clin Gastroenterol Hepatol.* 2009;7:234–238.
53. Sayiner M, Otgonsuren M, Cable R, Younossi I, Afendy M, Golabi P, et al. variables associated with inpatient and outpatient resource utilization among Medicare beneficiaries with nonalcoholic fatty liver disease with or without cirrhosis. *J Clin Gastroenterol.* 2017;51:254–260.
54. Adams LA, Lymp JF, St Sauver J, Sanderson SO, Lindor KD, Feldstein A, et al. The natural history of nonalcoholic fatty liver disease: a population-based cohort study. *Gastroenterology.* 2005;129:113–121.
55. Younossi Z, Henry L. Contribution of alcoholic and nonalcoholic fatty liver disease to the burden of liver-related morbidity and mortality. *Gastroenterology.* 2016;150:1778–1785.
56. Sayiner M, Koenig A, Henry L, Younossi ZM. Epidemiology of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis in the United States and the rest of the world. *Clin Liver Dis.* 2016;20:205–214.
57. GBD 2015 Mortality and Causes of Death Collaborators. Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980-2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet.* 2016;388:1459–1544.
58. Younossi ZM, Stepanova M, Rafiq N, Makhlof H, Younoszai Z, Agrawal R, et al. Pathologic criteria for nonalcoholic steatohepatitis: interprotocol agreement and ability to predict liver-related mortality. *Hepatology.* 2011;53:1874–1882.
59. Hossain N, Stepanova M, Afendy A, Nader F, Younossi Y, Rafiq N, et al. Non-alcoholic steatohepatitis (NASH) in patients with polycystic ovarian syndrome (PCOS). *Scand J Gastroenterol.* 2011;46:479–484.

60. Mohamad B, Shah V, Onyshchenko M, Elshamy M, Aucejo F, Lopez R, et al. Characterization of hepatocellular carcinoma (HCC) in non-alcoholic fatty liver disease (NAFLD) patients without cirrhosis. *Hepatol Int*. 2016;10:632–639.
61. Younossi ZM, Otgonsuren M, Henry L, Venkatesan C, Mishra A, Erario M, et al. Association of nonalcoholic fatty liver disease (NAFLD) with hepatocellular carcinoma (HCC) in the United States from 2004 to 2009. *Hepatology*. 2015;62:1723–1730.
62. Mittal S, El-Serag HB, Sada YH, Kanwal F, Duan Z, Temple S, et al. hepatocellular carcinoma in the absence of cirrhosis in United States veterans is associated with nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol*. 2016;14:124–131.e1.
63. Caldwell SH, Lee VD, Kleiner DE, Al-Osaimi AMS, Argo CK, Northup PG, et al. NASH and cryptogenic cirrhosis: a histological analysis. *Ann Hepatol*. 2009;8:346–352.
64. Brown GT, Kleiner DE. Histopathology of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. *Metabolism*. 2016;65:1080–1086.
65. Haga Y, Kanda T, Sasaki R, Nakamura M, Nakamoto S, Yokosuka O. Nonalcoholic fatty liver disease and hepatic cirrhosis: Comparison with viral hepatitis-associated steatosis. *World J Gastroenterol*. 2015;21:12989–12995.
66. Young K, Aguilar M, Gish R, Younossi Z, Saab S, Bhuket T, et al. lower rates of receiving model for end-stage liver disease exception and longer time to transplant among non-alcoholic steatohepatitis hepatocellular carcinoma. *Liver Transpl*. 2016;22:1356–1366.
67. Wong RJ, Aguilar M, Cheung R, Perumpail RB, Harrison SA, Younossi ZM, et al. Nonalcoholic steatohepatitis is the second leading etiology of liver disease among adults awaiting liver transplantation in the United States. *Gastroenterology*. 2015;148:547–555.
68. Sanyal AJ, Brunt EM, Kleiner DE, Kowdley KV, Chalasani N, Lavine JE, et al. Endpoints and clinical trial design for nonalcoholic steatohepatitis. *Hepatology*. 2011;54:344–353.
69. National Institute on Alcohol Abuse and Alcoholism. What is a standard drink? [Internet]. [cited 2016 Dec 12]; Available from: https://pubs.niaaa.nih.gov/publications/practitioner/pocketguide/pocket_guide2.htm
70. Liangpunsakul S, Chalasani N. What should we recommend to our patients with NAFLD regarding alcohol use? *Am J Gastroenterol*. 2012;107:976–978.
71. Wright AP, Desai AP, Bajpai S, King LY, Sahani DV, Corey KE. Gaps in recognition and evaluation of incidentally identified hepatic steatosis. *Dig Dis Sci*. 2015;60:333–338.
72. Portillo-Sanchez P, Bril F, Maximos M, Lomonaco R, Biernacki D, Orsak B, et al. High prevalence of nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus and normal plasma aminotransferase levels. *J Clin Endocrinol Metab*. 2015;100:2231–2238.

73. Koehler EM, Plompen EPC, Schouten JNL, Hansen BE, Darwish Murad S, Taimr P, et al. Presence of diabetes mellitus and steatosis is associated with liver stiffness in a general population: The Rotterdam study. *Hepatology*. 2016;63:138–147.
74. Kwok R, Choi KC, Wong GL-H, Zhang Y, Chan HL-Y, Luk AO-Y, et al. Screening diabetic patients for non-alcoholic fatty liver disease with controlled attenuation parameter and liver stiffness measurements: a prospective cohort study. *Gut*. 2016;65:1359–1368.
75. Corey KE, Klebanoff MJ, Tramontano AC, Chung RT, Hur C. Screening for nonalcoholic steatohepatitis in individuals with type 2 diabetes: a cost-effectiveness analysis. *Dig Dis Sci*. 2016;61:2108–2117.
76. Wong VW-S, Chalasani N. Not routine screening, but vigilance for chronic liver disease in patients with type 2 diabetes. *J Hepatol*. 2016;64:1211–1213.
77. Wagenknecht LE, Scherzinger AL, Stamm ER, Hanley AJG, Norris JM, Chen Y-DI, et al. Correlates and heritability of nonalcoholic fatty liver disease in a minority cohort. *Obesity (Silver Spring)*. 2009;17:1240–1246.
78. Abdelmalek MF, Liu C, Shuster J, Nelson DR, Asal NR. Familial aggregation of insulin resistance in first-degree relatives of patients with nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol*. 2006;4:1162–1169.
79. Struben VM, Hespeneide EE, Caldwell SH. Nonalcoholic steatohepatitis and cryptogenic cirrhosis within kindreds. *Am J Med*. 2000;108:9–13.
80. Willner IR, Waters B, Patil SR, Reuben A, Morelli J, Riely CA. Ninety patients with nonalcoholic steatohepatitis: insulin resistance, familial tendency, and severity of disease. *Am J Gastroenterol*. 2001;96:2957–2961.
81. Schwimmer JB, Celedon MA, Lavine JE, Salem R, Campbell N, Schork NJ, et al. Heritability of nonalcoholic fatty liver disease. *Gastroenterology*. 2009;136:1585–1592.
82. Tarnoki AD, Tarnoki DL, Bata P, Littvay L, Osztoivits J, Jermendy G, et al. Heritability of non-alcoholic fatty liver disease and association with abnormal vascular parameters: a twin study. *Liver Int*. 2012;32:1287–1293.
83. Makkonen J, Pietiläinen KH, Rissanen A, Kaprio J, Yki-Järvinen H. Genetic factors contribute to variation in serum alanine aminotransferase activity independent of obesity and alcohol: a study in monozygotic and dizygotic twins. *J Hepatol*. 2009;50:1035–1042.
84. Loomba R, Schork N, Chen C-H, Bettencourt R, Bhatt A, Ang B, et al. Heritability of hepatic fibrosis and steatosis based on a prospective twin study. *Gastroenterology*. 2015;149:1784–1793.
85. Kowdley KV, Belt P, Wilson LA, Yeh MM, Neuschwander-Tetri BA, Chalasani N, et al. Serum ferritin is an independent predictor of histologic severity and advanced fibrosis in patients with nonalcoholic fatty liver disease. *Hepatology*. 2012;55:77–85.

86. Valenti L, Fracanzani AL, Bugianesi E, Dongiovanni P, Galmozzi E, Vanni E, et al. HFE genotype, parenchymal iron accumulation, and liver fibrosis in patients with nonalcoholic fatty liver disease. *Gastroenterology*. 2010;138:905–912.
87. Vuppalanchi R, Gould RJ, Wilson LA, Unalp-Arida A, Cummings OW, Chalasani N, et al. Clinical significance of serum autoantibodies in patients with NAFLD: results from the nonalcoholic steatohepatitis clinical research network. *Hepatology*. 2012;56:379–385.
88. Chalasani N, Wilson L, Kleiner DE, Cummings OW, Brunt EM, Unalp A, et al. Relationship of steatosis grade and zonal location to histological features of steatohepatitis in adult patients with non-alcoholic fatty liver disease. *J Hepatol*. 2008;48:829–834.
89. Targher G, Marchesini G, Byrne CD. Risk of type 2 diabetes in patients with non-alcoholic fatty liver disease: Causal association or epiphenomenon? *Diabetes Metab*. 2016;42:142–156.
90. Li X, Xia M, Ma H, Hu Y, Yan H, He W, et al. Liver fat content, evaluated through semi-quantitative ultrasound measurement, is associated with impaired glucose profiles: a community-based study in Chinese. *PloS One*. 2013;8:e65210.
91. Shah RV, Allison MA, Lima JAC, Bluemke DA, Abbasi SA, Ouyang P, et al. Liver fat, statin use, and incident diabetes: the multi-ethnic study of atherosclerosis. *Atherosclerosis*. 2015;242:211–217.
92. Reeder SB, Cruite I, Hamilton G, Sirlin CB. Quantitative assessment of liver fat with magnetic resonance imaging and spectroscopy. *J Magn Reson Imaging*. 2011;34:spcone.
93. Idilman IS, Keskin O, Celik A, Savas B, Halil Elhan A, Idilman R, et al. A comparison of liver fat content as determined by magnetic resonance imaging-proton density fat fraction and MRS versus liver histology in non-alcoholic fatty liver disease. *Acta Radiol*. 2016;57:271–278.
94. Heba ER, Desai A, Zand KA, Hamilton G, Wolfson T, Schlein AN, et al. Accuracy and the effect of possible subject-based confounders of magnitude-based MRI for estimating hepatic proton density fat fraction in adults, using MR spectroscopy as reference. *J Magn Reson Imaging*. 2016;43:398–406.
95. Nouredin M, Lam J, Peterson MR, Middleton M, Hamilton G, Le T-A, et al. Utility of magnetic resonance imaging versus histology for quantifying changes in liver fat in nonalcoholic fatty liver disease trials. *Hepatology*. 2013;58:1930–1940.
96. De Lédinghen V, Wong GL-H, Vergniol J, Chan HL-Y, Hiriart J-B, Chan AW-H, et al. Controlled attenuation parameter for the diagnosis of steatosis in non-alcoholic fatty liver disease. *J Gastroenterol Hepatol*. 2016;31:848–855.
97. Vuppalanchi R, Chalasani N. Nonalcoholic fatty liver disease and nonalcoholic steatohepatitis: Selected practical issues in their evaluation and management. *Hepatology*. 2009;49:306–317.

98. Marchesini G, Bugianesi E, Forlani G, Cerrelli F, Lenzi M, Manini R, et al. Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. *Hepatology*. 2003;37:917–923.
99. Kang H, Greenson JK, Omo JT, Chao C, Peterman D, Anderson L, et al. Metabolic syndrome is associated with greater histologic severity, higher carbohydrate, and lower fat diet in patients with NAFLD. *Am J Gastroenterol*. 2006;101:2247–2253.
100. Ryan MC, Wilson AM, Slavin J, Best JD, Jenkins AJ, Desmond PV. Associations between liver histology and severity of the metabolic syndrome in subjects with nonalcoholic fatty liver disease. *Diabetes Care*. 2005;28:1222–1224.
101. Younossi ZM, Otgonsuren M, Venkatesan C, Mishra A. In patients with non-alcoholic fatty liver disease, metabolically abnormal individuals are at a higher risk for mortality while metabolically normal individuals are not. *Metabolism*. 2013;62:352–360.
102. Cusi K, Chang Z, Harrison S, Lomonaco R, Bril F, Orsak B, et al. Limited value of plasma cytokeratin-18 as a biomarker for NASH and fibrosis in patients with non-alcoholic fatty liver disease. *J Hepatol*. 2014;60:167–174.
103. Chen J, Zhu Y, Zheng Q, Jiang J. Serum cytokeratin-18 in the diagnosis of non-alcoholic steatohepatitis: A meta-analysis. *Hepatol Res*. 2014;44:854–862.
104. Kaswala DH, Lai M, Afdhal NH. Fibrosis assessment in nonalcoholic fatty liver disease (NAFLD) in 2016. *Dig Dis Sci*. 2016;61:1356–1364.
105. Imajo K, Kessoku T, Honda Y, Tomeno W, Ogawa Y, Mawatari H, et al. Magnetic resonance imaging more accurately classifies steatosis and fibrosis in patients with nonalcoholic fatty liver disease than transient elastography. *Gastroenterology*. 2016;150:626–637.e7.
106. 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;45:846–854.
107. Tapper EB, Challies T, Nasser I, Afdhal NH, Lai M. The performance of vibration controlled transient elastography in a US cohort of patients with nonalcoholic fatty liver disease. *Am J Gastroenterol*. 2016;111:677–684.
108. Nozaki Y, Fujita K, Wada K, Yoneda M, Shinohara Y, Imajo K, et al. Deficiency of eNOS exacerbates early-stage NAFLD pathogenesis by changing the fat distribution. *BMC Gastroenterol*. 2015;15:177.
109. Vuppalanchi R, Siddiqui MS, Hallinan EK, Abdelmalek MF, Neuschwander-Tetri B, Loomba R, et al. Transient elastography is feasible with high success rate for evaluation of non-alcoholic fatty liver disease (NAFLD) in a multicenter setting. *Hepatology*. 2015;62:1290A.

110. Kim D, Kim WR, Talwalkar JA, Kim HJ, Ehman RL. Advanced fibrosis in nonalcoholic fatty liver disease: noninvasive assessment with MR elastography. *Radiology*. 2013;268:411–419.
111. Loomba R, Wolfson T, Ang B, Hooker J, Behling C, Peterson M, et al. Magnetic resonance elastography predicts advanced fibrosis in patients with nonalcoholic fatty liver disease: a prospective study. *Hepatology*. 2014;60:1920–1928.
112. Kawaguchi T, Sumida Y, Umemura A, Matsuo K, Takahashi M, Takamura T, et al. Genetic polymorphisms of the human PNPLA3 gene are strongly associated with severity of non-alcoholic fatty liver disease in Japanese. *PloS One*. 2012;7:e38322.
113. Sookoian S, Pirola CJ. Meta-analysis of the influence of I148M variant of patatin-like phospholipase domain containing 3 gene (PNPLA3) on the susceptibility and histological severity of nonalcoholic fatty liver disease. *Hepatology*. 2011;53:1883–1894.
114. Rotman Y, Koh C, Zmuda JM, Kleiner DE, Liang TJ, NASH CRN. The association of genetic variability in patatin-like phospholipase domain-containing protein 3 (PNPLA3) with histological severity of nonalcoholic fatty liver disease. *Hepatology*. 2010;52:894–903.
115. Speliotes EK, Butler JL, Palmer CD, Voight BF, GIANT Consortium, MIGen Consortium, et al. PNPLA3 variants specifically confer increased risk for histologic nonalcoholic fatty liver disease but not metabolic disease. *Hepatology*. 2010;52:904–912.
116. Liu Y-L, Reeves HL, Burt AD, Tiniakos D, McPherson S, Leathart JBS, et al. TM6SF2 rs58542926 influences hepatic fibrosis progression in patients with non-alcoholic fatty liver disease. *Nat Commun*. 2014;5:4309.
117. Kozlitina J, Smagris E, Stender S, Nordestgaard BG, Zhou HH, Tybjaerg-Hansen A, et al. Exome-wide association study identifies a TM6SF2 variant that confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet*. 2014;46:352–356.
118. Dongiovanni P, Petta S, Maglio C, Fracanzani AL, Pipitone R, Mozzi E, et al. Transmembrane 6 superfamily member 2 gene variant disentangles nonalcoholic steatohepatitis from cardiovascular disease. *Hepatology*. 2015;61:506–514.
119. Donati B, Motta BM, Pingitore P, Meroni M, Pietrelli A, Alisi A, et al. The rs2294918 E434K variant modulates patatin-like phospholipase domain-containing 3 expression and liver damage. *Hepatology*. 2016;63:787–798.
120. Mancina RM, Dongiovanni P, Petta S, Pingitore P, Meroni M, Rametta R, et al. The MBOAT7-TMC4 Variant rs641738 Increases Risk of Nonalcoholic Fatty Liver Disease in Individuals of European Descent. *Gastroenterology*. 2016;150:1219–1230.e6.
121. Franzen R, Rashidisangary B, Ozturan S, Vanweersch L, Gutknecht N. Intrapulpal temperature changes during root surface irradiation with dual-wavelength laser (2780 and 940 nm): in vitro study. *J Biomed Opt*. 2015;20:018002.

122. Altamirano J, Miquel R, Katoonizadeh A, Abraldes JG, Duarte-Rojo A, Louvet A, et al. A histologic scoring system for prognosis of patients with alcoholic hepatitis. *Gastroenterology*. 2014;146:1231–1239.e1–6.
123. Patton HM, Lavine JE, Van Natta ML, Schwimmer JB, Kleiner D, Molleston J, et al. Clinical correlates of histopathology in pediatric nonalcoholic steatohepatitis. *Gastroenterology*. 2008;135:1961–1971.e2.
124. Schwimmer JB, Behling C, Newbury R, Deutsch R, Nievergelt C, Schork NJ, et al. Histopathology of pediatric nonalcoholic fatty liver disease. *Hepatology*. 2005;42:641–649.
125. Suzuki A, Abdelmalek MF, Schwimmer JB, Lavine JE, Scheimann AO, Unalp-Arida A, et al. Association between puberty and features of nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol*. 2012;10:786–794.
126. Kleiner DE, Brunt EM. Nonalcoholic fatty liver disease: pathologic patterns and biopsy evaluation in clinical research. *Semin Liver Dis*. 2012;32:3–13.
127. Yeh MM, Brunt EM. Pathological features of fatty liver disease. *Gastroenterology*. 2014;147:754–764.
128. Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology*. 2005;41:1313–1321.
129. Bedossa P, Poitou C, Veyrie N, Bouillot J-L, Basdevant A, Paradis V, et al. Histopathological algorithm and scoring system for evaluation of liver lesions in morbidly obese patients. *Hepatology*. 2012;56:1751–1759.
130. Bedossa P, the FLIP Pathology Consortium. Utility and appropriateness of the fatty liver inhibition of progression (FLIP) algorithm and steatosis, activity, and fibrosis (SAF) score in the evaluation of biopsies of nonalcoholic fatty liver disease. *Hepatology*. 2014;60:565–575.
131. Ratziu V, Charlotte F, Heurtier A, Gombert S, Giral P, Bruckert E, et al. Sampling variability of liver biopsy in nonalcoholic fatty liver disease. *Gastroenterology*. 2005;128:1898–1906.
132. Larson SP, Bowers SP, Palekar NA, Ward JA, Pulcini JP, Harrison SA. Histopathologic variability between the right and left lobes of the liver in morbidly obese patients undergoing Roux-en-Y bypass. *Clin Gastroenterol Hepatol*. 2007;5:1329–1332.
133. Vuppalanchi R, Unalp A, Van Natta ML, Cummings OW, Sandrasegaran KE, Hameed T, et al. Effects of liver biopsy sample length and number of readings on sampling variability in nonalcoholic Fatty liver disease. *Clin Gastroenterol Hepatol*. 2009;7:481–486.

134. Rockey DC, Caldwell SH, Goodman ZD, Nelson RC, Smith AD, American Association for the Study of Liver Diseases. Liver biopsy. *Hepatology*. 2009;49:1017–1044.
135. 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.
136. 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.
137. Fontana L, Meyer TE, Klein S, Holloszy JO. Long-term calorie restriction is highly effective in reducing the risk for atherosclerosis in humans. *Proc Natl Acad Sci U S A*. 2004;101:6659–6663.
138. Kirk E, Reeds DN, Finck BN, Mayurranjan SM, Mayurranjan MS, Patterson BW, et al. Dietary fat and carbohydrates differentially alter insulin sensitivity during caloric restriction. *Gastroenterology*. 2009;136:1552–1560.
139. Haufe S, Engeli S, Kast P, Böhnke J, Utz W, Haas V, et al. Randomized comparison of reduced fat and reduced carbohydrate hypocaloric diets on intrahepatic fat in overweight and obese human subjects. *Hepatology*. 2011;53:1504–1514.
140. Kistler KD, Brunt EM, Clark JM, Diehl AM, Sallis JF, Schwimmer JB, et al. Physical activity recommendations, exercise intensity, and histological severity of nonalcoholic fatty liver disease. *Am J Gastroenterol*. 2011;106:460–468.
141. Church TS, Kuk JL, Ross R, Priest EL, Biloft E, Biloft E, et al. Association of cardiorespiratory fitness, body mass index, and waist circumference to nonalcoholic fatty liver disease. *Gastroenterology*. 2006;130:2023–2030.
142. St George A, Bauman A, Johnston A, Farrell G, Chey T, George J. Independent effects of physical activity in patients with nonalcoholic fatty liver disease. *Hepatology*. 2009;50:68–76.
143. Sung K-C, Ryu S, Lee J-Y, Kim J-Y, Wild SH, Byrne CD. Effect of exercise on the development of new fatty liver and the resolution of existing fatty liver. *J Hepatol*. 2016;65:791–797.
144. Wu T, Gao X, Chen M, van Dam RM. Long-term effectiveness of diet-plus-exercise interventions vs. diet-only interventions for weight loss: a meta-analysis. *Obes Rev*. 2009;10:313–323.
145. Kurklinsky AK, McEachen JC, Friese JL. Bilateral traumatic chylothorax treated by thoracic duct embolization: a rare treatment for an uncommon problem. *Vasc Med*. 2011;16:284–287.

146. Marchesini G, Brizi M, Bianchi G, Tomassetti S, Zoli M, Melchionda N. Metformin in non-alcoholic steatohepatitis. *Lancet*. 2001;358:893–894.
147. Uygun A, Kadayifci A, Isik AT, Ozgurtas T, Deveci S, Tuzun A, et al. Metformin in the treatment of patients with non-alcoholic steatohepatitis. *Aliment Pharmacol Ther*. 2004;19:537–544.
148. Nair S, Diehl AM, Wiseman M, Farr GH, Perrillo RP. Metformin in the treatment of non-alcoholic steatohepatitis: a pilot open label trial. *Aliment Pharmacol Ther*. 2004;20:23–28.
149. Bugianesi E, Gentilcore E, Manini R, Natale S, Vanni E, Villanova N, et al. A randomized controlled trial of metformin versus vitamin E or prescriptive diet in nonalcoholic fatty liver disease. *Am J Gastroenterol*. 2005;100:1082–1090.
150. Loomba R, Lutchman G, Kleiner DE, Ricks M, Feld JJ, Borg BB, et al. Clinical trial: pilot study of metformin for the treatment of non-alcoholic steatohepatitis. *Aliment Pharmacol Ther*. 2009;29:172–182.
151. Shields WW, Thompson KE, Grice GA, Harrison SA, Coyle WJ. The effect of metformin and standard therapy versus standard therapy alone in nondiabetic patients with insulin resistance and nonalcoholic steatohepatitis (NASH): a pilot trial. *Ther Adv Gastroenterol*. 2009;2:157–163.
152. Haukeland JW, Konopski Z, Eggesbø HB, von Volkmann HL, Raschpichler G, Bjørø K, et al. Metformin in patients with non-alcoholic fatty liver disease: a randomized, controlled trial. *Scand J Gastroenterol*. 2009;44:853–860.
153. Idilman R, Mizrak D, Corapcioglu D, Bektas M, Doganay B, Sayki M, et al. Clinical trial: insulin-sensitizing agents may reduce consequences of insulin resistance in individuals with non-alcoholic steatohepatitis. *Aliment Pharmacol Ther*. 2008;28:200–208.
154. Duseja A, Das A, Dhiman RK, Chawla YK, Thumburu KT, Bhadada S, et al. Metformin is effective in achieving biochemical response in patients with nonalcoholic fatty liver disease (NAFLD) not responding to lifestyle interventions. *Ann. Hepatol*. 2007;6:222–226.
155. Nar A, Gedik O. The effect of metformin on leptin in obese patients with type 2 diabetes mellitus and nonalcoholic fatty liver disease. *Acta Diabetol*. 2009;46:113–118.
156. Omer Z, Cetinkalp S, Akyildiz M, Yilmaz F, Batur Y, Yilmaz C, et al. Efficacy of insulin-sensitizing agents in nonalcoholic fatty liver disease. *Eur J Gastroenterol Hepatol*. 2010;22:18–23.
157. Li Y, Liu L, Wang B, Wang J, Chen D. Metformin in non-alcoholic fatty liver disease: a systematic review and meta-analysis. *Biomed Rep*. 2013;1:57–64.
158. Musso G, Gambino R, Cassader M, Pagano G. A meta-analysis of randomized trials for the treatment of nonalcoholic fatty liver disease. *Hepatology*. 2010;52:79–104.

159. Soccio RE, Chen ER, Lazar MA. Thiazolidinediones and the promise of insulin sensitization in type 2 diabetes. *Cell Metab.* 2014;20:573–591.
160. Cusi K. Role of obesity and lipotoxicity in the development of nonalcoholic steatohepatitis: pathophysiology and clinical implications. *Gastroenterology.* 2012;142:711–725.e6.
161. Ratziu V, Giral P, Jacqueminet S, Charlotte F, Hartemann-Heurtier A, Serfaty L, et al. Rosiglitazone for nonalcoholic steatohepatitis: one-year results of the randomized placebo-controlled Fatty Liver Improvement with Rosiglitazone Therapy (FLIRT) trial. *Gastroenterology.* 2008;135:100–110.
162. Ratziu V, Charlotte F, Bernhardt C, Giral P, Halbron M, Lenaour G, et al. Long-term efficacy of rosiglitazone in nonalcoholic steatohepatitis: results of the fatty liver improvement by rosiglitazone therapy (FLIRT 2) extension trial. *Hepatology.* 2010;51:445–453.
163. Hiatt WR, Kaul S, Smith RJ. The cardiovascular safety of diabetes drugs—insights from the rosiglitazone experience. *N Engl J Med.* 2013;369:1285–1287.
164. Belfort R, Harrison SA, Brown K, Darland C, Finch J, Hardies J, et al. A placebo-controlled trial of pioglitazone in subjects with nonalcoholic steatohepatitis. *N Engl J Med.* 2006;355:2297–2307.
165. Cusi K, Orsak B, Bril F, Lomonaco R, Hecht J, Ortiz-Lopez C, et al. Long-term pioglitazone treatment for patients with nonalcoholic steatohepatitis and prediabetes or type 2 diabetes mellitus: a randomized trial. *Ann Intern Med.* 2016;165:305–315.
166. Aithal GP, Thomas JA, Kaye PV, Lawson A, Ryder SD, Spendlove I, et al. Randomized, placebo-controlled trial of pioglitazone in nondiabetic subjects with nonalcoholic steatohepatitis. *Gastroenterology.* 2008;135:1176–1184.
167. 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.* 2010;362:1675–1685.
168. Williams CD, Stengel J, Asike MI, Torres DM, Shaw J, Contreras M, et al. Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: a prospective study. *Gastroenterology.* 2011;140:124–131.
169. Yau H, Rivera K, Lomonaco R, Cusi K. The future of thiazolidinedione therapy in the management of type 2 diabetes mellitus. *Curr Diab Rep.* 2013;13:329–341.
170. Levin D, Bell S, Sund R, Hartikainen SA, Tuomilehto J, Pukkala E, et al. Pioglitazone and bladder cancer risk: a multipopulation pooled, cumulative exposure analysis. *Diabetologia.* 2015;58:493–504.

171. Tuccori M, Filion KB, Yin H, Yu OH, Platt RW, Azoulay L. Pioglitazone use and risk of bladder cancer: population based cohort study. *BMJ*. 2016;352:i1541.
172. Lewis JD, Habel LA, Quesenberry CP, Strom BL, Peng T, Hedderson MM, et al. Pioglitazone use and risk of bladder cancer and other common cancers in persons with diabetes. *JAMA*. 2015;314:265–277.
173. Eguchi Y, Kitajima Y, Hyogo H, Takahashi H, Kojima M, Ono M, et al. Pilot study of liraglutide effects in non-alcoholic steatohepatitis and non-alcoholic fatty liver disease with glucose intolerance in Japanese patients (LEAN-J). *Hepatol Res*. 2015;45:269–278.
174. Armstrong MJ, Gaunt P, Aithal GP, Barton D, Hull D, Parker R, et al. Liraglutide safety and efficacy in patients with non-alcoholic steatohepatitis (LEAN): a multicentre, double-blind, randomised, placebo-controlled phase 2 study. *Lancet*. 2016;387:679–690.
175. Armstrong MJ, Houlihan DD, Rowe IA, Clausen WHO, Elbrønd B, Gough SCL, et al. Safety and efficacy of liraglutide in patients with type 2 diabetes and elevated liver enzymes: individual patient data meta-analysis of the LEAD program. *Aliment Pharmacol Ther*. 2013;37:234–242.
176. Kenny PR, Brady DE, Torres DM, Ragozzino L, Chalasani N, Harrison SA. Exenatide in the treatment of diabetic patients with non-alcoholic steatohepatitis: a case series. *Am J Gastroenterol*. 2010;105:2707–2709.
177. Buse JB, Klonoff DC, Nielsen LL, Guan X, Bowlus CL, Holcombe JH, et al. Metabolic effects of two years of exenatide treatment on diabetes, obesity, and hepatic biomarkers in patients with type 2 diabetes: an interim analysis of data from the open-label, uncontrolled extension of three double-blind, placebo-controlled trials. *Clin Ther*. 2007;29:139–153.
178. Hasegawa T, Yoneda M, Nakamura K, Makino I, Terano A. Plasma transforming growth factor-beta1 level and efficacy of alpha-tocopherol in patients with non-alcoholic steatohepatitis: a pilot study. *Aliment Pharmacol Ther*. 2001;15:1667–1672.
179. Harrison SA, Torgerson S, Hayashi P, Ward J, Schenker S. Vitamin E and vitamin C treatment improves fibrosis in patients with nonalcoholic steatohepatitis. *Am. J. Gastroenterol*. 2003;98:2485–2490.
180. Dufour J-F, Oneta CM, Gonvers J-J, Bihl F, Cerny A, Cereda J-M, et al. Randomized placebo-controlled trial of ursodeoxycholic acid with vitamin e in nonalcoholic steatohepatitis. *Clin Gastroenterol Hepatol*. 2006;4:1537–1543.
181. Sanyal AJ, Mofrad PS, Contos MJ, Sargeant C, Luketic VA, Sterling RK, et al. A pilot study of vitamin E versus vitamin E and pioglitazone for the treatment of nonalcoholic steatohepatitis. *Clin Gastroenterol Hepatol*. 2004;2:1107–1115.
182. Yakaryilmaz F, Guliter S, Savas B, Erdem O, Ersoy R, Erden E, et al. Effects of vitamin E treatment on peroxisome proliferator-activated receptor-alpha expression and insulin

- resistance in patients with non-alcoholic steatohepatitis: results of a pilot study. *Intern Med J*. 2007;37:229–235.
183. Lavine JE, Schwimmer JB, Van Natta ML, Molleston JP, Murray KF, Rosenthal P, et al. Effect of vitamin E or metformin for treatment of nonalcoholic fatty liver disease in children and adolescents: the TONIC randomized controlled trial. *JAMA*. 2011;305:1659–1668.
 184. Sato K, Gosho M, Yamamoto T, Kobayashi Y, Ishii N, Ohashi T, et al. Vitamin E has a beneficial effect on nonalcoholic fatty liver disease: a meta-analysis of randomized controlled trials. *Nutrition*. 2015;31:923–930.
 185. Xu R, Tao A, Zhang S, Deng Y, Chen G. Association between vitamin E and non-alcoholic steatohepatitis: a meta-analysis. *Int J Clin Exp Med*. 2015;8:3924–3934.
 186. Miller ER, Pastor-Barriuso R, Dalal D, Riemersma RA, Appel LJ, Guallar E. Meta-analysis: high-dosage vitamin E supplementation may increase all-cause mortality. *Ann Intern Med*. 2005;142:37–46.
 187. Gerss J, Köpcke W. The questionable association of vitamin E supplementation and mortality—inconsistent results of different meta-analytic approaches. *Cell Mol Biol*. 2009;55 Suppl:OL1111–1120.
 188. Abner EL, Schmitt FA, Mendiondo MS, Marcum JL, Kryscio RJ. Vitamin E and all-cause mortality: a meta-analysis. *Curr Aging Sci*. 2011;4:158–170.
 189. Klein EA, Thompson IM, Tangen CM, Crowley JJ, Lucia MS, Goodman PJ, et al. Vitamin E and the risk of prostate cancer: the Selenium and Vitamin E Cancer Prevention Trial (SELECT). *JAMA*. 2011;306:1549–1556.
 190. Kristal AR, Darke AK, Morris JS, Tangen CM, Goodman PJ, Thompson IM, et al. Baseline selenium status and effects of selenium and vitamin e supplementation on prostate cancer risk. *J Natl Cancer Inst*. 2014;106:djt456.
 191. Chan JM, Darke AK, Penney KL, Tangen CM, Goodman PJ, Lee G-SM, et al. Selenium- or vitamin E-related gene variants, interaction with supplementation, and risk of high-grade prostate cancer in SELECT. *Cancer Epidemiol Biomark Prev*. 2016;25:1050–1058.
 192. Sjöström L, Peltonen M, Jacobson P, Sjöström CD, Karason K, Wedel H, et al. Bariatric surgery and long-term cardiovascular events. *JAMA*. 2012;307:56–65.
 193. Pontiroli AE, Morabito A. Long-term prevention of mortality in morbid obesity through bariatric surgery. a systematic review and meta-analysis of trials performed with gastric banding and gastric bypass. *Ann Surg*. 2011;253:484–487.
 194. Adams TD, Gress RE, Smith SC, Halverson RC, Simper SC, Rosamond WD, et al. Long-term mortality after gastric bypass surgery. *N Engl J Med*. 2007;357:753–761.

195. Sjöström L, Narbro K, Sjöström CD, Karason K, Larsson B, Wedel H, et al. Effects of bariatric surgery on mortality in Swedish obese subjects. *N Engl J Med*. 2007;357:741–752.
196. Mathurin P, Hollebecque A, Arnalsteen L, Buob D, Leteurtre E, Caiazzo R, et al. Prospective study of the long-term effects of bariatric surgery on liver injury in patients without advanced disease. *Gastroenterology*. 2009;137:532–540.
197. Lassailly G, Caiazzo R, Buob D, Pigeyre M, Verkindt H, Labreuche J, et al. Bariatric surgery reduces features of nonalcoholic steatohepatitis in morbidly obese patients. *Gastroenterology*. 2015;149:379–388.
198. Bower G, Toma T, Harling L, Jiao LR, Efthimiou E, Darzi A, et al. Bariatric surgery and non-alcoholic fatty liver disease: a systematic review of liver biochemistry and histology. *Obes Surg*. 2015;25:2280–2289.
199. Mosko JD, Nguyen GC. Increased perioperative mortality following bariatric surgery among patients with cirrhosis. *Clin Gastroenterol Hepatol*. 2011;9:897–901.
200. Jan A, Narwaria M, Mahawar KK. A systematic review of bariatric surgery in patients with liver cirrhosis. *Obes Surg*. 2015;25:1518–1526.
201. Laurin J, Lindor KD, Crippin JS, Gossard A, Gores GJ, Ludwig J, et al. Ursodeoxycholic acid or clofibrate in the treatment of non-alcohol-induced steatohepatitis: a pilot study. *Hepatology*. 1996;23:1464–1467.
202. Leuschner UFH, Lindenthal B, Herrmann G, Arnold JC, Rössle M, Cordes H-J, et al. High-dose ursodeoxycholic acid therapy for nonalcoholic steatohepatitis: a double-blind, randomized, placebo-controlled trial. *Hepatology*. 2010;52:472–479.
203. Lindor KD, Kowdley KV, Heathcote EJ, Harrison ME, Jorgensen R, Angulo P, et al. Ursodeoxycholic acid for treatment of nonalcoholic steatohepatitis: results of a randomized trial. *Hepatology*. 2004;39:770–778.
204. Ratziu V, de Ledinghen V, Oberti F, Mathurin P, Wartelle-Bladou C, Renou C, et al. A randomized controlled trial of high-dose ursodesoxycholic acid for nonalcoholic steatohepatitis. *J Hepatol*. 2011;54:1011–1019.
205. Capanni M, Calella F, Biagini MR, Genise S, Raimondi L, Bedogni G, et al. Prolonged n-3 polyunsaturated fatty acid supplementation ameliorates hepatic steatosis in patients with non-alcoholic fatty liver disease: a pilot study. *Aliment Pharmacol Ther*. 2006;23:1143–1151.
206. Masterton GS, Plevris JN, Hayes PC. Review article: omega-3 fatty acids—a promising novel therapy for non-alcoholic fatty liver disease. *Aliment Pharmacol Ther*. 2010;31:679–692.

207. Scorletti E, Bhatia L, McCormick KG, Clough GF, Nash K, Hodson L, et al. Effects of purified eicosapentaenoic and docosahexaenoic acids in nonalcoholic fatty liver disease: results from the Welcome* study. *Hepatology*. 2014;60:1211–1221.
208. Sanyal AJ, Abdelmalek MF, Suzuki A, Cummings OW, Chojkier M, EPE-A Study Group. No significant effects of ethyl-eicosapentanoic acid on histologic features of nonalcoholic steatohepatitis in a phase 2 trial. *Gastroenterology*. 2014;147:377–384.e1.
209. What's at-risk or heavy drinking? - rethinking drinking - NIAAA [Internet]. [cited 2016 Sep 6]; Available from: <http://rethinkingdrinking.niaaa.nih.gov/How-much-is-too-much/Is-your-drinking-pattern-risky/whats-At-Risk-Or-Heavy-drinking.aspx>
210. Dunn W, Xu R, Schwimmer JB. Modest wine drinking and decreased prevalence of suspected nonalcoholic fatty liver disease. *Hepatology*. 2008;47:1947–1954.
211. Gunji T, Matsushashi N, Sato H, Fujibayashi K, Okumura M, Sasabe N, et al. Light and moderate alcohol consumption significantly reduces the prevalence of fatty liver in the Japanese male population. *Am J Gastroenterol*. 2009;104:2189–2195.
212. Suzuki A, Angulo P, St Sauver J, Muto A, Okada T, Lindor K. Light to moderate alcohol consumption is associated with lower frequency of hypertransaminasemia. *Am J Gastroenterol*. 2007;102:1912–1919.
213. Moriya A, Iwasaki Y, Ohguchi S, Kayashima E, Mitsumune T, Taniguchi H, et al. Alcohol consumption appears to protect against non-alcoholic fatty liver disease. *Aliment Pharmacol Ther*. 2011;33:378–388.
214. Dixon JB, Bhathal PS, O'Brien PE. Nonalcoholic fatty liver disease: predictors of nonalcoholic steatohepatitis and liver fibrosis in the severely obese. *Gastroenterology*. 2001;121:91–100.
215. Cotrim HP, Freitas LA, Alves E, Almeida A, May DS, Caldwell S. Effects of light-to-moderate alcohol consumption on steatosis and steatohepatitis in severely obese patients. *Eur J Gastroenterol Hepatol*. 2009;21:969–972.
216. Dunn W, Sanyal AJ, Brunt EM, Unalp-Arida A, Donohue M, McCullough AJ, et al. Modest alcohol consumption is associated with decreased prevalence of steatohepatitis in patients with non-alcoholic fatty liver disease (NAFLD). *J Hepatol*. 2012;57:384–391.
217. Sookoian S, Pirola CJ. How safe is moderate alcohol consumption in overweight and obese individuals? *Gastroenterology*. 2016;150:1698–1703.e2.
218. Ekstedt M, Franzén LE, Mathiesen UL, Thorelius L, Holmqvist M, Bodemar G, et al. Long-term follow-up of patients with NAFLD and elevated liver enzymes. *Hepatology*. 2006;44:865–873.
219. Pais R, Giral P, Khan J-F, Rosenbaum D, Housset C, Poynard T, et al. Fatty liver is an independent predictor of early carotid atherosclerosis. *J Hepatol*. 2016;65:95–102.

220. Francque SM, van der Graaff D, Kwanten WJ. Non-alcoholic fatty liver disease and cardiovascular risk: Pathophysiological mechanisms and implications. *J Hepatol*. 2016;65:425–443.
221. Corey KE, Misdraji J, Gelrud L, Zheng H, Chung RT, Krauss RM. Nonalcoholic steatohepatitis is associated with an atherogenic lipoprotein subfraction profile. *Lipids Health Dis*. 2014;13:100.
222. Siddiqui MS, Fuchs M, Idowu MO, Luketic VA, Boyett S, Sargeant C, et al. Severity of nonalcoholic fatty liver disease and progression to cirrhosis are associated with atherogenic lipoprotein profile. *Clin Gastroenterol Hepatol*. 2015;13:1000–1008.e3.
223. Bril F, Sninsky JJ, Baca AM, Superko HR, Portillo Sanchez P, Biernacki D, et al. Hepatic steatosis and insulin resistance, but not steatohepatitis, promote atherogenic dyslipidemia in NAFLD. *J Clin Endocrinol Metab*. 2016;101:644–652.
224. Athyros VG, Tziomalos K, Gossios TD, Griva T, Anagnostis P, Kargiotis K, et al. Safety and efficacy of long-term statin treatment for cardiovascular events in patients with coronary heart disease and abnormal liver tests in the Greek Atorvastatin and Coronary Heart Disease Evaluation (GREACE) Study: a post-hoc analysis. *Lancet*. 2010;376:1916–1922.
225. Tikkanen MJ, Fayyad R, Faergeman O, Olsson AG, Wun C-C, Laskey R, et al. Effect of intensive lipid lowering with atorvastatin on cardiovascular outcomes in coronary heart disease patients with mild-to-moderate baseline elevations in alanine aminotransferase levels. *Int J Cardiol*. 2013;168:3846–3852.
226. Stone NJ, Robinson JG, Lichtenstein AH, Bairey Merz CN, Blum CB, Eckel RH, et al. 2013 ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *Circulation*. 2015;129:S1–45.
227. Chalasani N, Aljadhey H, Kesterson J, Murray MD, Hall SD. Patients with elevated liver enzymes are not at higher risk for statin hepatotoxicity. *Gastroenterology*. 2004;126:1287–1292.
228. Lewis JH, Mortensen ME, Zweig S, Fusco MJ, Medoff JR, Belder R, et al. Efficacy and safety of high-dose pravastatin in hypercholesterolemic patients with well-compensated chronic liver disease: results of a prospective, randomized, double-blind, placebo-controlled, multicenter trial. *Hepatology*. 2007;46:1453–1463.
229. Foster T, Budoff MJ, Saab S, Ahmadi N, Gordon C, Guerci AD. Atorvastatin and antioxidants for the treatment of nonalcoholic fatty liver disease: the St Francis Heart Study randomized clinical trial. *Am J Gastroenterol*. 2011;106:71–77.
230. Kimura Y, Hyogo H, Yamagishi S, Takeuchi M, Ishitobi T, Nabeshima Y, et al. Atorvastatin decreases serum levels of advanced glycation endproducts (AGEs) in

- nonalcoholic steatohepatitis (NASH) patients with dyslipidemia: clinical usefulness of AGEs as a biomarker for the attenuation of NASH. *J Gastroenterol*. 2010;45:750–757.
231. Ekstedt M, Franzén LE, Mathiesen UL, Holmqvist M, Bodemar G, Kechagias S. Statins in non-alcoholic fatty liver disease and chronically elevated liver enzymes: a histopathological follow-up study. *J Hepatol*. 2007;47:135–141.
232. Hyogo H, Tazuma S, Arihiro K, Iwamoto K, Nabeshima Y, Inoue M, et al. Efficacy of atorvastatin for the treatment of nonalcoholic steatohepatitis with dyslipidemia. *Metabolism*. 2008;57:1711–1718.
233. Nelson A, Torres DM, Morgan AE, Fincke C, Harrison SA. A pilot study using simvastatin in the treatment of nonalcoholic steatohepatitis: a randomized placebo-controlled trial. *J Clin Gastroenterol*. 2009;43:990–994.
234. Neuschwander-Tetri BA, Loomba R, Sanyal AJ, Lavine JE, Van Natta ML, Abdelmalek MF, et al. Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial. *Lancet*. 2015;385:956–965.
235. Ratziu V, Harrison SA, Francque S, Bedossa P, Lehert P, Serfaty L, et al. Elafibranor, an agonist of the peroxisome proliferator-activated receptor- α and - δ , induces resolution of nonalcoholic steatohepatitis without fibrosis worsening. *Gastroenterology*. 2016;150:1147–1159.e5.
236. Berzigotti A, Garcia-Tsao G, Bosch J, Grace ND, Burroughs AK, Morillas R, et al. Obesity is an independent risk factor for clinical decompensation in patients with cirrhosis. *Hepatology*. 2011;54:555–561.
237. O’Leary JG, Landaverde C, Jennings L, Goldstein RM, Davis GL. Patients with NASH and cryptogenic cirrhosis are less likely than those with hepatitis C to receive liver transplants. *Clin Gastroenterol Hepatol*. 2011;9:700–704.e1.
238. Nair S, Verma S, Thuluvath PJ. Obesity and its effect on survival in patients undergoing orthotopic liver transplantation in the United States. *Hepatology*. 2002;35:105–109.
239. Hakeem AR, Cockbain AJ, Raza SS, Pollard SG, Toogood GJ, Attia MA, et al. Increased morbidity in overweight and obese liver transplant recipients: a single-center experience of 1325 patients from the United Kingdom. *Liver Transpl*. 2013;19:551–562.
240. Leonard J, Heimbach JK, Malinchoc M, Watt K, Charlton M. The impact of obesity on long-term outcomes in liver transplant recipients-results of the NIDDK liver transplant database. *Am J Transplant*. 2008;8:667–672.
241. Heimbach JK, Watt KDS, Poterucha JJ, Ziller NF, Cecco SD, Charlton MR, et al. Combined liver transplantation and gastric sleeve resection for patients with medically complicated obesity and end-stage liver disease. *Am J Transplant*. 2013;13:363–368.

242. Kim TN, Park MS, Lim KI, Choi HY, Yang SJ, Yoo HJ, et al. Relationships between sarcopenic obesity and insulin resistance, inflammation, and vitamin D status: the Korean Sarcopenic Obesity Study. *Clin Endocrinol (Oxf)*. 2013;78:525–532.
243. Tandon P, Ney M, Irwin I, Ma MM, Gramlich L, Bain VG, et al. Severe muscle depletion in patients on the liver transplant wait list: its prevalence and independent prognostic value. *Liver Transpl*. 2012;18:1209–1216.
244. Hong HC, Hwang SY, Choi HY, Yoo HJ, Seo JA, Kim SG, et al. Relationship between sarcopenia and nonalcoholic fatty liver disease: the Korean Sarcopenic Obesity Study. *Hepatology*. 2014;59:1772–1778.
245. Abellan van Kan G, Houles M, Vellas B. Identifying sarcopenia. *Curr Opin Clin Nutr Metab Care*. 2012;15:436–441.
246. Montano-Loza AJ, Meza-Junco J, Baracos VE, Prado CMM, Ma M, Meeberg G, et al. Severe muscle depletion predicts postoperative length of stay but is not associated with survival after liver transplantation. *Liver Transpl*. 2014;20:640–648.
247. Durand F, Buyse S, Francoz C, Laouénan C, Bruno O, Belghiti J, et al. Prognostic value of muscle atrophy in cirrhosis using psoas muscle thickness on computed tomography. *J Hepatol*. 2014;60:1151–1157.
248. Alvares-da-Silva MR, Reverbel da Silveira T. Comparison between handgrip strength, subjective global assessment, and prognostic nutritional index in assessing malnutrition and predicting clinical outcome in cirrhotic outpatients. *Nutrition*. 2005;21:113–117.
249. Figueiredo FA, Dickson ER, Pasha TM, Porayko MK, Therneau TM, Malinchoc M, et al. Utility of standard nutritional parameters in detecting body cell mass depletion in patients with end-stage liver disease. *Liver Transpl*. 2000;6:575–581.
250. Targher G, Bertolini L, Padovani R, Rodella S, Zoppini G, Pichiri I, et al. Prevalence of non-alcoholic fatty liver disease and its association with cardiovascular disease in patients with type 1 diabetes. *J Hepatol*. 2010;53:713–718.
251. Oni ET, Agatston AS, Blaha MJ, Fialkow J, Cury R, Sposito A, et al. A systematic review: burden and severity of subclinical cardiovascular disease among those with nonalcoholic fatty liver; should we care? *Atherosclerosis*. 2013;230:258–267.
252. Lentine KL, Costa SP, Weir MR, Robb JF, Fleisher LA, Kasiske BL, et al. Cardiac disease evaluation and management among kidney and liver transplantation candidates: a scientific statement from the American Heart Association and the American College of Cardiology Foundation. *J Am Coll Cardiol*. 2012;60:434–480.
253. Singal AK, Hasanin M, Kaif M, Wiesner R, Kuo Y-F. Nonalcoholic steatohepatitis is the most rapidly growing indication for simultaneous liver kidney transplantation in the United States. *Transplantation*. 2016;100:607–612.

254. Inker LA, Schmid CH, Tighiouart H, Eckfeldt JH, Feldman HI, Greene T, et al. Estimating glomerular filtration rate from serum creatinine and cystatin C. *N Engl J Med*. 2012;367:20–29.
255. Maor-Kendler Y, Batts KP, Burgart LJ, Wiesner RH, Krom RA, Rosen CB, et al. Comparative allograft histology after liver transplantation for cryptogenic cirrhosis, alcohol, hepatitis C, and cholestatic liver diseases. *Transplantation*. 2000;70:292–297.
256. Dumortier J, Giostra E, Belbouab S, Morard I, Guillaud O, Spahr L, et al. Non-alcoholic fatty liver disease in liver transplant recipients: another story of “seed and soil.” *Am J Gastroenterol*. 2010;105:613–620.
257. Bhati C, Idowu MO, Sanyal AJ, Rivera M, Driscoll C, Stravitz RT, et al. Long term outcomes in patients undergoing liver transplantation for nonalcoholic steatohepatitis related cirrhosis. *Transplantation*. 2017;[Epub ahead of print].
258. Everhart JE, Lombardero M, Lake JR, Wiesner RH, Zetterman RK, Hoofnagle JH. Weight change and obesity after liver transplantation: incidence and risk factors. *Liver Transpl Surg*. 1998;4:285–296.
259. Laish I, Braun M, Mor E, Sulkes J, Harif Y, Ben Ari Z. Metabolic syndrome in liver transplant recipients: prevalence, risk factors, and association with cardiovascular events. *Liver Transpl*. 2011;17:15–22.
260. Schäcke H, Döcke WD, Asadullah K. Mechanisms involved in the side effects of glucocorticoids. *Pharmacol Ther*. 2002;96:23–43.
261. Øzbay LA, Smidt K, Mortensen DM, Carstens J, Jørgensen KA, Rungby J. Cyclosporin and tacrolimus impair insulin secretion and transcriptional regulation in INS-1E beta-cells. *Br J Pharmacol*. 2011;162:136–146.
262. Garcia-Tsao G, Abraldes JG, Berzigotti A, Bosch J. Portal hypertensive bleeding in cirrhosis: Risk stratification, diagnosis, and management: 2016 practice guidance by the American Association for the Study of Liver Diseases. *Hepatology*. 2017;65:310–335.
263. Heimbach J, Kulik LM, Finn R, Sirlin CB, Abecassis M, Roberts LR, et al. AASLD guidelines for the treatment of hepatocellular carcinoma. *Hepatology* [Internet]. 2017 [cited 2017 Apr 5]; Available from: <http://onlinelibrary.wiley.com/doi/10.1002/hep.29086/abstract>
264. Schwimmer JB, Deutsch R, Kahen T, Lavine JE, Stanley C, Behling C. Prevalence of fatty liver in children and adolescents. *Pediatrics*. 2006;118:1388–1393.
265. Anderson EL, Howe LD, Jones HE, Higgins JPT, Lawlor DA, Fraser A. The prevalence of non-alcoholic fatty liver disease in children and adolescents: a systematic review and meta-analysis. *PloS One*. 2015;10:e0140908.

266. Sundaram SS, Sokol RJ, Capocelli KE, Pan Z, Sullivan JS, Robbins K, et al. Obstructive sleep apnea and hypoxemia are associated with advanced liver histology in pediatric nonalcoholic fatty liver disease. *J Pediatr*. 2014;164:699–706.e1.
267. Molleston JP, Schwimmer JB, Yates KP, Murray KF, Cummings OW, Lavine JE, et al. Histological abnormalities in children with nonalcoholic fatty liver disease and normal or mildly elevated alanine aminotransferase levels. *J Pediatr*. 2014;164:707–713.e3.
268. Schwimmer JB, Newton KP, Awai HI, Choi LJ, Garcia MA, Ellis LL, et al. Paediatric gastroenterology evaluation of overweight and obese children referred from primary care for suspected non-alcoholic fatty liver disease. *Aliment Pharmacol Ther*. 2013;38:1267–1277.
269. Feldstein AE, Charatcharoenwitthaya P, Treeprasertsuk S, Benson JT, Enders FB, Angulo P. The natural history of non-alcoholic fatty liver disease in children: a follow-up study for up to 20 years. *Gut*. 2009;58:1538–1544.
270. Lavine JE, Yates KP, Brunt EM, Kleiner DE, Schwimmer D, Murray KF, et al. The natural history of nonalcoholic fatty liver disease in children and adolescents assessed in placebo recipients in the TONIC trial. *Hepatology*. 2012;56:905A.
271. Riley MR, Bass NM, Rosenthal P, Merriman RB. Underdiagnosis of pediatric obesity and underscreening for fatty liver disease and metabolic syndrome by pediatricians and pediatric subspecialists. *J Pediatr*. 2005;147:839–842.
272. Barlow SE, Expert Committee. Expert committee recommendations regarding the prevention, assessment, and treatment of child and adolescent overweight and obesity: summary report. *Pediatrics*. 2007;120 Suppl 4:S164–192.
273. Loomba R, Sirlin CB, Schwimmer JB, Lavine JE. Advances in pediatric nonalcoholic fatty liver disease. *Hepatology*. 2009;50:1282–1293.
274. Mansoor S, Collyer E, Alkhoury N. A comprehensive review of noninvasive liver fibrosis tests in pediatric nonalcoholic fatty liver disease. *Curr Gastroenterol Rep*. 2015;17:23.
275. Alkhoury N, Carter-Kent C, Lopez R, Rosenberg WM, Pinzani M, Bedogni G, et al. A combination of the pediatric NAFLD fibrosis index and enhanced liver fibrosis test identifies children with fibrosis. *Clin. Gastroenterol. Hepatol*. 2011;9:150–155.
276. Nobili V, Parkes J, Bottazzo G, Marcellini M, Cross R, Newman D, et al. Performance of ELF serum markers in predicting fibrosis stage in pediatric non-alcoholic fatty liver disease. *Gastroenterology*. 2009;136:160–167.
277. Vuppalanchi R, Jain AK, Deppe R, Yates K, Comerford M, Masuoka HC, et al. Relationship between changes in serum levels of keratin 18 and changes in liver histology in children and adults with nonalcoholic fatty liver disease. *Clin. Gastroenterol. Hepatol*. 2014;12:2121–2130.e1–2.

278. Carter-Kent C, Yerian LM, Brunt EM, Angulo P, Kohli R, Ling SC, et al. Nonalcoholic steatohepatitis in children: a multicenter clinicopathological study. *Hepatology*. 2009;50:1113–1120.
279. Africa JA, Behling CA, Brunt EM, Zhang N, Luo Y, Wells A, et al. In children with nonalcoholic fatty liver disease, zone 1 steatosis is associated with advanced fibrosis. *Clin. Gastroenterol. Hepatol*. 2017;[Epub ahead of print].
280. Nobili V, Manco M, Devito R, Ciampalini P, Piemonte F, Marcellini M. Effect of vitamin E on aminotransferase levels and insulin resistance in children with non-alcoholic fatty liver disease. *Aliment Pharmacol Ther*. 2006;24:1553–1561.
281. Nobili V, Manco M, Devito R, Di Ciommo V, Comparcola D, Sartorelli MR, et al. Lifestyle intervention and antioxidant therapy in children with nonalcoholic fatty liver disease: a randomized, controlled trial. *Hepatology*. 2008;48:119–128.
282. Koot BGP, van der Baan-Slootweg OH, Vinke S, Bohte AE, Tamminga-Smeulders CLJ, Jansen PLM, et al. Intensive lifestyle treatment for non-alcoholic fatty liver disease in children with severe obesity: inpatient versus ambulatory treatment. *Int J Obes (Lond)*. 2016;40:51–57.
283. Barlow SE, Dietz WH. Management of child and adolescent obesity: summary and recommendations based on reports from pediatricians, pediatric nurse practitioners, and registered dietitians. *Pediatrics*. 2002;110:236–238.
284. Schwimmer JB, Lavine JE, Wilson LA, Neuschwander-Tetri BA, Xanthakos SA, Kohli R, et al. In children with nonalcoholic fatty liver disease, cysteamine bitartrate delayed release improves liver enzymes but does not reduce disease activity scores. *Gastroenterology*. 2016;151:1141–1154.
285. Janczyk W, Lebensztejn D, Wierzbicka-Rucińska A, Mazur A, Neuhoﬀ-Murawska J, Matusik P, et al. Omega-3 Fatty acids therapy in children with nonalcoholic fatty liver disease: a randomized controlled trial. *J Pediatr*. 2015;166:1358–1363.e1–3.
286. Pacifico L, Bonci E, Di Martino M, Versacci P, Andreoli G, Silvestri LM, et al. A double-blind, placebo-controlled randomized trial to evaluate the efficacy of docosahexaenoic acid supplementation on hepatic fat and associated cardiovascular risk factors in overweight children with nonalcoholic fatty liver disease. *Nutr Metab Cardiovasc Dis NMCD*. 2015;25:734–741.
287. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, et al. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute scientific statement. *Circulation*. 2005;112:2735–2752.