LIVER DISEASE

The intersection of nonalcoholic fatty liver disease and obesity

Jennifer A. Woo Baidal and Joel E. Lavine*

Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease worldwide and recently emerged as the most rapidly increasing indication for liver transplant. Although obesity is a risk factor for NAFLD, overlap between these two entities is incompletely understood. We highlight recent insights into the pathogenesis of human NAFLD in relation to obesity and discuss advances in the diagnosis and treatment of NAFLD.

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is a common cause of chronic liver disease and has emerged as the most rapidly growing cause of hepatocellular carcinoma requiring liver transplant in the United States (*1*). Today's historically high prevalence of obesity at all ages is accompanied by related health complications, including NAFLD (*2*, *3*). From a global perspective, countries with higher obesity prevalence tend to have a higher prevalence of NAFLD (*2*). In addition to obesity, NAFLD is associated with type 2 diabetes mellitus and dyslipidemia. Although closely related, obesity is neither necessary nor sufficient for development of NAFLD. NAFLD impacts an estimated 20 to 30% of adults and 10% of children (*3*–*5*).

The term NAFLD describes a spectrum of liver disease that can range from isolated steatosis to steatosis with lobular inflammation and evidence of cellular injury (termed nonalcoholic steatohepatitis or NASH). Generally, patients with NASH also demonstrate perisinusoidal fibrosis in the liver. NAFLD is diagnosed by the appearance of excessive hepatic fat (hepatocellular macrovesicular steatosis) in the absence of alternative causes. Such alternative causes could be excessive alcohol consumption or use of medication, such as glucocorticoids or tamoxifen, that are known to promote fatty liver (*6*), or conditions such as autoimmune hepatitis, hepatitis C virus infection, or Wilson's disease. Hepatic steatosis is defined as macrovesicular steatosis in greater than 5% of hepatocytes on histology or greater than 5.5% hepatic fat fraction measured by magnetic resonance imaging (MRI) (*6*). In individuals with NASH, death of liver cells (hepatocytes) may lead to cirrhosis, liver failure, and hepatocellular carcinoma. There is uncertainty regarding the natural history and prognosis of isolated hepatic steatosis. A recent longitudinal study of 108 adults with hepatic steatosis with no or mild inflammation at baseline found that 44% progressed to NASH over a median of 6.6 years (*7*). However, other evidence in patients followed for decades suggests that those with hepatic steatosis are unlikely to develop liver complications.

Efficient energy storage as fat once served primarily as a protective mechanism, but now culminates in an unprecedented burden of obesity-related diseases including NAFLD. Ingested calories that are not immediately needed for energy consumption can be stored as triglycerides in adipose tissue. In times of food scarcity, these triglycerides are mobilized to provide fuel. However, much of the world now faces an abundance of calorically dense foods that are high in fat and refined sugar and lack essential micronutrients. With an excess of calories and a sedentary life-style, the evolutionary mechanism that preserves energy as fat changes from protective to maladaptive, resulting in accumulation of lipid-laden material, primarily triglycerides, in the liver. Day and James conceptualized such an accumulation as a necessary first "hit" in the downstream processes that lead to NASH, with subsequent oxidant stress and lipid peroxidation acting as a second "hit" in the etiology of NASH (*8*). However, an alternate view that challenges the "two-hit hypothesis" has since gained traction. In line with the suggestion that triglyceride accumulation is an innocent bystander in the origins of NASH as originally proposed by Thaler in 1975 (*9*), the "lipotoxic liver injury" hypothesis points to certain free fatty acids and metabolites, such as diacylglycerols and ceramide, as the central players in the pathogenesis of NASH (*10*). In this hypothesis, these lipids flux through the liver, leading to hepatocellular injury, endoplasmic reticulum (ER) stress, and inflammation, whereas alterations in intestinal microbiota, hypoxia (for example, in the setting of sleep apnea), aberrant adipocyte signaling, and reduced antioxidant defenses modify the risk of NASH and liver fibrosis.

Given the increasing prevalence and morbidity associated with NASH, efforts to understand its etiology and to identify potential therapeutic targets for NASH have intensified. Translation of basic science discoveries has resulted in new diagnostic and therapeutic investigations, with noninvasive diagnostic tools and new pharmacological therapies appearing on the horizon.

THE CONNECTION BETWEEN NAFLD AND OBESITY

Obesity is a major risk factor for NAFLD. Although obesity correlates with NAFLD, not all patients with obesity develop NAFLD. Furthermore, a minority of patients with NAFLD are lean. In the National Health and Nutrition Examination Survey III, 7.4% of lean adults and 27.8% of overweight/obese adults had hepatic steatosis that could be detected by ultrasound (*11*). Compared to counterparts in the same body mass index (BMI) category without NAFLD, adults with ultrasound-defined NAFLD were more likely to be older, of Hispanic ethnicity, have more features of metabolic syndrome, and have higher amounts of aminotransferases in serum. Compared to adults with both obesity and NAFLD, lean adults with NAFLD were more likely to be younger and female and less likely to have insulin resistance and hypercholesterolemia. In contrast, in a study of almost 30,000 Korean adults, the associations of ultrasound-based NAFLD with hypertension, impaired fasting glucose levels, low HDL (high-density lipoprotein) cholesterol, and elevated

Division of Pediatric Gastroenterology, Hepatology and Nutrition, Department of Pediatrics, Columbia University Medical Center, New York, NY 10032, USA.
*Corresponding author. E-mail: jl3553@cumc.columbia.edu

triglycerides were stronger in female adults with obesity than in those with normal weight or who were overweight (*12*).

One reason for this incomplete overlap between obesity and NAFLD is related to the use of BMI to define obesity. Although BMI cutoffs have good specificity for detecting excess adiposity, they lack sensitivity (*13*). BMI also does not distinguish between total body fat mass or lean mass, nor does it provide information about the distribution or types of body fat. Visceral adipose tissue is a well-recognized mediator of lipid metabolism and is linked to adverse health outcomes, including NAFLD. In a cross-sectional study of adults undergoing MRI and liver biopsy, visceral adipose tissue had a strong direct association with liver inflammation and fibrosis and more strongly predicted histological severity than did subcutaneous fat (*14*).

Although once thought of as either visceral or subcutaneous and white or brown, adipose biology has come to be seen as much more complex than previously appreciated (*15*). Differences in location of adipose tissue (fat pads), types of adipose tissue (white or brown), and types of adipocytes (white, beige, or brown) within adipose are important determinants of metabolic disease (Fig. 1). For example, the visceral adipose tissue depot can be further partitioned into perigonadal, mesenteric, omental, and retroperitoneal fat pads. Epicardial fat may act like visceral fat but might not be captured by some imaging techniques. Whether these fat depots differ in metabolic signaling functions is unknown. The hormonal and biological activity of adipose tissue through secretion of adipokines by white adipocytes, release of inflammatory cytokines by macrophages within adipose tissue, and release of free fatty acids from adipocytes are increasingly recognized as contributing to insulin resistance and metabolic disease, including NASH (Fig. 1).

White adipose tissue is composed of loose connective tissue with adipocytes surrounded by extracellular matrix. White adipocytes are unilocular cells that store nutrients as lipids and release them as fatty acids during food scarcity, which can contribute to metabolic disease. In the setting of chronic overnutrition, white adipose tissue becomes engorged with lipid. This leads to adipocyte hyperplasia or hypertrophy. Remodeling of white adipose tissue helps to accommodate adipocyte hypertrophy, but eventually, impaired innervation and vascularization result in hypoxia and adipocyte dysfunction (*16*). This dysfunction is hypothesized to lead to c-Jun NH2-terminal kinase 1 (JNK1)–mediated adipocyte secretion of inflammatory cytokines (*17*), resulting in increases and shifts in the immune cell population in adipose tissue, and eventual systemic metabolic stress with mitochondrial dysfunction, lipolysis, decreased lipid storage capacity, and disruption of insulin signaling (*18*). Traditionally, it was thought that brown adipocytes were present in human infants, but not in adult humans (*15*). Recent findings on positron emission tomography and tissue biopsy have shown that adult humans do in fact have functional brown fat in the supraclavicular and spinal regions (*19*, *20*). Brown adipocytes have a multilocular lipid droplet structure and are filled with mitochondria, and they dissipate stored energy in the form of heat via the actions of a mitochondrial protein, UCP-1. UCP-1 causes a proton leak across inner mitochondrial membranes that leads to uncoupling of oxidation from ATP (adenosine 5′-triphosphate) synthesis, resulting in nonshivering thermogenesis (*15*). Another type of UCP-1–positive adipocyte, the "beige" or "brite" cell, was very recently identified within white adipose tissue (*21*) and found to contribute to basal energy expenditure (*22*). Two pathways for beige adipocyte development have been identified: de novo recruitment from progenitor cells and differentiation of white adipocytes (*23*). Given their thermogenic properties and ability to drive energy expenditure, activation of brown and beige adipocytes may be a potential strategy for treating obesity, NAFLD, and insulin resistance, but research in this area is still at the preclinical stage.

The crosstalk between adipocytes and hepatocytes is an active area of investigation, and better understanding of these signaling pathways will contribute to elucidating NAFLD pathogenesis and how it relates to systemic metabolic disease (Fig. 1). Recent attention has focused on organokines for their potential role in organ crosstalk. Organokines are proteins that are predominantly secreted by a specific tissue and have a paracrine or endocrine action. Hepatokines, such as FGF21 and fetuin A, are produced mainly by the liver in humans. Adipokines, such as adiponectin and leptin, are released by adipose tissue. In mouse models of obesity, FGF21 overexpression has beneficial effects on body weight, liver fat, and insulin resistance, potentially through modulation of adipocyte signaling activities that result in up-regulated expression of UCP-1 and downstream energy dissipation. In humans, FGF21 is mainly produced by the liver, and its function is less clear because higher FGF21 levels correlate with poorer metabolic outcomes (*24*). Conversely, impaired adipocyte release of adiponectin has been linked to worse metabolic

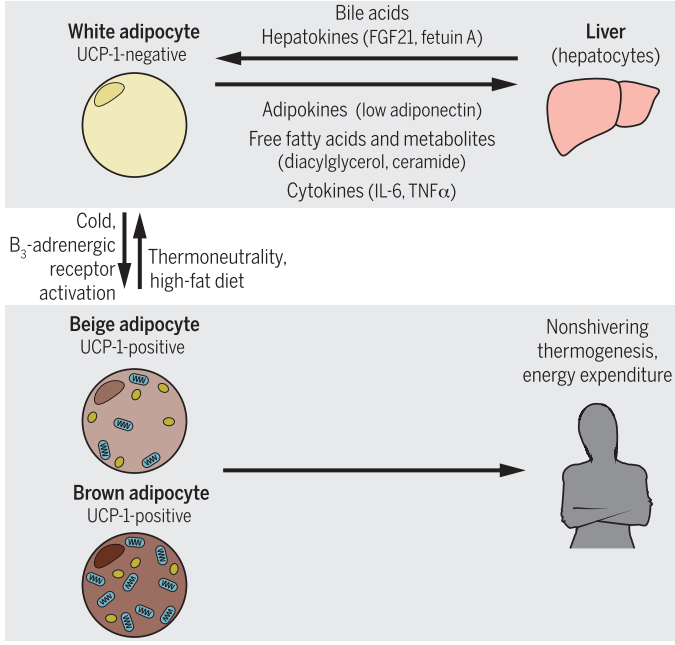

Fig. 1. Crosstalk between adipocytes and hepatocytes. White adipocytes store nutrients as lipids and release them as fatty acids during food scarcity. Recently described beige (also called inducible brown or brite) adipocytes are predominantly found in white adipose tissue during conditions of energy expenditure, and they may contribute to heat production and energy dissipation. The reversible, adaptive process that leads to development of beige adipocytes in white adipose tissue is called "browning" of adipocytes. Brown adipocytes contain many mitochondria and are capable of rapid energy expenditure. The role of proteins (hepatokines), bile acids, and other substances secreted by the liver in adipocyte homeostasis and browning is unclear. White adipocytes release proteins (adipokines) that may influence liver health. Release of free fatty acids and metabolites by adipocytes and secretion of cytokines by macrophages in adipose tissue contribute to the development of NAFLD and NASH. UCP-1, uncoupling protein-1; FGF21, fibroblast growth factor 21; IL-6, interleukin-6; TNFα, tumor necrosis factor–α.

health (*25*). A better understanding of how perturbations in adipocyte-hepatocyte signaling contribute to systemic metabolic disease could yield insight into new diagnostic and therapeutic opportunities to improve metabolic health.

NAFLD: DIFFERENT ETIOLOGIES, DIFFERENT OUTCOMES

Lipotoxicity, inflammation, and stress pathways

According to the lipotoxic liver injury hypothesis, certain free fatty acids and their metabolites flux through the liver and provoke NASH. Dietary intake plays a major role in generation of free fatty acids. Excess carbohydrate consumption, particularly fructose, leads to de novo lipogenesis. Excess calories and fat can result in lipid accumulation in adipose tissue, and ultimately, stored fatty acids are released via lipolysis. Free fatty acids generated by lipogenesis, lipolysis, or other mechanisms have three potential fates: triglyceride formation, oxidation and disposal, or lipotoxic lipid intermediate formation. The role of these pathways in the pathogenesis of NAFLD has been previously reviewed (*10*). Triglyceride accumulation appears to be an innocent bystander of metabolic perturbations that lead to NASH. Oxidation pathways in mitochondria, peroxisomes, and ER facilitate disposal of free fatty acids. Certain free fatty acids and metabolites such as ceramide and diacylglycerol are noxious to biological systems, and the exact mechanism mediating cellular death in lipotoxicity likely involves oxidative stress and apoptosis.

In the setting of obesity, a state of inflammation, excess lipids, and accumulation of reactive oxygen species (ROS) may impair organelle function. The ER is an organelle responsible for protein folding, maturation, and trafficking [reviewed in (*26*)]. Under certain conditions such as accumulation of unfolded proteins, energy deprivation, or hypoxia, ER stress may ensue. ER stress activates the unfolded protein response (UPR), which decreases protein synthesis and increases protein clearance, thus facilitating reduction of ER stress. If the UPR is not successful, cellular apoptosis may occur. ER stress and the UPR are linked to activation of inflammatory pathways with downstream release of inflammatory cytokines, as well as generation of ROS. Not all cell types respond similarly to UPR, but ER stress represents a major mechanistic underpinning to the development of hepatocyte injury in NAFLD.

Hepatic mitochondria play a major role in fatty acid oxidation and ATP synthesis. Mitochondrial β-oxidation is one pathway for disposal of fatty acids, but results in generation of ROS. Under most circumstances, endogenous antioxidant mechanisms are able to protect against cellular injury from ROS. However, in the setting of impaired mitochondrial function related to obesity and chronic lipid overload, ROS leads to fatty acid peroxidation that further interferes with mitochondrial function through oxidative damage to mitochondrial DNA and proteins (*27*). There are few human studies of the role of hepatic mitochondria in obesity and its complications. Why some humans with obesity are able to up-regulate mitochondrial function and compensate for lipid overload whereas others do not remains unclear. Multiple pathways involving a complex interplay between excess lipids, systemic inflammation, and cellular stress likely contribute to the development and progression of NAFLD.

Genetics

The rapid increase in NAFLD prevalence suggests that environmental and behavioral factors underlie its origins, but understanding genetic susceptibility could provide clues to pathogenesis. In a cohort of adult twins in Southern California, the degree of hepatic steatosis and fibrosis, as measured by MRI, correlated between twins who were monozygotic, but not dizygotic (*28*). This suggests that both hepatic steatosis and fibrosis are heritable.

Many candidate genes or genomewide association studies have been examined to identify genetic factors predisposing to NAFLD or NASH. However, a recent systematic review and meta-analysis found that only six genes (*PNPLA3*, *APOE*, *SOD2*, *TNF*, *TM6SF2*, and *GCKR*) had been studied in more than one cohort with liver histology outcomes (*29*). I148M is a common variant in the gene encoding PNPLA3 (patatin-like phospholipase domain–containing protein 3, also known as adiponutrin) and is the strongest genetic predictor of hepatic steatosis (*30*). Its effects appear to be independent of insulin resistance and obesity (*30*–*32*). Also, the I148M polymorphism is associated with NASH and alcoholic cirrhosis (*33*, *34*). The *PNPLA3* variant was first identified in Europeans but may help to explain higher NAFLD prevalence in Hispanics, particularly those of Mexican origin (*30*). Additionally, an environmental interaction with the *PNPLA3* variant seems to exist. In one study of Hispanic adolescents who were overweight or obese, higher carbohydrate and total sugar intake were associated with higher hepatic fat fractions on MRI only among those with the *PNPLA3* variant (*35*). Given the increased consumption of fructose and other sugars (*36*), these data support the role of environmental factors contributing to rapid increases in NAFLD in the context of a predisposing and common genetic variant.

The mechanism by which the *PNPLA3* variant contributes to NAFLD is not fully understood. Researchers are divided on whether reduced hepatic triglyceride catabolism or gain of function in triglyceride synthesis leads to increased genetic risk for NAFLD in the setting of the *PNPLA3* variant, and evidence exists to support both possibilities (*37*, *38*). A recent study describes differences in serum triglyceride composition among obese adults with NAFLD compared to lean adults with *PNPLA3* variant–related hepatic steatosis (*39*). In adults with the *PNPLA3* variant, saturated and monounsaturated fatty acids were lower, and long-chain polyunsaturated fatty acids were higher compared to those with obesity-related NAFLD (*39*). These data support the multifactorial etiology of NAFLD and suggest that NAFLD is really an umbrella term for an array of histopathological findings.

Understanding the different relationships between genetic variants and NAFLD, insulin resistance, and lipid abnormalities could help to more accurately define disease phenotypes and ultimately advance personalized therapeutic approaches for NAFLD and cardiometabolic disorders. For example, the *PNPLA3* variant seems to confer risk for NAFLD without adverse impacts on lipid metabolism. Conversely, the TM6SF2 variant that decreases hepatic very-low-density lipoprotein (VLDL) excretion leads to a higher risk of hepatic steatosis, NASH, hepatic fibrosis, and type 2 diabetes mellitus, but also confers a reduced risk of cardiovascular disease likely through reduced excretion of hepatic VLDL and lower concentrations of serum triglycerides and low-density lipoproteins (LDLs) (*40*–*42*).

Whereas the role of hepatic steatosis in later development of NASH and hepatocellular carcinoma remains unclear, emerging data suggest that the degree of hepatic steatosis continues to deserve attention for at least two reasons. First, recent data in adults show that hepatic steatosis can progress to NASH in some instances. Although many cases of steatosis are commonly thought to be "benign," a systematic review and meta-analysis of adults with biopsy-proven NAFLD found

that patients with hepatic steatosis at baseline showed progression of fibrosis at a rate of one stage per 14.3 years [95% confidence interval (CI), 9.1 to 50 years] (*43*). Second, the quantity of hepatic steatosis is associated with cardiovascular complications independent of NASH and BMI. Hepatic steatosis may contribute to the development of diabetes and adverse cardiovascular outcomes (*44*–*46*).

DIAGNOSTIC ADVANCES FOR NAFLD

Reliable, noninvasive tests to quantify hepatic steatosis, NASH, and fibrosis in patients with NAFLD in the clinical setting do not yet exist (*6*). Thus, liver biopsy remains the gold standard for NAFLD diagnosis and severity staging despite its invasiveness, associated risks, and cost (Table 1). Additionally, liver biopsy sampling and interpretation by pathologists may be subject to variability (*47*, *48*). The primary outcome of NASH treatment trials in adults and children often involves changes in a histological scoring system that includes measures of steatosis, inflammation, and hepatocyte ballooning, the summation of which is the NAFLD activity score (NAS) (*47*). Stage of fibrosis is also measured. Other scoring systems exist but do not account for common histological findings in NASH that are unique to pediatrics.

Lack of a reliable, noninvasive method to diagnose and stage NAFLD precludes evidence-based recommendations for screening and is a major barrier to understanding disease prevalence, incidence, and natural history. In clinical practice and large-scale epidemiological studies, serum alanine aminotransferase (ALT) concentrations often are used for screening of NAFLD despite suboptimal sensitivity and specificity. Although sustained ALT elevation may signal liver disease, adults with NAFLD may have bridging fibrosis and cirrhosis even in the presence of normal ALT concentrations (*49*). The current normal values for ALT used as laboratory reference ranges are likely too high for adults and children, and no threshold level for ALT elevation has been defined (*50*, *51*). Given the large burden of NAFLD among patients with excessive weight gain, identifying reliable noninvasive testing to assess steatosis, NASH, and fibrosis in adults and children who are overweight or obese is a clinical and research priority.

Liver ultrasound is a widely available imaging modality that can detect hepatic steatosis when there is moderate to severe infiltration of the liver with fat, but it cannot accurately quantify steatosis. Ultrasound sensitivity has been reported to be as low as 60% but increases to 94% sensitivity when steatosis is moderate or above; specificity ranges between 84 and 100% (*52*–*54*). New quantitative ultrasound methods are under development for diagnosis of steatosis and quantification of liver fat but require further evaluation before determining their clinical utility (*55*). The controlled attenuation parameter (CAP) is a new method for quantification of hepatic steatosis that is based on the attenuation of ultrasound waves generated during transient elastography (described below) without requiring interpretation by a radiologist. Sensitivity of CAP for discriminating between steatosis grades in adults has been reported as 89 to 100%, and specificity as 78 to 86% (*56*, *57*). Computed tomography is highly sensitive and specific, but its cost together with radiation exposure prevents its widespread application. MRI techniques accurately quantify hepatic steatosis (*58*, *59*), and magnetic resonance spectroscopy (MRS) is able to detect hepatic fat in small quantities. Cost and limited availability are major barriers to the widespread use of MRI and MRS in research and clinical settings.

Current biomarkers and imaging do not reliably allow for detection of NASH. Cytokeratin 18 fragments are generated during caspase 3–mediated apoptosis and have been studied as markers of NASH severity alone and in combination with other markers of apoptosis (*60*–*63*). One study of cytokeratin 18 found 58% sensitivity and 68% specificity for the detection of NASH, but an analysis of how well cytokeratin 18 detects changes in liver biopsy histology found that it did not perform better than measurement of ALT concentrations (*64*, *65*). MRI techniques with newer contrast agents may make it possible to detect NASH by imaging, but further validation is needed (*66*). Liver biopsy is still the only reliable way to detect improvement or worsening of NASH, which is a crucial component of therapeutic trial outcomes and clinical decision-making. For example, the presence of NASH is an indication to initiate

Table 1. Noninvasive modalities for diagnosis and staging of NAFLD.

	Hepatic steatosis	NASH	Fibrosis
Imaging	Magnetic resonance imaging		Magnetic resonance elastography
	Magnetic resonance spectroscopy		Transient elastography
	Controlled attenuation parameter		
Serum markers		Oxidative stress markers:	Hyaluronic acid
		Thiobarbituric acid (TBARS), oxidized LDLs	Type IV collagen S
		Inflammation:	Procollagen III N-terminal peptide
		C-reactive protein, TNFα, adipokines (adiponectin, leptin), soluble CD14, retinol binding protein-4	Tissue inhibitor of metalloproteinase 1
		Hepatocellular apoptosis:	Fibronectin
		Cytokeratin fragment 18	Laminin
			YKL-40

vitamin E therapy and informs risk for progressive hepatic, metabolic, and cardiovascular outcomes.

In a prospective study of adults with NAFLD, fibrosis predicted future progression of liver disease and death (*67*). Perhaps the greatest diagnostic advance for NAFLD in the past few years has been the development of imaging techniques to noninvasively detect hepatic fibrosis. Although a number of predictive models have been developed to detect severe fibrosis using clinical and laboratory data, in a study of 102 patients with biopsy-proven NAFLD, magnetic resonance elastography (MRE) performed better than eight clinical prediction rules for diagnosing advanced fibrosis (*68*). Two-dimensional MRE had 86% sensitivity and 91% specificity for accurate prediction of patients with stage 3 (bridging fibrosis) or stage 4 (cirrhosis) fibrosis on liver biopsy. MRE is costly and not widely available outside of academic settings, but further validation of its ability to detect fibrosis could lead to more widespread use in the future.

Transient elastography is a technique to quantify fibrosis that can be performed in an office setting. A probe transducer is used to both generate a low-frequency vibration and calculate the speed of the generated wave as it propagates through the liver. The wave travels more quickly through stiffer tissue; thus, the speed of the wave directly reflects tissue stiffness and fibrosis (*69*, *70*). Transient elastography is less accurate in obese patients, perhaps owing to the thickness of subcutaneous tissue and greater wave attenuation in that tissue (*71*). Because fibrosis measurements with transient elastography can be impacted by a greater degree of steatosis, further validation in patients with NAFLD is required before transient elastography can be considered for use as an outcome in NAFLD treatment trials.

THERAPEUTIC OPTIONS FOR TREATING NAFLD AND NASH

Weight loss, diet, and physical activity

Weight loss is the target of obesity therapy and is the most effective therapy currently available for NAFLD and NASH. Loss of 3 to 5% total body weight decreases hepatic steatosis. Weight loss of 10% or more can reduce hepatocellular inflammation and may even reverse NASH and fibrosis (*6*, *72*, *73*). Chronic excessive caloric intake leads to lipid storage in adipocytes, which can ultimately promote adipocyte insulin resistance and increase production of free fatty acids and their metabolites, thus promulgating the development of NASH. Because most weight reduction interventions target multiple behaviors, the effects of a hypocaloric diet alone in patients with NASH are unknown. Dietary composition likely plays a role in NAFLD development and severity. In an observational study of children with biopsy-proven NAFLD, lower vitamin E intake was associated with a higher grade of steatosis. In the same study, uric acid, which is a biomarker for fructose intake, was higher in children with NASH (*74*). Thus, simply limiting calories may not be as effective as targeting healthy intake of macronutrients (carbohydrates, fats, and protein), limiting sugar (particularly fructose) intake, and promoting antioxidant (for example, vitamin E) intake that aligns with current intake recommendations.

Physical activity can reduce weight, decrease white adipose tissue volume and associated lipolysis, as well as improve glucose uptake in skeletal tissue. Ectopic fat deposition and insulin resistance in skeletal muscle contribute to the development of hepatic steatosis and other obesity-related comorbidities by diverting consumed carbohydrates down the pathway that leads to hepatic de novo lipogenesis (Fig. 2). Moderate intensity exercise can reverse this deviant energy storage pattern (*75*, *76*). The optimal episode duration, frequency, and type of exercise for NAFLD treatment are not known, but improvement in peripheral and hepatic insulin resistance and a decrease in hepatic fat fraction on MRS seem to be achievable within 3 to 4 months of sustained moderate physical activity, regardless of whether the activity is aerobic or resistance training (*77*).

Pharmacological treatments for NAFLD and NASH

The high prevalence and associated morbidity and mortality of NASH make it an intense area for pharmacological research, and an accelerated regulatory pathway exists for drug approval for NASH treatments (*78*). Although the U.S. Food and Drug Administration (FDA) has yet to approve any medications for use in NASH, intense research focuses on pharmacological interventions, some of which have demonstrated efficacy in phase 1 and 2 clinical trials. Pharmacological targets can be broadly categorized as having one or more actions (*10*). Reduction of lipolysis can be achieved through improved insulin sensitivity or through increased activation of TGR5 (G-protein coupled bile acid receptor 1) and FXR by bile acids (Fig. 2). Increased activation of oxidative pathways to promote fatty acid disposal could be boosted by ligands for peroxisome proliferator–activated receptors (PPAR) or the "beiging" of adipocytes. FXR activation can reduce de novo lipogenesis and decrease hepatic triglyceride synthesis. Reducing hepatocellular injury could be achieved using antioxidants, polyunsaturated fat supplements, or probiotics. Inflammation could be reduced through treatment with anti-TNFα or other anti-inflammatory agents. Fibrosis might be reduced by inhibition of LOXL2 or CCR2/CXCR5 (*10*). Most of these targets not only have the potential to benefit NASH but could also have applications in weight management or the treatment of metabolic diseases such as type 2 diabetes mellitus.

Insulin resistance appears to promote peripheral lipolysis and de novo lipogenesis and is closely linked to NASH. Thus, reducing insulin resistance has been a target of pharmacological interventions to treat NASH. Pioglitazone is a PPARγ agonist involved in carbohydrate and lipid metabolism. In the multicenter, placebo-controlled Pioglitazone versus Vitamin E versus Placebo for the Treatment of Nonalcoholic Steatohepatitis (PIVENS) trial, 247 adult patients without diabetes were randomized to pioglitazone (30 mg/day), D-α-tocopheral (800 IU/day), or placebo for 96 weeks. Compared to placebo, more pioglitazone recipients achieved the primary endpoint of reducing NAS by ≥2 points without worsening fibrosis [34% of pioglitazone recipients compared to 19% of controls ($P = 0.04$, not significant for multiple testing)], had significant improvement in hepatic steatosis and lobular inflammation, and had resolution of NASH compared to controls despite weight gain associated with pioglitazone treatment (*79*). Pioglitazone has not been labeled for use in children. Metformin, another insulin sensitizer, is not considered a potential NAFLD therapy given the lack of clinically meaningful histological improvement in adults and children (*80*–*82*). Elafibranor, or GFT505, is a dual PPARα and PPARδ agonist that may reduce insulin resistance, improve lipid profiles, and treat NASH. PPARα is expressed in hepatocytes, and its activation leads to a reduction in plasma triglycerides and increases in HDL cholesterol. PPARδ reduces insulin resistance, and may stimulate fatty acid oxidation and reduce circulating free fatty acids with a resultant reduction in hepatic

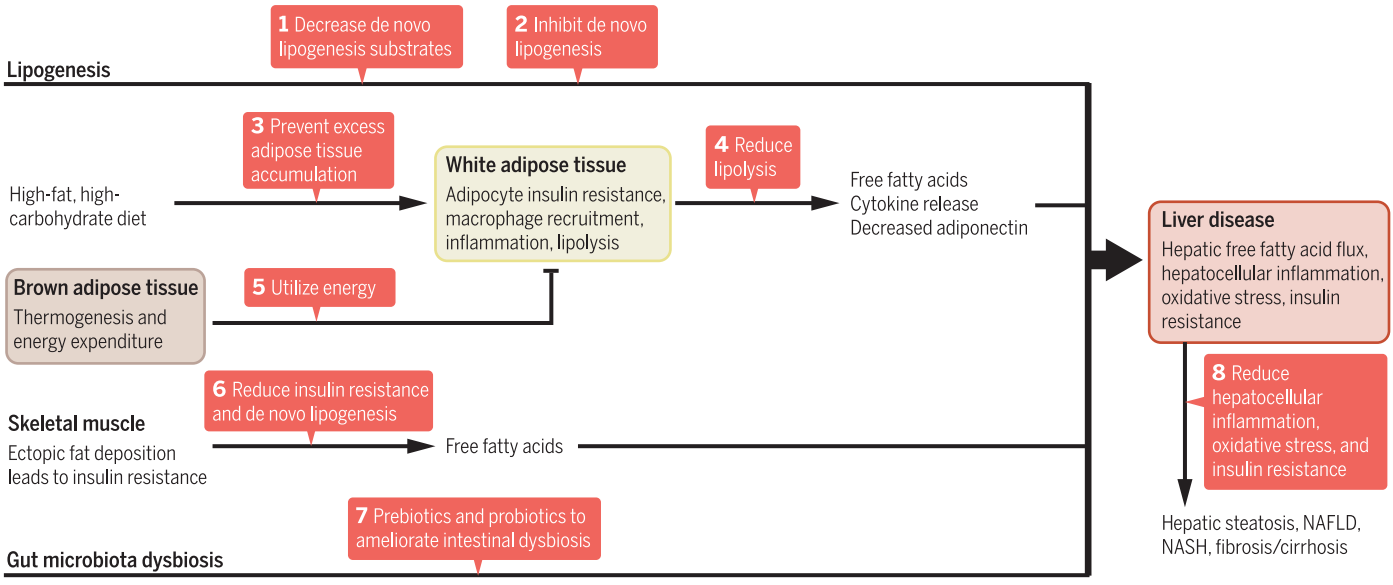

Fig. 2. Therapeutic interventions for the treatment of NAFLD and obesity. Fructose intake contributes to de novo lipogenesis. Avoidance of high-fructose foods and beverages decreases intake of substrates for de novo lipogenesis (1). De novo lipogenesis leads to free fatty acid production, which can be inhibited by activation of farnesoid X receptor (FXR), a bile acid receptor (2). Avoidance of a high-fat, high-carbohydrate diet and participation in routine moderate to vigorous physical activity prevent accumulation of excess adipose tissue (3). Excess fat accumulation leads to adipocyte hypertrophy and adipose tissue dysfunction resulting in insulin resistance of adipocytes, macrophage recruitment, inflammation, and lipolysis with the generation of free fatty acids. Lipolysis could be reduced by improving adipocyte insulin sensitivity using agents such as pioglitazone or glucagon-like peptide 1 (GLP-1) agonists (4). Harnessing mitochondrial uncoupling for thermogenesis and energy expenditure by activating brown adipose tissue (that is, "browning" of white adipocytes) could reduce adipose tissue mass and decrease de novo lipogenesis (5). Moderate to vigorous exercise could reduce skeletal muscle insulin resistance and de novo lipogenesis resulting in decreased release of free fatty acids (6). Intestinal dysbiosis due to alterations in the gut microbiota could be improved using prebiotics and probiotics (7). In the liver, targets include reducing hepatocellular inflammation with omega-3 fatty acids or phosphodiesterase inhibitors, decreasing oxidative stress with vitamin E or cysteamine, and decreasing hepatic insulin resistance by activating FXRs with bile acids such as obeticholic acid (8).

fat on MRI (*83*). Initial results were recently presented from a multicenter phase 2B randomized controlled trial to examine the safety and efficacy of GFT505 for reversing NASH without worsening fibrosis over 52 weeks. After controlling for baseline severity, participants who received 120 mg daily of GFT505, but not those who received 80 mg daily, had a relative risk of 1.94 (CI, 1.08 to 3.48; $P = 0.027$) to meet the primary endpoint of NASH resolution (*84*).

GLP-1 analogs stimulate insulin secretion, reduce appetite, and are FDA-approved for the treatment of type 2 diabetes mellitus. They also have demonstrated efficacy for weight loss in several trials (*85*). A recent multisite, randomized placebo-controlled trial of the GLP-1 mimetic liraglutide was conducted among nondiabetic adults with either (i) BMI $\geq$27 kg/m^2 and hypertension and/or dyslipidemia or (ii) obesity. Administration of liraglutide at a high dose of 3.0 mg subcutaneously once daily for 56 weeks resulted in greater weight loss compared to placebo (*86*). The FDA recently approved liraglutide for weight loss in adults, making it the first GLP-1 agonist approved for weight loss. Initial randomized controlled trials of liraglutide at a dose of 1.8 mg, which is the dosing used for type 2 diabetes mellitus treatment, are underway for exploring its utility in the treatment of NASH.

Bile acids are well known for their role in dietary lipid absorption and cholesterol homeostasis, but their ability to increase insulin sensitivity, decrease hepatic gluconeogenesis, and reduce circulating triglycerides has led to a focus on their potential for NASH treatment. Bile acids serve as ligands for the membrane-bound TGR5 G protein–coupled bile acid receptor and activate nucleic acid receptors including FXR, the vitamin D receptor, and the liver X receptor (*87*). When bile acids activate FXR, they bind to its response elements in target gene promoter regions. FXR can act as a monomer or a heterodimer with retinoid X receptor, inducing expression of multiple genes (*88*). The downstream effects of FXR activation include decreased hepatic lipid synthesis and enhanced peripheral VLDL clearance leading to improved insulin sensitivity and decreased triglyceride concentrations (*88*). Activation of FXR also provides negative feedback on bile acid uptake and production through inhibition of CYP7a1. Inhibition of CYP7a1 blocks conversion of cholesterol to bile acids, which is a major mechanism for cholesterol disposal. FXR activation also decreases HDL cholesterol levels (*87*).

Obeticholic acid is a synthetic bile acid that has a potent agonist effect on FXR. The FLINT (Farnesoid X Receptor Ligand Obeticholic Acid in NASH Treatment) study was a phase 2B randomized placebo-controlled trial of obeticholic acid (25 mg/day) treatment for 72 weeks in 283 adults with NASH (*89*). The primary endpoint was improvement in the NAS of $\geq$2 points and no worsening in fibrosis. In the obeticholic acid group, 45% of recipients experienced improvement in NAS compared to 21% in the placebo group ($P < 0.001$). All of the histological features of NASH improved in the treatment group. However, no statistically significant difference in NASH resolution existed between the two groups. In the treatment group, small decreases in HDL cholesterol and increases in LDL and total cholesterol were noted, but the clinical significance of these augmented lipid levels is not known. Agonists of FXR remain a target of interest for liver disease treatment, and longer-duration trials may help to determine outcomes over longer treatment periods.

Oxidative stress contributes to hepatocellular injury; thus, boosting activation of pathways that protect against oxidative stress is a treatment strategy for NASH. The antioxidant vitamin E (D-α-tocopherol) was studied in arms of the PIVENS and TONIC (Treatment of Nonalcoholic Fatty Liver Disease in Children) trials. In the PIVENS trial of nondiabetic adults with NASH, the primary endpoint was improvement in histological findings with a decrease of at least two points in NAS and at least one-point improvement in hepatocyte ballooning, a histological sign of cell death. Vitamin E treatment (800 IU daily) for 96 weeks improved NASH on the basis of liver biopsy histology among adults, achieving the primary endpoint (*79*). In the TONIC trial, children with NAFLD were randomized to placebo, metformin, or vitamin E (800 IU daily). Here, too, vitamin E improved hepatocyte ballooning and resulted in NASH resolution (58%) compared to placebo (28%), although the primary outcome measure of sustained ALT reduction did not differ between treatment and control groups (*82*). In both the PIVENS and TONIC trials, vitamin E did not result in a substantial improvement in fibrosis. Together, these findings demonstrate that vitamin E effectively improves NASH on the basis of liver biopsy histology. The long-term effectiveness of these doses of vitamin E requires further evaluation.

Cysteamine bitartrate is another antioxidant under investigation for NAFLD treatment. Cysteamine bitartrate reduces cysteine accumulation in lysosomes and is FDA-approved for the treatment of cystinosis in children. By reacting with cystine to produce cysteine, cysteamine bitartrate can increase glutathione (*90*), thus exerting antioxidant and antiapoptotic effects (*91*, *92*). A pilot study of 13 children with biopsy-proven NAFLD and ALT ≥60 IU/l found that treatment with enteric-coated cysteamine bitartrate was associated with a ≥50% reduction in ALT from baseline to 24 weeks among 7 of the 11 children who completed the study. Cysteamine bitartrate use was associated with an increase in adiponectin and a decrease in leptin and cytokeratin 18 fragments. Children had no change in BMI (*93*). The results of the NASH Clinical Research Network's multisite, placebo-controlled CyNCH (Cysteamine Bitartrate Delayed-Release for the Treatment of NAFLD in Children) trial of extended-release cysteamine bitartrate in 169 pediatric patients with NAFLD are anticipated in 2016. The primary outcome of the CyNCH trial is improvement in NAS over 52 weeks, making it the first pediatric liver trial to use liver histology as the primary endpoint. Results reported at the 2015 Annual Meeting of the American Association for the Study of Liver Disease (AASLD) did not show a difference between treatment and placebo groups for primary outcome but did show improvements in serum ALT concentrations and lobular inflammation.

Alterations in intestinal microbial composition occur in animals and humans with obesity. Diet, genetics, weight loss, and use of antibiotics or other medications can alter the microbiome. In obesity, the intestinal microbiota show an increased proportion of Firmicutes and decreased Bacteroidetes. Intestinal dysbiosis and bacterial overgrowth may contribute to development of NAFLD and insulin resistance in a variety of ways (*94*, *95*). One mechanism may be through fermentation of otherwise indigestible carbohydrates to short-chain fatty acids (SCFAs). SCFAs provide energy to intestinal epithelial cells, are metabolized by the liver, and promote intestinal permeability and lipopolysaccharide (LPS) translocation into the systemic circulation. LPS stimulation of Toll-like receptor 4 generates inflammatory cytokines (for example, IL-6 and TNFα) that may increase hepatic inflammation and insulin resistance. A second mechanism may involve other metabolites of carbohydrate fermentation, including choline and butyrate. Low choline concentrations are associated with a decrease in Proteobacteria and hepatic steatosis. Butyrate stimulates leptin production by adipocytes and GLP-1 secretion. In a third possible mechanism, alterations in intestinal microbiota alter the bile acid pool. Thus, modulating the intestinal microbiota through the use of probiotics or prebiotics could alter bile acid signaling via FXR, TGR5, and other bile acid signaling pathways (*96*, *97*) (Fig. 2). In animal models, probiotics reduce ALT concentrations, improve insulin resistance, and decrease steatosis and fibrosis as observed in liver histology sections. Multiple randomized controlled trials show the potential for probiotics and prebiotics as therapeutic options for treating obesity, NASH, and insulin resistance, but long-term follow-up with histological evaluations is needed.

Inflammation may play a role in hepatocellular injury, and an increase in inflammatory cytokines such as IL-6 and TNFα exists in NAFLD (*98*). Omega-3 fatty acids and phosphodiesterase inhibitors that antagonize TNFα are two anti-inflammatory therapies that have been studied in NAFLD. Although animal models and human studies demonstrate promise for the ability of omega-3 fatty acids to reduce hepatic steatosis and improve ALT, triglyceride, and insulin resistance, insufficient long-term randomized controlled trials exist to recommend its use in NAFLD treatment (*99*–*101*). Pentoxifylline, a phosphodiesterase inhibitor, has been studied in randomized placebo-controlled trials in adults (*102*, *103*). In a trial of 55 adults with NASH, those receiving 400 mg of pentoxifylline three times daily for 1 year met the primary endpoint of a ≥2-point decrease in NAS (*103*). These improvements were related to decreased steatosis and lobular inflammation, but not decreased hepatocyte ballooning. TNFα concentrations did not differ between placebo and treatment groups. In a follow-up analysis, a decrease in oxidized lipid products was found, suggesting that the effects of pentoxifylline in this trial may have been mediated in part by decreased lipid oxidation rather than a decrease in TNFα (*104*).

Surgical interventions for NAFLD and NASH

Severely obese adults and adolescents who are unable to reduce weight with life-style changes and medical management may be candidates for bariatric surgery. In studies with outcomes beyond 2 years for laparoscopic adjustable gastric banding (LAGB), Roux-en-Y gastric bypass (RYGB), vertical-banded gastroplasty, sleeve gastrectomy, or other procedures, bariatric surgery reduced weight by 16 to 31% (*105*, *106*). Few studies have examined the impact of bariatric surgery on NAFLD, but there is some evidence for an improvement in NAFLD and NASH. The exact mechanism for improvement in liver disease is unknown, but weight loss alone may not account for improvement in NAFLD and NASH among patients who underwent bariatric surgery. For example, the Lille Bariatric Cohort is an ongoing, open cohort of adults undergoing bariatric surgery with liver biopsy histology results at the time of surgery, 1 year after surgery, and 5 years after surgery. In an analysis of 381 patients, prevalence of steatosis decreased from 34 to 16%, and probable NASH decreased from 27 to 14% at 5 years (*107*). In a later analysis of 1201 patients in the Lille Bariatric Cohort with baseline liver biopsies, 578 and 413 had biopsies at 1 and 5 years, respectively. At both time points, steatosis and NAS scores improved after surgery, but patients who underwent RYGB had greater weight loss and greater improvement in steatosis and NAS scores than those who underwent LAGB (*108*). Among 109 patients with NASH at baseline, NASH resolved in 85% of patients 1 year after surgery. Persistent NASH occurred more often in LAGB than in RYGB patients (30.4% versus 7.6%, $P = 0.015$) at 1 year, and these differences were accounted for in part by greater weight loss in RYGB patients (*109*). Bariatric surgery is postulated

to reduce weight and improve metabolism through restrictive and malabsorptive effects. Additionally, lower hunger from decreased ghrelin secretion, enhanced satiety through increased GLP-1 and peptide tyrosine-tyrosine secretion, and alterations in the intestinal microbiota may all contribute to weight loss, particularly after RYGB (*110*). Although these initial data for the effects of bariatric surgery on NASH are promising, prospective studies in diverse populations, including pediatrics, are lacking.

NAFLD AND OBESITY: FUTURE PERSPECTIVES

Obesity prevalence among adults and children remains at historically high rates. Given the high prevalence of obesity and its multisystem complications, the ability to identify individual-level risk for progressive liver, metabolic, and cardiovascular complications would enable tailored diagnostic evaluation and therapeutic intervention. Currently, liver biopsy with histological examination of liver tissue is the reference standard for diagnosing and staging NAFLD, NASH, and liver fibrosis. Given the large proportion of individuals with obesity who are at risk for developing NAFLD and NASH, taking single or serial liver biopsies from at-risk individuals is not practical. In addition, there are potential complications, sampling errors, costs, and variability associated with liver biopsies. Thus, noninvasive strategies that can monitor serial changes in features of NAFLD would improve clinical care and enable elucidation of the pathogenesis, natural history, and epidemiology of NAFLD, NASH, and fibrosis. Noninvasive measurements over time of hepatic steatosis, steatohepatitis, and fibrosis also could help to untangle the mechanisms by which the various phenotypes of NAFLD correlate with obesity, metabolic disease, and cardiovascular complications. Imaging modalities such as ultrasound or MRI ultimately may be able to quantify hepatic steatosis and fibrosis; however, noninvasive detection of NASH remains elusive. Among patients with NAFLD, differentiating between those with and without NASH is particularly important because patients with NASH are more likely to progress to liver disease and obesity complications.

The need for noninvasive methods for detection of NASH has boosted interest in biomarker discovery. MicroRNAs (miRNAs) are small noncoding RNAs that play a role in gene expression via degradation or translational inhibition of mRNA and have emerged as potential diagnostic and therapeutic targets in NASH. This focus on miRNAs is due to their role in a variety of physiological processes related to NASH, as well as their ability to be readily modified and measured in serum. In animal models and humans, miRNAs have been implicated in the regulation of inflammation, cellular proliferation, hepatocyte apoptosis, and liver fibrosis (*111*). Some miRNAs are emerging as potential candidates for future diagnostic tools and therapeutic interventions, but research is still in the earliest stages. For example, miR-122 regulates genes involved in hepatic cholesterol and lipid metabolism. In both animal models of NASH and human patients, a reduction in miR-122 has been implicated in development of steatohepatitis. Mice lacking miR-122 developed steatohepatitis and hepatocellular carcinoma despite lower cholesterol, LDL, and serum triglycerides compared to wild-type mice (*112*, *113*). In humans, a cross-sectional case-control study found detectable differences in the hepatic expression of miRNAs using miRNA microarray assay, with lower miR-122 expression in patients with NASH and metabolic disease compared to patients with metabolic disease alone (*114*).

miRNAs are being studied as potential therapeutic targets in preclinical studies. Both rodent models and humans with NASH showed increased expression of miR-21 within biliary and inflammatory cells (*115*). Inhibition of miR-21 decreased liver injury, inflammation, and fibrosis in rodent models of NASH by restoring PPARα expression, which had apparently been suppressed by miR-21. Advances in miRNA research could yield opportunities for understanding the various links among obesity, cardiometabolic disease, and NAFLD and could help to identify new diagnostic and intervention approaches.

Harnessing mitochondrial uncoupling to dissipate energy through increased uncoupling or "browning" of adipocytes holds tremendous interest as a potential therapy for NAFLD and obesity. For example, the mitochondrial protonophore 2,4-dinitrophenol was used in the 1930s for weight loss but was pulled by the FDA after reports of deaths in individuals using this drug. However, recent preclinical studies of a controlled-release mitochondrial pronotophore, a form of 2,4-dinitrophenol altered to target hepatic mitochondrial uncoupling with lower peak plasma concentrations and sustained-release pharmacokinetics, have renewed interest in mitochondrial protonophores as a potential pharmacological agent (*116*). In these studies, administration of a controlled-release mitochondrial pronotophore reduced triglycerides, reduced hepatic and skeletal muscle insulin resistance, and improved hepatic steatosis without systemic toxicity in rat models of NAFLD and insulin resistance. In rat models of NASH, the controlled-release mitochondrial pronotophore reduced liver fibrosis. Whether targeting mitochondrial uncoupling will prove fruitful in human studies as it has in rodent models remains an unanswered question and will require additional safety and efficacy testing to determine its clinical utility.

Given the recent advances in diagnostic and treatment strategies for NAFLD and NASH, the near future should see approval for noninvasive measures of steatosis and fibrosis as well as several therapeutics for treating NASH. Ultimately, genomic, proteomic, and metabolomic profiling of patients should enable personalized approaches to therapy. For example, a patient with a normal BMI but a known *PNPLA3* risk variant may be started on therapy to prevent adverse liver and cardiometabolic outcomes, whereas a patient with obesity and the *TM6SF2* risk variant may be counseled on his or her heightened risk of NAFLD but lower risk of cardiovascular disease. In the future, the decision to start vitamin E therapy in a patient with NASH might be informed by metabolomics profiling, and the patient might be dosed on the basis of his or her cytochrome P450 polymorphism profile (*117*, *118*). In addition to improving diet and physical activity, pharmacological therapy with one or more agents ultimately will be tailored to the patient's genotype and phenotype. Further identification and understanding of mechanisms that correlate obesity to NAFLD and NASH will inform specific approaches for personalized medicine.

REFERENCES AND NOTES

1. R. J. Wong, R. Cheung, A. Ahmed, Nonalcoholic steatohepatitis is the most rapidly growing indication for liver transplantation in patients with hepatocellular carcinoma in the U.S. *Hepatology* **59**, 2188–2195 (2014).
2. R. Loomba, A. J. Sanyal, The global NAFLD epidemic. *Nat. Rev. Gastroenterol. Hepatol.* **10**, 686–690 (2013).
3. M. E. Rinella, Nonalcoholic fatty liver disease: A systematic review. *JAMA* **313**, 2263–2273 (2015).
4. S. K. Satapathy, A. J. Sanyal, Epidemiology and natural history of nonalcoholic fatty liver disease. *Semin. Liver Dis.* **35**, 221–235 (2015).

5. J. B. Schwimmer, R. Deutsch, T. Kahen, J. E. Lavine, C. Stanley, C. Behling, Prevalence of fatty liver in children and adolescents. *Pediatrics* **118**, 1388–1393 (2006).
6. N. Chalasani, Z. Younossi, J. E. Lavine, A. M. Diehl, E. M. Brunt, K. Cusi, M. Charlton, A. J. Sanyal, The diagnosis and management of non-alcoholic fatty liver disease: Practice guideline by the American Gastroenterological Association, American Association for the Study of Liver Diseases, and American College of Gastroenterology. *Gastroenterology* **142**, 1592–1609 (2012).
7. S. McPherson, T. Hardy, E. Henderson, A. D. Burt, C. P. Day, Q. M. Anstee, Evidence of NAFLD progression from steatosis to fibrosing-steatohepatitis using paired biopsies: Implications for prognosis and clinical management. *J. Hepatol.* **62**, 1148–1155 (2015).
8. C. P. Day, O. F. W. James, Steatohepatitis: A tale of two "hits"? *Gastroenterology* **114**, 842–845 (1998).
9. H. Thaler, Relation of steatosis to cirrhosis. *Clin. Gastroenterol.* **4**, 273–280 (1975).
10. B. A. Neuschwander-Tetri, Hepatic lipotoxicity and the pathogenesis of nonalcoholic steatohepatitis: The central role of nontriglyceride fatty acid metabolites. *Hepatology* **52**, 774–788 (2010).
11. Z. M. Younossi, M. Stepanova, F. Negro, S. Hallaji, Y. Younossi, B. Lam, M. Srishord, Nonalcoholic fatty liver disease in lean individuals in the United States. *Medicine* **91**, 319–327 (2012).
12. Y.-M. Kwon, S.-W. Oh, S.-s. Hwang, C. Lee, H. Kwon, G. E. Chung, Association of nonalcoholic fatty liver disease with components of metabolic syndrome according to body mass index in Korean adults. *Am. J. Gastroenterol.* **107**, 1852–1858 (2012).
13. D. O. Okorodudu, M. F. Jumean, V. M. Montori, A. Romero-Corral, V. K. Somers, P. J. Erwin, F. Lopez-Jimenez, Diagnostic performance of body mass index to identify obesity as defined by body adiposity: A systematic review and meta-analysis. *Int. J. Obes.* **34**, 791–799 (2010).
14. D. van der Poorten, K.-L. Milner, J. Hui, A. Hodge, M. I. Trenell, J. G. Kench, R. London, T. Peduto, D. J. Chisholm, J. George, Visceral fat: A key mediator of steatohepatitis in metabolic liver disease. *Hepatology* **48**, 449–457 (2008).
15. E. D. Rosen, B. M. Spiegelman, What we talk about when we talk about fat. *Cell* **156**, 20–44 (2014).
16. F. Badoud, M. Perreault, M. A. Zulyniak, D. M. Mutch, Molecular insights into the role of white adipose tissue in metabolically unhealthy normal weight and metabolically healthy obese individuals. *FASEB J.* **29**, 748–758 (2015).
17. G. Sabio, M. Das, A. Mora, Z. Zhang, J. Y. Jun, H. J. Ko, T. Barrett, J. K. Kim, R. J. Davis, A stress signaling pathway in adipose tissue regulates hepatic insulin resistance. *Science* **322**, 1539–1543 (2008).
18. R. Stinkens, G. H. Goossens, J. W. E. Jocken, E. E. Blaak, Targeting fatty acid metabolism to improve glucose metabolism. *Obes. Rev.* **16**, 715–757 (2015).
19. W. D. van Marken Lichtenbelt, J. W. Vanhommerig, N. M. Smulders, J. M. A. F. L. Drossaerts, G. J. Kemerink, N. D. Bouvy, P. Schrauwen, G. J. Jaap Teule, Cold-activated brown adipose tissue in healthy men. *N. Engl. J. Med.* **360**, 1500–1508 (2009).
20. K. A. Virtanen, M. E. Lidell, J. Orava, M. Heglind, R. Westergren, T. Niemi, M. Taittonen, J. Laine, N.-J. Savisto, S. Enerbäck, P. Nuutila, Functional brown adipose tissue in healthy adults. *N. Engl. J. Med.* **360**, 1518–1525 (2009).
21. J. Wu, P. Boström, L. M. Sparks, L. Ye, J. H. Choi, A.-H. Giang, M. Khandekar, K. A. Virtanen, P. Nuutila, G. Schaart, K. Huang, H. Tu, W. D. van Marken Lichtenbelt, J. Hoeks, S. Enerbäck, P. Schrauwen, B. M. Spiegelman, Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. *Cell* **150**, 366–376 (2012).
22. M. Claussnitzer, S. N. Dankel, K.-H. Kim, G. Quon, W. Meuleman, C. Haugen, V. Glunk, I. S. Sousa, J. L. Beaudry, V. Puviindran, N. A. Abdennur, J. Liu, P.-A. Svensson, Y.-H. Hsu, D. J. Drucker, G. Mellgren, C.-C. Hui, H. Hauner, M. Kellis, *FTO* obesity variant circuitry and adipocyte browning in humans. *N. Engl. J. Med.* **373**, 895–907 (2015).
23. A. Bartelt, J. Heeren, Adipose tissue browning and metabolic health. *Nat. Rev. Endocrinol.* **10**, 24–36 (2014).
24. B. Angelin, T. E. Larsson, M. Rudling, Circulating fibroblast growth factors as metabolic regulators—A critical appraisal. *Cell Metab.* **16**, 693–705 (2012).
25. L. Berti, M. Irmler, M. Zdichavsky, T. Meile, A. Böhm, N. Stefan, A. Fritsche, J. Beckers, A. Königsrainer, H.-U. Häring, M. H. de Angelis, H. Staiger, Fibroblast growth factor 21 is elevated in metabolically unhealthy obesity and affects lipid deposition, adipogenesis, and adipokine secretion of human abdominal subcutaneous adipocytes. *Mol. Metab.* **4**, 519–527 (2015).
26. G. S. Hotamisligil, Endoplasmic reticulum stress and the inflammatory basis of metabolic disease. *Cell* **140**, 900–917 (2010).
27. C. Koliaki, M. Roden, Hepatic energy metabolism in human diabetes mellitus, obesity and non-alcoholic fatty liver disease. *Mol. Cell. Endocrinol.* **379**, 35–42 (2013).
28. R. Loomba, N. Schork, C.-H. Chen, R. Bettencourt, A. Bhatt, B. Ang, P. Nguyen, C. Hernandez, L. Richards, J. Salotti, S. Lin, E. Seki, K. E. Nelson, C. B. Sirlin, D. Brenner; Genetics of NAFLD in Twins Consortium, Heritability of hepatic fibrosis and steatosis based on a prospective twin study. *Gastroenterology* **149**, 1784–1793 (2015).
29. K. L. Wood, M. H. Miller, J. F. Dillon, Systematic review of genetic association studies involving histologically confirmed non-alcoholic fatty liver disease. *BMJ Open Gastroenterol.* **2**, e000019 (2015).
30. S. Romeo, J. Kozlitina, C. Xing, A. Pertsemlidis, D. Cox, L. A. Pennacchio, E. Boerwinkle, J. C. Cohen, H. H. Hobbs, Genetic variation in *PNPLA3* confers susceptibility to nonalcoholic fatty liver disease. *Nat. Genet.* **40**, 1461–1465 (2008).
31. K. Kantartzis, A. Peter, F. Machicao, J. Machann, S. Wagner, I. Konigsrainer, A. Königsrainer, F. Schick, A. Fritsche, H.-U. Haring, N. Stefan, Dissociation between fatty liver and insulin resistance in humans carrying a variant of the patatin-like phospholipase 3 gene. *Diabetes* **58**, 2616–2623 (2009).
32. A. Kotronen, L. E. Johansson, L. M. Johansson, C. Roos, J. Westerbacka, A. Hamsten, R. Bergholm, P. Arkkila, J. Arola, T. Kiviluoto, R. M. Fisher, E. Ehrenborg, M. Orho-Melander, M. Ridderstråle, L. Groop, H. Yki-Järvinen, A common variant in *PNPLA3*, which encodes adiponutrin, is associated with liver fat content in humans. *Diabetologia* **52**, 1056–1060 (2009).
33. E. K. Speliotes, J. L. Butler, C. D. Palmer, B. F. Voight; GIANT Consortium, MIGen Consortium, NASH CRN, J. N. Hirschhorn, *PNPLA3* variants specifically confer increased risk for histologic nonalcoholic fatty liver disease but not metabolic disease. *Hepatology* **52**, 904–912 (2010).
34. F. Stickel, S. Buch, K. Lau, H. Meyer zu Schwabedissen, T. Berg, M. Ridinger, M. Rietschel, C. Schafmayer, F. Braun, H. Hinrichsen, R. Günther, A. Arlt, M. Seeger, S. Müller, H. K. Seitz, M. Soyka, M. Lerch, F. Lammert, C. Sarrazin, R. Kubitz, D. Häussinger, C. Hellerbrand, D. Bröring, S. Schreiber, F. Kiefer, R. Spanagel, K. Mann, C. Datz, M. Krawczak, N. Wodarz, H. Völzke, J. Hampe, Genetic variation in the *PNPLA3* gene is associated with alcoholic liver injury in caucasians. *Hepatology* **53**, 86–95 (2011).
35. J. N. Davis, K.-A. Lê, R. W. Walker, S. Vikman, D. Spruijt-Metz, M. J. Weigensberg, H. Allayee, M. I. Goran, Increased hepatic fat in overweight Hispanic youth influenced by interaction between genetic variation in *PNPLA3* and high dietary carbohydrate and sugar consumption. *Am. J. Clin. Nutr.* **92**, 1522–1527 (2010).
36. M. B. Vos, J. E. Kimmons, C. Gillespie, J. Welsh, H. M. Blanck, Dietary fructose consumption among US children and adults: The Third National Health and Nutrition Examination Survey. *Medscape J. Med.* **10**, 160 (2008).
37. S. He, C. McPhaul, J. Z. Li, R. Garuti, L. Kinch, N. V. Grishin, J. C. Cohen, H. H. Hobbs, A sequence variation (I148M) in PNPLA3 associated with nonalcoholic fatty liver disease disrupts triglyceride hydrolysis. *J. Biol. Chem.* **285**, 6706–6715 (2010).
38. M. Kumari, G. Schoiswohl, C. Chitraju, M. Paar, I. Cornaciu, A. Y. Rangrez, N. Wongsiriroj, H. M. Nagy, P. T. Ivanova, S. A. Scott, O. Knittelfelder, G. N. Rechberger, R. Birner-Gruenberger, S. Eder, H. A. Brown, G. Haemmerle, M. Oberer, A. Lass, E. E. Kershaw, R. Zimmermann, R. Zechner, Adiponutrin functions as a nutritionally regulated lysophosphatidic acid acyltransferase. *Cell Metab.* **15**, 691–702 (2012).
39. J. Hyysalo, P. Gopalacharyulu, H. Bian, T. Hyötyläinen, M. Leivonen, N. Jaser, A. Juuti, M.-J. Honka, P. Nuutila, V. M. Olkkonen, M. Oresic, H. Yki-Järvinen, Circulating triacylglycerol signatures in nonalcoholic fatty liver disease associated with the I148M variant in PNPLA3 and with obesity. *Diabetes* **63**, 312–322 (2014).
40. J. Kozlitina, E. Smagris, S. Stender, B. G. Nordestgaard, H. H. Zhou, A. Tybjærg-Hansen, T. F. Vogt, H. H. Hobbs, J. C. Cohen, Exome-wide association study identifies a *TM6SF2* variant that confers susceptibility to nonalcoholic fatty liver disease. *Nat. Genet.* **46**, 352–356 (2014).
41. P. Dongiovanni, S. Petta, C. Maglio, A. L. Fracanzani, R. Pipitone, E. Mozzi, B. M. Motta, D. Kaminska, R. Rametta, S. Grimaudo, S. Pelusi, T. Montalcini, A. Alisi, M. Maggioni, V. Kärjä, J. Borén, P. Käkelä, V. Di Marco, C. Xing, V. Nobili, B. Dallapiccola, A. Craxi, J. Pihlajamäki, S. Fargion, L. Sjöström, L. M. Carlsson, S. Romeo, L. Valenti, Transmembrane 6 superfamily member 2 gene variant disentangles nonalcoholic steatohepatitis from cardiovascular disease. *Hepatology* **61**, 506–514 (2015).
42. O. L. Holmen, H. Zhang, Y. Fan, D. H. Hovelson, E. M. Schmidt, W. Zhou, Y. Guo, J. Zhang, A. Langhammer, M.-L. Løchen, S. K. Ganesh, L. Vatten, F. Skorpen, H. Dalen, J. Zhang, S. Pennathur, J. Chen, C. Platou, E. B. Mathiesen, T. Wilsgaard, I. Njølstad, M. Boehnke, Y. E. Chen, G. R. Abecasis, K. Hveem, C. J. Willer, Systematic evaluation of coding variation identifies a candidate causal variant in *TM6SF2* influencing total cholesterol and myocardial infarction risk. *Nat. Genet.* **46**, 345–351 (2014).
43. S. Singh, A. M. Allen, Z. Wang, L. J. Prokop, M. H. Murad, R. Loomba, Fibrosis progression in nonalcoholic fatty liver vs nonalcoholic steatohepatitis: A systematic review and meta-analysis of paired-biopsy studies. *Clin. Gastroenterol. Hepatol.* **13**, 643–654.e1-9 (2015).
44. A. Arulanandan, B. Ang, R. Bettencourt, J. Hooker, C. Behling, G. Y. Lin, M. A. Valasek, J. H. Ix, B. Schnabl, C. B. Sirlin, R. Loomba, Association between quantity of liver fat and cardiovascular risk in patients with nonalcoholic fatty liver disease independent of nonalcoholic steatohepatitis. *Clin. Gastroenterol. Hepatol.* **13**, 1513–1520.e1 (2015).
45. R. Jin, N.-A. Le, R. Cleeton, X. Sun, J. Cruz Muños, J. Otvos, M. B. Vos, Amount of hepatic fat predicts cardiovascular risk independent of insulin resistance among Hispanic-American adolescents. *Lipids Health Dis.* **14**, 39 (2015).
46. Y. Chang, H.-S. Jung, K. E. Yun, J. Cho, Y. K. Cho, S. Ryu, Cohort study of non-alcoholic fatty liver disease, NAFLD fibrosis score, and the risk of incident diabetes in a Korean population. *Am. J. Gastroenterol.* **108**, 1861–1868 (2013).
47. D. E. Kleiner, E. M. Brunt, M. Van Natta, C. Behling, M. J. Contos, O. W. Cummings, L. D. Ferrell, Y.-C. Liu, M. S. Torbenson, A. Unalp-Arida, M. Yeh, A. J. McCullough, A. J. Sanyal, Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* **41**, 1313–1321 (2005).

48. V. Ratziu, E. Bugianesi, J. Dixon, E. Fassio, M. Ekstedt, F. Charlotte, S. Kechagias, T. Poynard, R. Olsson, Histological progression of non-alcoholic fatty liver disease: A critical reassessment based on liver sampling variability. *Aliment. Pharmacol. Ther.* **26**, 821–830 (2007).
49. P. Mofrad, M. J. Contos, M. Haque, C. Sargeant, R. A. Fisher, V. A. Luketic, R. K. Sterling, M. L. Shiffman, R. T. Stravitz, A. J. Sanyal, Clinical and histologic spectrum of nonalcoholic fatty liver disease associated with normal ALT values. *Hepatology* **37**, 1286–1292 (2003).
50. W.-C. Wu, C.-Y. Wu, Y.-J. Wang, H.-H. Hung, H.-I. Yang, W.-Y. Kao, C.-W. Su, J.-C. Wu, W.-L. Chan, H.-C. Lin, F.-Y. Lee, S.-D. Lee, Updated thresholds for serum alanine aminotransferase level in a large-scale population study composed of 34 346 subjects. *Aliment. Pharmacol. Ther.* **36**, 560–568 (2012).
51. J. B. Schwimmer, W. Dunn, G. J. Norman, P. E. Pardee, M. S. Middleton, N. Kerkar, C. B. Sirlin, SAFETY study: Alanine aminotransferase cutoff values are set too high for reliable detection of pediatric chronic liver disease. *Gastroenterology* **138**, 1357–1364.e1-2 (2010).
52. A. Shannon, N. Alkhouri, C. Carter-Kent, L. Monti, R. Devito, R. Lopez, A. E. Feldstein, V. Nobili, Ultrasonographic quantitative estimation of hepatic steatosis in children with NAFLD. *J. Pediatr. Gastroenterol. Nutr.* **53**, 190–195 (2011).
53. S. Saadeh, Z. M. Younossi, E. M. Remer, T. Gramlich, J. P. Ong, M. Hurley, K. D. Mullen, J. N. Cooper, M. J. Sheridan, The utility of radiological imaging in nonalcoholic fatty liver disease. *Gastroenterology* **123**, 745–750 (2002).
54. S. Dasarathy, J. Dasarathy, A. Khiyami, R. Joseph, R. Lopez, A. J. McCullough, Validity of real time ultrasound in the diagnosis of hepatic steatosis: A prospective study. *J. Hepatol.* **51**, 1061–1067 (2009).
55. S. C. Lin, E. Heba, T. Wolfson, B. Ang, A. Gamst, A. Han, J. W. Erdman Jr., W. D. O'Brien Jr., M. P. Andre, C. B. Sirlin, R. Loomba, Noninvasive diagnosis of nonalcoholic fatty liver disease and quantification of liver fat using a new quantitative ultrasound technique. *Clin. Gastroenterol. Hepatol.* **13**, 1337–1345.e6 (2015).
56. M. Sasso, V. Miette, L. Sandrin, M. Beaugrand, The controlled attenuation parameter (CAP): A novel tool for the non-invasive evaluation of steatosis using Fibroscan. *Clin. Res. Hepatol. Gastroenterol.* **36**, 13–20 (2012).
57. M. Sasso, M. Beaugrand, V. de Ledinghen, C. Douvin, P. Marcellin, R. Poupon, L. Sandrin, V. Miette, Controlled attenuation parameter (CAP): A novel VCTE™ guided ultrasonic attenuation measurement for the evaluation of hepatic steatosis: Preliminary study and validation in a cohort of patients with chronic liver disease from various causes. *Ultrasound Med. Biol.* **36**, 1825–1835 (2010).
58. A. Tang, J. Tan, M. Sun, G. Hamilton, M. Bydder, T. Wolfson, A. C. Gamst, M. Middleton, E. M. Brunt, R. Loomba, J. E. Lavine, J. B. Schwimmer, C. B. Sirlin, Nonalcoholic fatty liver disease: MR imaging of liver proton density fat fraction to assess hepatic steatosis. *Radiology* **267**, 422–431 (2013).
59. J. B. Schwimmer, M. S. Middleton, C. Behling, K. P. Newton, H. I. Awai, M. N. Paiz, J. Lam, J. C. Hooker, G. Hamilton, J. Fontanesi, C. B. Sirlin, Magnetic resonance imaging and liver histology as biomarkers of hepatic steatosis in children with nonalcoholic fatty liver disease. *Hepatology* **61**, 1887–1895 (2015).
60. A. Wieckowska, N. N. Zein, L. M. Yerian, A. R. Lopez, A. J. McCullough, A. E. Feldstein, In vivo assessment of liver cell apoptosis as a novel biomarker of disease severity in non-alcoholic fatty liver disease. *Hepatology* **44**, 27–33 (2006).
61. A. E. Feldstein, A. Wieckowska, A. R. Lopez, Y.-C. Liu, N. N. Zein, A. J. McCullough, Cytokeratin-18 fragment levels as noninvasive biomarkers for nonalcoholic steatohepatitis: A multicenter validation study. *Hepatology* **50**, 1072–1078 (2009).
62. T. I. Abu-Rajab Tamimi, H. M. Elgouhari, N. Alkhouri, L. M. Yerian, M. P. Berk, R. Lopez, P. R. Schauer, N. N. Zein, A. E. Feldstein, An apoptosis panel for nonalcoholic steatohepatitis diagnosis. *J. Hepatol.* **54**, 1224–1229 (2011).
63. J. Shen, H. L.-Y. Chan, G. L.-H. Wong, P. C.-L. Choi, A. W.-H. Chan, H.-Y. Chan, A. M.-L. Chim, D. K.-W. Yeung, F. K.-L. Chan, J. Woo, J. Yu, W. C.-W. Chu, V. W.-S. Wong, Non-invasive diagnosis of non-alcoholic steatohepatitis by combined serum biomarkers. *J. Hepatol.* **56**, 1363–1370 (2012).
64. K. Cusi, Z. Chang, S. Harrison, R. Lomonaco, F. Bril, B. Orsak, C. Ortiz-Lopez, J. Hecht, A. E. Feldstein, A. Webb, C. Louden, M. Goros, F. Tio, Limited value of plasma cytokeratin-18 as a biomarker for NASH and fibrosis in patients with non-alcoholic fatty liver disease. *J. Hepatol.* **60**, 167–174 (2014).
65. R. Vuppalanchi, A. K. Jain, R. Deppe, K. Yates, M. Comerford, H. C. Masuoka, B. A. Neuschwander-Tetri, R. Loomba, E. M. Brunt, D. E. Kleiner, J. P. Molleston, J. B. Schwimmer, J. E. Lavine, J. Tonascia, N. Chalasani, 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.* **12**, 2121–2130.e1–2 (2014).
66. N. Bastati, D. Feier, A. Wibmer, S. Traussnigg, C. Balassy, D. Tamandl, H. Einspieler, F. Wrba, M. Trauner, C. Herold, A. Ba-Ssalamah, Noninvasive differentiation of simple steatosis and steatohepatitis by using gadoxetic acid–enhanced MR imaging in patients with non-alcoholic fatty liver disease: A proof-of-concept study. *Radiology* **271**, 739–747 (2014).
67. P. Angulo, D. E. Kleiner, S. Dam-Larsen, L. A. Adams, E. S. Bjornsson, P. Charatcharoenwitthaya, P. R. Mills, J. C. Keach, H. D. Lafferty, A. Stahler, S. Haflidadottir, F. Bendtsen, Liver fibrosis, but no other histologic features, is associated with long-term outcomes of patients with non-alcoholic fatty liver disease. *Gastroenterology* **149**, 389–397.e10 (2015).
68. J. Cui, B. Ang, W. Haufe, C. Hernandez, E. C. Verna, C. B. Sirlin, R. Loomba, Comparative diagnostic accuracy of magnetic resonance elastography vs. eight clinical prediction rules for non-invasive diagnosis of advanced fibrosis in biopsy-proven non-alcoholic fatty liver disease: A prospective study. *Aliment. Pharmacol. Ther.* **41**, 1271–1280 (2015).
69. L. Sandrin, B. Fourquet, J.-M. Hasquenoph, S. Yon, C. Fournier, F. Mal, C. Christidis, M. Ziol, B. Poulet, F. Kazemi, M. Beaugrand, R. Palau, Transient elastography: A new noninvasive method for assessment of hepatic fibrosis. *Ultrasound Med. Biol.* **29**, 1705–1713 (2003).
70. R. Kwok, Y.-K. Tse, G. L.-H. Wong, Y. Ha, A. U. Lee, M. C. Ngu, H. L.-Y. Chan, V. W.-S. Wong, Systematic review with meta-analysis: Non-invasive assessment of non-alcoholic fatty liver disease–the role of transient elastography and plasma cytokeratin-18 fragments. *Aliment. Pharmacol. Ther.* **39**, 254–269 (2014).
71. L. Castéra, J. Foucher, P.-H. Bernard, F. Carvalho, D. Allaix, W. Merrouche, P. Couzigou, V. de Lédinghen, Pitfalls of liver stiffness measurement: A 5-year prospective study of 13,369 examinations. *Hepatology* **51**, 828–835 (2010).
72. E. Vilar-Gomez, Y. Martinez-Perez, L. Calzadilla-Bertot, A. Torres-Gonzalez, B. Gra-Oramas, L. Gonzalez-Fabian, S. L. Friedman, M. Diago, M. Romero-Gomez, Weight loss through lifestyle modification significantly reduces features of nonalcoholic steatohepatitis. *Gastroenterology* **149**, 367–378.e5 (2015).
73. K. Promrat, D. E. Kleiner, H. M. Niemeier, E. Jackvony, M. Kearns, J. R. Wands, J. L. Fava, R. R. Wing, Randomized controlled trial testing the effects of weight loss on nonalcoholic steatohepatitis. *Hepatology* **51**, 121–129 (2010).
74. M. B. Vos, R. Colvin, P. Belt, J. P. Molleston, K. F. Murray, P. Rosenthal, J. B. Schwimmer, J. Tonascia, A. Unalp, J. E. Lavine; NASH CRN Research Group, Correlation of vitamin E, uric acid, and diet composition with histologic features of pediatric NAFLD. *J. Pediatr. Gastroenterol. Nutr.* **54**, 90–96 (2012).
75. G. Perseghin, T. B. Price, K. F. Petersen, M. Roden, G. W. Cline, K. Gerow, D. L. Rothman, G. I. Shulman, Increased glucose transport–phosphorylation and muscle glycogen synthesis after exercise training in insulin-resistant subjects. *N. Engl. J. Med.* **335**, 1357–1362 (1996).
76. R. Rabøl, K. F. Petersen, S. Dufour, C. Flannery, G. I. Shulman, Reversal of muscle insulin resistance with exercise reduces postprandial hepatic de novo lipogenesis in insulin resistant individuals. *Proc. Natl. Acad. Sci. U.S.A.* **108**, 13705–13709 (2011).
77. A. Berzigotti, U. Saran, J.-F. Dufour, Physical activity and liver diseases. *Hepatology*, 10.1002/hep.28132 (2015).
78. V. Ratziu, Z. Goodman, A. Sanyal, Current efforts and trends in the treatment of NASH. *J. Hepatol.* **62**, S65–S75 (2015).
79. A. J. Sanyal, N. Chalasani, K. V. Kowdley, A. McCullough, A. M. Diehl, N. M. Bass, B. A. Neuschwander-Tetri, J. E. Lavine, J. Tonascia, A. Unalp, M. Van Natta, J. Clark, E. M. Brunt, D. E. Kleiner, J. H. Hoofnagle, P. R. Robuck; NASH CRN, Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. *N. Engl. J. Med.* **362**, 1675–1685 (2010).
80. E. B. Mitchel, J. E. Lavine, Review article: The management of paediatric nonalcoholic fatty liver disease. *Aliment. Pharmacol. Ther.* **40**, 1155–1170 (2014).
81. M. O. Rakoski, A. G. Singal, M. A. M. Rogers, H. Conjeevaram, Meta-analysis: Insulin sensitizers for the treatment of non-alcoholic steatohepatitis. *Aliment. Pharmacol. Ther.* **32**, 1211–1221 (2010).
82. J. E. Lavine, J. B. Schwimmer, M. L. Van Natta, J. P. Molleston, K. F. Murray, P. Rosenthal, S. H. Abrams, A. O. Scheimann, A. J. Sanyal, N. Chalasani, J. Tonascia, A. Ünalp, J. M. Clark, E. M. Brunt, D. E. Kleiner, J. H. Hoofnagle, P. R. Robuck; Nonalcoholic Steatohepatitis Clinical Research Network, Effect of vitamin E or metformin for treatment of nonalcoholic fatty liver disease in children and adolescents: The TONIC randomized controlled trial. *JAMA* **305**, 1659–1668 (2011).
83. U. Risérus, D. Sprecher, T. Johnson, E. Olson, S. Hirschberg, A. Liu, Z. Fang, P. Hegde, D. Richards, L. Sarov-Blat, J. C. Strum, S. Basu, J. Cheeseman, B. A. Fielding, S. M. Humphreys, T. Danoff, N. R. Moore, P. Murgatroyd, S. O'Rahilly, P. Sutton, T. Willson, D. Hassall, K. N. Frayn, F. Karpe, Activation of peroxisome proliferator-activated receptor (PPAR)δ promotes reversal of multiple metabolic abnormalities, reduces oxidative stress, and increases fatty acid oxidation in moderately obese men. *Diabetes* **57**, 332–339 (2008).
84. V. Ratziu, S. A. Harrison, S. M. Francque, P. Bedossa, L. Serfaty, M. Romero-Gomez, P. Cales, M. F. Abdelmalek, S. H. Caldwell, J. Drenth, Q. M. Anstee, D. W. Hum, R. Hanf, A. Roudot, S. Megnien, B. Staels, A. Sanyal, An international, phase 2 randomized controlled trial of the dual PPAR α-δ agonist GFT505 in adult patients with NASH, The 66th Annual Meeting of the American Association for the Study of Liver Diseases: The Liver Meeting, San Francisco, CA, 16 November 2015.
85. T. Vilsbøll, M. Christensen, A. E. Junker, F. K. Knop, L. L. Gluud, Effects of glucagon-like peptide-1 receptor agonists on weight loss: Systematic review and meta-analyses of randomised controlled trials. *BMJ* **344**, d7771 (2012).
86. X. Pi-Sunyer, A. Astrup, K. Fujioka, F. Greenway, A. Halpern, M. Krempf, D. C. W. Lau, C. W. le Roux, R. Violante Ortiz, C. B. Jensen, J. P. H. Wilding; SCALE Obesity and Prediabetes NN8022-1839 Study Group, A randomized, controlled trial of 3.0 mg of liraglutide in weight management. *N. Engl. J. Med.* **373**, 11–22 (2015).
87. G. Porez, J. Prawitt, B. Gross, B. Staels, Bile acid receptors as targets for the treatment of dyslipidemia and cardiovascular disease. *J. Lipid Res.* **53**, 1723–1737 (2012).

88. A. C. Calkin, P. Tontonoz, Transcriptional integration of metabolism by the nuclear sterol-activated receptors LXR and FXR. *Nat. Rev. Mol. Cell Biol.* **13**, 213–224 (2012).
89. B. A. Neuschwander-Tetri, R. Loomba, A. J. Sanyal, J. E. Lavine, M. L. Van Natta, M. F. Abdelmalek, N. Chalasani, S. Dasarathy, A. M. Diehl, B. Hameed, K. V. Kowdley, A. McCullough, N. Terrault, J. M. Clark, J. Tonascia, E. M. Brunt, D. E. Kleiner, E. Doo; NASH Clinical Research Network, Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): A multicentre, randomised, placebo-controlled trial. *Lancet* **385**, 956–965 (2015).
90. S. C. Lu, Regulation of glutathione synthesis. *Mol. Aspects Med.* **30**, 42–59 (2009).
91. W. A. Gahl, N. Bashan, F. Tietze, I. Bernardini, J. D. Schulman, Cystine transport is defective in isolated leukocyte lysosomes from patients with cystinosis. *Science* **217**, 1263–1265 (1982).
92. A. Kessler, M. Biasibetti, D. A. da Silva Melo, M. Wajner, C. S. Dutra-Filho, Â. T. de Souza Wyse, C. M. D. Wannmacher, Antioxidant effect of cysteamine in brain cortex of young rats. *Neurochem. Res.* **33**, 737–744 (2008).
93. R. Dohil, S. Schmeltzer, B. L. Cabrera, T. Wang, J. Durelle, K. B. Duke, J. B. Schwimmer, J. E. Lavine, Enteric-coated cysteamine for the treatment of paediatric non-alcoholic fatty liver disease. *Aliment. Pharmacol. Ther.* **33**, 1036–1044 (2011).
94. L.-C. Kong, J. Tap, J. Aron-Wisnewsky, V. Pelloux, A. Basdevant, J.-L. Bouillot, J.-D. Zucker, J. Doré, K. Clément, Gut microbiota after gastric bypass in human obesity: Increased richness and associations of bacterial genera with adipose tissue genes. *Am. J. Clin. Nutr.* **98**, 16–24 (2013).
95. R. E. Ley, F. Bäckhed, P. Turnbaugh, C. A. Lozupone, R. D. Knight, J. I. Gordon, Obesity alters gut microbial ecology. *Proc. Natl. Acad. Sci. U.S.A.* **102**, 11070–11075 (2005).
96. C. Thomas, A. Gioiello, L. Noriega, A. Strehle, J. Oury, G. Rizzo, A. Macchiarulo, H. Yamamoto, C. Mataki, M. Pruzanski, R. Pellicciari, J. Auwerx, K. Schoonjans, TGR5-mediated bile acid sensing controls glucose homeostasis. *Cell Metab.* **10**, 167–177 (2009).
97. M. Watanabe, S. M. Houten, C. Mataki, M. A. Christoffolete, B. W. Kim, H. Sato, N. Messaddeq, J. W. Harney, O. Ezaki, T. Kodama, K. Schoonjans, A. C. Bianco, J. Auwerx, Bile acids induce energy expenditure by promoting intracellular thyroid hormone activation. *Nature* **439**, 484–489 (2006).
98. H. Tilg, The role of cytokines in non-alcoholic fatty liver disease. *Dig. Dis.* **28**, 179–185 (2010).
99. M. Capanni, F. Calella, M. R. Biagini, S. Genise, L. Raimondi, G. Bedogni, G. Svegliati-Baroni, F. Sofi, S. Milani, R. Abbate, C. Surrenti, A. Casini, Prolonged n-3 polyunsaturated fatty acid supplementation ameliorates hepatic steatosis in patients with non-alcoholic fatty liver disease: A pilot study. *Aliment. Pharmacol. Ther.* **23**, 1143–1151 (2006).
100. F. Sofi, I. Giangrandi, F. Cesari, I. Corsani, R. Abbate, G. F. Gensini, A. Casini, Effects of a 1-year dietary intervention with n-3 polyunsaturated fatty acid-enriched olive oil on non-alcoholic fatty liver disease patients: A preliminary study. *Int. J. Food Sci. Nutr.* **61**, 792–802 (2010).
101. L. Spadaro, O. Magliocco, D. Spampinato, S. Piro, C. Oliveri, C. Alagona, G. Papa, A. M. Rabuazzo, F. Purrello, Effects of n-3 polyunsaturated fatty acids in subjects with nonalcoholic fatty liver disease. *Dig. Liver Dis.* **40**, 194–199 (2008).
102. L. B. Van Wagner, S. W. P. Koppe, E. M. Brunt, J. Gottstein, K. Gardikiotes, R. M. Green, M. E. Rinella, Pentoxifylline for the treatment of non-alcoholic steatohepatitis: A randomized controlled trial. *Ann. Hepatol.* **10**, 277–286 (2011).
103. C. O. Zein, L. M. Yerian, P. Gogate, R. Lopez, J. P. Kirwan, A. E. Feldstein, A. J. McCullough, Pentoxifylline improves nonalcoholic steatohepatitis: A randomized placebo-controlled trial. *Hepatology* **54**, 1610–1619 (2011).
104. C. O. Zein, R. Lopez, X. Fu, J. P. Kirwan, L. M. Yerian, A. J. McCullough, S. L. Hazen, A. E. Feldstein, Pentoxifylline decreases oxidized lipid products in nonalcoholic steatohepatitis: New evidence on the potential therapeutic mechanism. *Hepatology* **56**, 1291–1299 (2012).
105. L. Sjöström, Review of the key results from the Swedish Obese Subjects (SOS) trial-a prospective controlled intervention study of bariatric surgery. *J. Intern. Med.* **273**, 219–234 (2013).
106. A. P. Courcoulas, N. J. Christian, S. H. Belle, P. D. Berk, D. R. Flum, L. Garcia, M. Horlick, M. A. Kalarchian, W. C. King, J. E. Mitchell, E. J. Patterson, J. R. Pender, A. Pomp, W. J. Pories, R. C. Thirlby, S. Z. Yanovski, B. M. Wolfe; Longitudinal Assessment of Bariatric Surgery (LABS) Consortium, Weight change and health outcomes at 3 years after bariatric surgery among individuals with severe obesity. *JAMA* **310**, 2416–2425 (2013).
107. P. Mathurin, A. Hollebecque, L. Arnalsteen, D. Buob, E. Leteurtre, R. Caiazzo, M. Pigeyre, H. Verkindt, S. Dharancy, A. Louvet, M. Romon, F. Pattou, Prospective study of the long-term effects of bariatric surgery on liver injury in patients without advanced disease. *Gastroenterology* **137**, 532–540 (2009).
108. R. Caiazzo, G. Lassailly, E. Leteurtre, G. Baud, H. Verkindt, V. Raverdy, D. Buob, M. Pigeyre, P. Mathurin, F. Pattou, Roux-en-Y gastric bypass versus adjustable gastric banding to reduce nonalcoholic fatty liver disease: A 5-year controlled longitudinal study. *Ann. Surg.* **260**, 893–898 (2014).
109. G. Lassailly, R. Caiazzo, D. Buob, M. Pigeyre, H. Verkindt, J. Labreuche, V. Raverdy, E. Leteurtre, S. Dharancy, A. Louvet, M. Romon, A. Duhamel, F. Pattou, P. Mathurin, Bariatric surgery reduces features of nonalcoholic steatohepatitis in morbidly obese patients. *Gastroenterology* **149**, 379–388 (2015).
110. N. E. Aguilar-Olivos, P. Almeda-Valdes, C. A. Aguilar-Salinas, M. Uribe, N. Méndez-Sánchez, The role of bariatric surgery in the management of nonalcoholic fatty liver disease and metabolic syndrome. *Metabolism* 10.1016/j.metabol.2015.09.004 (2015).
111. G. Szabo, S. Bala, MicroRNAs in liver disease. *Nat. Rev. Gastroenterol. Hepatol.* **10**, 542–552 (2013).
112. W.-C. Tsai, S.-D. Hsu, C.-S. Hsu, T.-C. Lai, S.-J. Chen, R. Shen, Y. Huang, H.-C. Chen, C.-H. Lee, T.-F. Tsai, M.-T. Hsu, J.-C. Wu, H.-D. Huang, M.-S. Shiao, M. Hsiao, A.-P. Tsou, MicroRNA-122 plays a critical role in liver homeostasis and hepatocarcinogenesis. *J. Clin. Invest.* **122**, 2884–2897 (2012).
113. D. Iliopoulos, K. Drosatos, Y. Hiyama, I. J. Goldberg, V. I. Zannis, MicroRNA-370 controls the expression of microRNA-122 and Cpt1α and affects lipid metabolism. *J. Lipid Res.* **51**, 1513–1523 (2010).
114. O. Cheung, P. Puri, C. Eicken, M. J. Contos, F. Mirshahi, J. W. Maher, J. M. Kellum, H. Min, V. A. Luketic, A. J. Sanyal, Nonalcoholic steatohepatitis is associated with altered hepatic microRNA expression. *Hepatology* **48**, 1810–1820 (2008).
115. X. Loyer, V. Paradis, C. Hénique, A.-C. Vion, N. Colnot, C. L. Guerin, C. Devue, S. On, J. Scetbun, M. Romain, J.-L. Paul, M. E. Rothenberg, P. Marcellin, F. Durand, P. Bedossa, C. Prip-Buus, E. Baugé, B. Staels, C. M. Boulanger, A. Tedgui, P.-E. Rautou, Liver microRNA-21 is overexpressed in non-alcoholic steatohepatitis and contributes to the disease in experimental models by inhibiting PPARα expression. *Gut*, pii: gutjnl-2014-308883 (2015).
116. R. J. Perry, D. Zhang, X.-M. Zhang, J. L. Boyer, G. I. Shulman, Controlled-release mitochondrial protonophore reverses diabetes and steatohepatitis in rats. *Science* **347**, 1253–1256 (2015).
117. S. Athinarayanan, R. Wei, M. Zhang, S. Bai, M. G. Traber, K. Yates, O. W. Cummings, J. Molleston, W. Liu, N. Chalasani, Genetic polymorphism of cytochrome P450 4F2, vitamin E level and histological response in adults and children with nonalcoholic fatty liver disease who participated in PIVENS and TONIC clinical trials. *PLOS One* **9**, e95366 (2014).
118. J. Cheng, A. Joyce, K. Yates, B. Aouizerat, A. J. Sanyal, Metabolomic profiling to identify predictors of response to vitamin E for non-alcoholic steatohepatitis (NASH). *PLOS One* **7**, e44106 (2012).

Acknowledgments: J.E.L. is co-chair and principal investigator for the National Institute of Diabetes and Digestive and Kidney Diseases NASH Clinical Research Network, and is a co-investigator on the FLINT, PIVENS, TONIC, and CYNCH clinical trials. J.E.L. consults for Merck, Pfizer, Alexion/Synageva, Synta, Prestige Brands, Poseida, Magellan Health, and Humana.

Submitted 9 November 2015
Accepted 7 January 2016
Published 27 January 2016
10.1126/scitranslmed.aad8390

Citation: J. A. Woo Baidal, J. E. Lavine, The intersection of nonalcoholic fatty liver disease and obesity. *Sci. Transl. Med.* **8**, 323rv1 (2016).

The intersection of nonalcoholic fatty liver disease and obesity

Jennifer A. Woo Baidal and Joel E. Lavine (January 27, 2016)

Science Translational Medicine **8** (323), 323rv1. [doi: 10.1126/scitranslmed.aad8390]