Diabetes and Nonalcoholic Fatty Liver Disease: A Pathogenic Duo

K. H. Williams, N. A. Shackel, M. D. Gorrell, S. V. McLennan, and S. M. Twigg

Sydney Medical School and the Bosch Institute (K.H.W., S.V.M., S.M.T.); Department of Endocrinology, Royal Prince Alfred Hospital (K.H.W., S.V.M., S.M.T.); A. W. Morrow Gastroenterology and Liver Centre, Royal Prince Alfred Hospital (N.A.S., M.D.G.); Liver Injury and Cancer, Centenary Institute (N.A.S., M.D.G.); and Sydney Medical School, The University of Sydney (K.H.W., N.A.S., M.D.G., S.V.M., S.M.T.), Sydney, New South Wales 2006, Australia

Recent data increasingly support a complex interplay between the metabolic condition diabetes mellitus and the pathologically defined nonalcoholic fatty liver disease (NAFLD). NAFLD predicts the development of type 2 diabetes and vice versa, and each condition may serve as a progression factor for the other. Although the association of diabetes and NAFLD is likely to be partly the result of a "common soil," it is also probable that diabetes interacts with NAFLD through specific pathogenic mechanisms. In particular, through interrelated metabolic pathways currently only partly understood, diabetes appears to accelerate the progression of NAFLD to nonalcoholic steatohepatitis, defined by the presence of necroinflammation, with varying degrees of liver fibrosis. In the research setting, obstacles that have made the identification of clinically significant NAFLD, and particularly nonalcoholic steatohepatitis, difficult are being addressed with the use of new imaging techniques combined with risk algorithms derived from peripheral blood profiling. These techniques are likely to be used in the diabetes population in the near future. This review examines the pathogenic links between NAFLD and diabetes by exploring the epidemiological evidence in humans and also through newer animal models. Emerging technology to help screen noninvasively for differing pathological forms of NAFLD and the potential role of preventive and therapeutic approaches for NAFLD in the setting of diabetes are also examined. **(*Endocrine Reviews* 34: 84–129, 2013)**

ISSN Print 0163-769X ISSN Online 1945-7189
Printed in U.S.A.
Copyright © 2013 by The Endocrine Society
doi: 10.1210/er.2012–1009 Received February 8, 2012. Accepted September 10, 2012.
First Published Online December 13, 2012

Abbreviations: AGE, Advanced glycosylation end-products; ALT, alanine aminotransferase; ApoC3, apolipoprotein C3; ARFI, acoustic radiation force impulse; AST, aspartate aminotransferase; AUROC, area under the receiver operating characteristic curve; BMI, body mass index; CI, confidence interval; CKD, chronic kidney disease; CT, computed tomography; CTGF, connective tissue growth factor; DAG, diacylglycerol; DPP-IV, dipeptidyl peptidase-IV; eGFR, estimated glomerular filtration rate; ELF, European liver fibrosis (panel); ER, endoplasmic reticulum; F, fibrosis stage; FFA, free fatty acid; FXR, farnesoid X receptor; GLP-1, glucagon-like peptide-1; GLUT, glucose transporter; HbA1c, glycosylated hemoglobin; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HOMA-IR, homeostasis model of assessment for insulin resistance; HR, hazard ratio; HSC, hepatic stellate cell; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; IRS, insulin receptor substrate; LDL, low-density lipoprotein; LPS, lipopolysaccharide; LV, left ventricular; M, medium (probe for FibroScan); MET, metabolic equivalent; MR, magnetic resonance; MRE, MR elastography; MRI, MR imaging; MRS, MR spectroscopy; MTP, microsomal triglyceride transfer protein; NAFLD, nonalcoholic fatty liver disease; NAS, NAFLD activity score; NASH, nonalcoholic steatohepatitis; NF-κB, nuclear factor κB; NFS, NAFLD fibrosis score; OGTT, oral glucose tolerance test; OR, odds ratio; PIIINP, procollagen-3 N-terminal peptide; PKC, protein kinase C; PNPLA3, adiponutrin/patatin-like phospholipase domain-containing 3 (gene); PPAR, peroxisome proliferator-activated receptor; RAS, renin-angiotensin system; RCT, randomized controlled trial; ROS, reactive oxygen species; SOD2, superoxide dismutase 2; SREBP, sterol regulatory element-binding protein; TIMP-1, tissue inhibitor of metalloproteinase-1; TLR, toll-like receptor; TZD, thiazolidinedione; UDCA, ursodeoxycholic acid; XL, extra large (probe for FibroScan).

Downloaded from https://academic.oup.com/edrv/article/34/1/84/2354662 by U.S. Department of Justice user on 16 August 2022

I. Introduction

There is a clear association between diabetes and nonalcoholic fatty liver disease (NAFLD). Studies over recent years have shown that NAFLD predicts the development of diabetes and vice versa (1) and that each condition serves as a progression factor for the other (1–5). Although this association is likely to be partly the result of a "common soil," it is also probable that diabetes interacts with NAFLD via specific pathogenic mechanisms (6). In this context, the future challenges for the researcher and clinician alike will include increasing awareness of the association between diabetes and NAFLD, understanding pathogenic factors linking these conditions, screening for and diagnosing NAFLD progression noninvasively and cost effectively in those at risk, and establishing effective interventions for NAFLD in diabetes to prevent both hepatic and systemic sequelae. This review will explore these issues by further examining the relationship between the increasingly common and well-established disease entities of diabetes and NAFLD.

In type 2 diabetes, the metabolic derangements of insulin resistance and hyperinsulinemia, with a relative insulin deficiency causing changes to lipid and protein catabolism, as well as hyperglycemia, have recently been reviewed extensively in articles addressing diabetes pathogenesis (7, 8). NAFLD has also been reviewed extensively in other contemporary series (9, 10), as have links of hepatitis C virus (HCV) to type 2 diabetes (11). In contrast, the purpose of this review is to address the multifaceted relationship of NAFLD with diabetes mellitus.

II. Methodology

Literature review has been conducted with the use of Scopus and Web of Knowledge medical databases using the search terms "NASH," "NAFLD," "fatty liver," "diabetes and liver," and "diabetes and hepatic insulin resistance" up to and including June 7, 2012. Articles were selected from search results if they were considered to be relevant to the topic of "diabetes and nonalcoholic fatty liver disease."

III. Background: an Established Relationship between Diabetes and the Liver

A. Hepatic insulin resistance and type 2 diabetes

The liver, together with skeletal muscle and adipose tissue, displays exquisite sensitivity to insulin (8), with classical insulin signaling occurring mainly through specific cell surface receptors and series of postreceptor signaling pathways (12, 13). By insulin-dependent processes, the liver dynamically regulates glucose flux and metabolism and, consequently, glycemia (14).

Hepatic insulin resistance is a common feature that predisposes to compensatory hyperinsulinemia, often followed by consequent pancreatic β-cell dysfunction and development of type 2 diabetes (15, 16). In recent years, the role of neural and endocrine signaling between the liver and brain in the pathophysiology of diabetes has also been more clearly defined (17, 18).

B. Diabetes in liver disease

The prevalence of type 2 diabetes is increased not only in NAFLD, but also in chronic liver disease of any etiology, including: chronic viral hepatitis, hemochromatosis, alcoholic liver disease, and cirrhosis from any cause (19). Dysglycemia in chronic liver disease is associated with increased insulin resistance in skeletal muscle, hepatic, and adipose tissues, and compensatory hyperinsulinemia with consequent pancreatic endocrine (β-cell) dysfunction (20). Impaired glucose metabolism becomes more significant as liver pathology progresses, with up to 96% of patients with cirrhosis having impaired glucose tolerance (IGT) (21). The term "hepatogenous diabetes" has been used to describe the presentation of diabetes in patients with cirrhosis (22). The clinical course of hepatogenous diabetes, thought to be present in 30–60% of patients with cirrhosis, differs from classical type 2 diabetes, in that it is less frequently associated with the microangiopathic complications that are featured in diabetes, whereas it is more frequently associated with complications secondary to cirrhosis (20).

The HCV has a particular association with dysglycemia and type 2 diabetes. A prospective study by Mehta *et al.* (23) after 9 yr of follow-up found that in individuals at high risk of developing type 2 diabetes, there was an 11-

Downloaded from https://academic.oup.com/edrv/article/34/1/84/2354662 by U.S. Department of Justice user on 16 August 2022

fold increased risk of diabetes diagnosis in those infected with HCV *vs.* those not infected. This finding has been supported by other epidemiological data, including those from the Third National Health and Nutrition Examination Survey III (NHANES III 1988–1994) where a 3.77-fold increased risk of diabetes was found for patients at least 40 yr old with HCV (24), and also from a study comparing 1117 individuals with similar hepatic dysfunction due to hepatitis B virus *vs.* HCV, demonstrating a near doubling of diabetes prevalence in the patients with HCV (25). Interestingly, the association between diabetes and HCV described in NHANES III disappeared on analysis of subsequent NHANES cycles (1999–2004 and 2005–2008), a finding that is likely to be related to increasing rates of insulin resistance and diabetes in the general population, coupled with increasing rates of obesity and NAFLD (26).

The pathogenic factors behind the relationship between HCV and diabetes are multiple. In summary, insulin resistance in both the liver and skeletal muscle has been documented in several studies in patients with HCV (27–29), with altered glucose uptake by hepatocytes. HCV appears to have direct effects on insulin-signaling pathways (30). One mechanism through which this may occur is through the induction of suppressor of cytokine signaling 3 that can lead to the degradation of insulin receptor substrate (IRS)-1 and -2 by ubiquitination (31). Increased production of proinflammatory cytokines (32) and reactive oxygen species (ROS) (33) has also been found in HCV and is likely to contribute to diabetes risk, as is the promotion of truncal obesity (34). Direct effects of HCV on the pancreatic β-cell have also been found (35). Of clinical importance to liver disease secondary to HCV, the presence of diabetes is associated with a decrease in sustained response to antiviral therapy and an increased progression of fibrosis in both the pre- and posttransplant setting (20, 36). Studies have demonstrated that insulin resistance in HCV, primarily measured via homeostasis model of assessment for insulin resistance (HOMA-IR), has been linked to increased fibrosis, and also to increased rates of hepatic complications and a poorer response to antiviral therapy (37). Furthermore, steatosis is also strongly associated with HCV, particularly genotype 3, and with poor response to antiviral therapy and increased fibrosis progression. Although it is difficult to determine cause and effect, NAFLD is likely to be important in exacerbating recurrent HCV after transplant and in increasing rates of graft failure (38, 39).

IV. NAFLD and NASH Definitions

NAFLD incorporates a spectrum of pathology from simple steatosis, to nonalcoholic steatohepatitis (NASH), to fibrosis and cirrhosis (1). Patients with NAFLD are also at increased risk of hepatocellular carcinoma (HCC) (40). Although NAFLD is likely to have an impact on the prognosis of a variety of other liver diseases (41, 42), the diagnosis is generally reserved for patients without other significant causes of liver pathology, particularly excluding those with significant alcohol intake.

NAFLD has been recognized in the medical literature across some decades. In 1980, Ludwig *et al.* (43) described a small patient series (n = 20) with liver histology characterized not only by fat accumulation but also by the presence of hepatic necroinflammation and, in most cases, fibrosis in the absence of a history of excessive alcohol consumption. They coined the term "nonalcoholic steatohepatitis" (or NASH). With time, the need to define differing stages of NAFLD, its activity, and related outcomes, became increasingly apparent. Subsequently, definitions of NAFLD based on liver biopsy findings were developed; in 1999, Matteoni *et al.* (44) proposed pathological NAFLD subtypes based on long-term outcome studies, and Brunt *et al.* (45) proposed a system specifically for NASH based on methods accepted in other forms of nonbiliary chronic liver disease, using separate assessment for necroinflammatory lesions (grade) and fibrosis (stage). Subsequently, the U.S. Pathology Committee of the NASH Clinical Research Network designed and validated a histological scoring system of specific lesions that addresses the full spectrum of NAFLD (46). In addition, a separate scoring system known as the NAFLD activity score (NAS) has been developed for use in clinical trials to enable systematic documentation of changes in activity of NAFLD prospectively within individuals (46). Importantly, the NAS is not intended for the diagnosis of NASH because it underdiagnoses patients with NAFLD who will subsequently die of liver-related causes (47, 48). Furthermore, considerable debate continues regarding the diagnosis and predictive value of this system and other widely used scoring systems in NAFLD (47).

Despite the reservations listed above, the U.S. Pathology Committee system remains the most widely used classification system for NAFLD and includes four features that are scored semiquantitatively: steatosis, lobular inflammation, hepatocyte ballooning, and fibrosis. Another nine features are recorded as "present" or "absent" (46). Significant steatosis is defined as fat accumulation in more than 5% of hepatocytes. Steatosis, coupled with evidence of necroinflammation, including the features of hepatocyte ballooning and inflammatory infiltrates with or without fibrosis, is defined as steatohepatitis. Fibrosis is staged on histology from 0–4, with a score of at least 2 consistent with advanced fibrosis and a score of 4 consistent with cirrhosis (46). The NAS uses features of active injury that are potentially reversible in the short term only. It is the

Downloaded from https://academic.oup.com/edrv/article/34/1/84/2354662 by U.S. Department of Justice user on 16 August 2022

sum of the semiquantitated steatosis (0–3), lobular inflammation (0–3), and hepatocellular ballooning (0–2) scores. Fibrosis is not included in the NAS. A NAS of less than 3 is diagnosed as "not NASH" (46). Higher NAS cutoffs should not be used to exclude NASH, but a score of more than 5 is consistent with a diagnosis of NASH (46–48). This classification system requires only routine histochemical stains (hematoxylin and eosin and Masson trichrome stains), and so it can be used by an experienced practicing pathologist working in the clinical care or clinical research setting, and also by research pathologists assessing NASH in preclinical animal models (6, 49, 50).

V. Epidemiology, Natural History of Liver Morbidity, and Risk Factors for Fibrosis Development in the General Population

A. Prevalence of nonalcoholic liver disease

NAFLD is thought to be present in over 30% of the U.S. population and approximately 25% of the Asian population, making it the commonest liver disease in the world (51, 52). The prevalence of NASH is difficult to estimate in the general adult population because a liver biopsy is required for a definitive diagnosis, but it is commonly quoted at around 3–5% in the United States, including in a recent U.S. position guideline (1, 53, 54). This prevalence figure for NASH is likely to be a significant underestimate of disease burden. Certainly the rates of NASH in different study cohorts, which exhibit heterogeneity in risk factors for NASH, vary greatly and are often significantly more than 3–5%. For example, in a recent prospective study of 400 U.S. military personnel and their families with a mean age of 55 yr, 46% were found to have evidence of steatosis on ultrasound, and NASH was present at biopsy in 30% of these ultrasound positive patients, resulting in 12% of the entire study cohort having NASH (55). Furthermore, larger studies are required to assess for NASH in the general population.

By examining data trends from liver transplant registries, it is apparent that NAFLD is becoming increasingly important as a cause of significant morbidity due to chronic liver disease. Data obtained from the Scientific Transplant Registry on 35,781 adult patients undergoing a liver transplant from 2001 to 2009 show that NASH is currently the third most common indication for liver transplantation in the United States and is on a trajectory to become the most common. The percentage of patients undergoing a liver transplant for NASH on this registry increased every year from 1.2% in 2001 to 9.7% in 2009. No other indication for liver transplantation increased in frequency during the study period. Furthermore, these statistics are likely to underestimate the prevalence of NASH as an indication for liver transplant because the changes associated with fatty liver may become less pronounced on biopsy as fibrosis increases. They also neglect the likely impact that NASH has on the other defined chronic liver diseases (56).

B. Natural history of liver morbidity in NAFLD in the general population—focus on liver biopsy findings

Understanding the burden of NASH in the NAFLD population is important because, whereas hepatic steatosis alone is thought to be relatively benign from a liver perspective, with a 0–3% liver related mortality rate over 10–20 yr, NASH presence was associated with a 17.5% risk of liver-related mortality over approximately 20 yr of follow-up in a series of 131 subjects (1, 51, 57). A significant minority of people with simple steatosis will also develop NASH over time, as illustrated by one comprehensive follow-up series using serial liver biopsy, where 23% of patients with simple steatosis were found to progress to NASH in a short, 3-yr period (58).

The presence and degree of fibrosis found on liver biopsy due to NASH is also required to assess prognosis (59). In a small Swedish cohort of patients with a mean follow-up of 13.7 yr, 0% of patients with stage 0–1 fibrosis, 14% of those with stage 2 fibrosis, and 25% of those with stage 3 or 4 fibrosis developed end-stage liver disease (60). In addition, in a meta-analysis by Musso *et al.* (1), the odds ratio (OR) of liver-related mortality in the presence of advanced fibrosis ($F \geq 3$) was 10.06 [95% confidence interval (CI), 4.35–23.25; $P = 0.00001$], compared with milder fibrosis stages ($F = 0–2$).

C. A focus on clinical markers of NAFLD progression beyond liver biopsy findings

Defining clinical risk factors and markers for NAFLD presence, severity, and particularly progression to significant fibrosis is of major clinical importance. This is required to aid the screening of subgroups at high risk for progression to significant liver disease, to increase our understanding of NAFLD and NASH pathogenesis, and to identify potential therapies for NAFLD, including the targeting of reversible pathogenic risk factors.

To date, independent risk factors for NASH presence and progression to its more fibrotic forms have been defined in only relatively small series. In a study of NAFLD in 105 morbidly obese individuals undergoing bariatric surgery, in whom 25% had NASH, a calculated index of insulin resistance and systemic hypertension, as well as elevated alanine aminotransferase (ALT), were each independently associated with NASH (61). In that study,

Downloaded from https://academic.oup.com/edrv/article/34/1/84/2354662 by U.S. Department of Justice user on 16 August 2022

known type 2 diabetes was positively associated with advanced fibrosis (stage 3 or 4) in NASH with OR of 4.4 (95% CI, 1.6–16.2; $P = 0.018$), although on logistic regression the only independent predictors of advanced fibrosis were systemic hypertension, elevated ALT enzyme level, and an increased serum C-peptide (61). In a cross-sectional study of 733 cases of NAFLD where liver biopsy was undertaken, including a validation cohort, independent indicators of advanced liver fibrosis were greater age, hyperglycemia, body mass index (BMI), lower platelet count, and elevated serum albumin and aspartate aminotransferase (AST)/ALT ratio (62). Smoking history (at or above 10 pack years) has also been associated with severe fibrosis (stages 3 or 4) in NASH (5). Longitudinal studies have implicated male gender, obesity, and, as will be explored in this review, preexisting diabetes at study baseline as independent risk factors of NASH fibrosis progression (63). Although potential biomarkers such as circulating adiponectin (64) and TNF-α gene polymorphisms (65) have added little value in defining the risk of NAFLD presence or progression, some markers for hepatic fibrosis in NASH, such as procollagen-3 N-terminal peptide (PIIINP), hyaluronic acid, tissue inhibitor of metalloproteinase-1 (TIMP-1), and cytokeratin 18, contribute useful information (66, 67) and, as described in *Section IX.B* and Table 4 have been included in algorithms defining risk of NASH with fibrosis.

VI. NAFLD and Diabetes

A. NAFLD in diabetes and prediabetes

NAFLD prevalence is increased in people with type 2 diabetes and has been estimated at around 70% using ultrasound techniques (1). This figure is likely to be an underestimate because ultrasound has reduced sensitivity to detect NAFLD presence at levels of liver steatosis below 33% (68) and also in more obese patients (69). Data on NAFLD in type 1 diabetes are far less comprehensive. A study by Targher *et al.* (70) of 250 patients with type 1 diabetes estimated the prevalence of NAFLD by ultrasound at 44.4%.

Although studies in patients with type 2 diabetes examining the spectrum of pathology within NAFLD utilizing liver biopsy are limited by small numbers, they collectively estimate the prevalence of NASH at 63–87% and moderate-severe fibrosis at 22–60% (71). In a recent study of patients with type 2 diabetes, with an average BMI of 36 kg/m^2, over 60% of patients who underwent weight reduction surgery (n = 64 of 92) had moderate to severe NAFLD on liver biopsy (72).

Diabetes presence is particularly associated with fibrosis in NASH (Table 1). Angulo *et al.* (73) found that when diabetes and obesity coexisted, 66% of patients with NAFLD had advanced fibrosis. This figure is much higher than those estimated for patients without diabetes or obesity (1). Furthermore, a study by Leite *et al.* (71) in patients with type 2 diabetes found that three of the 92 patients had histological evidence of cirrhosis secondary to NAFLD without clinical evidence of liver disease.

Diabetes has not only been associated with fibrosis in NAFLD in cross-sectional studies but also has been shown to be a marker of progression for fibrosis in longitudinal series. In a study of histologically proven NAFLD in 432 patients, type 2 diabetes was found to be an independent risk factor for fibrosis progression to moderate and severe fibrosis (2). In another clinical cohort of 103 patients with average age of 45 ± 11 yr who underwent serial liver biopsy at an average interval of 3.2 ± 3.0 yr (range, 0.3–21.3 yr), and in whom 42% had diabetes, preexisting diabetes was found to be a predictor of fibrosis progression on multivariate analysis with a regression coefficient of 0.35 ($P = 0.007$) (63). As previously discussed, the increased rates of NASH and fibrosis and also apparent increases in fibrosis progression observed in populations with diabetes are of great importance because NASH, particularly with increasing fibrosis, appears to be far more significant than simple steatosis in leading to adverse mor-

TABLE 1. Summary of relative strength of associations of NAFLD and its subtypes with prediabetes and types of diabetes based on human studies

NAFLD pathological subcategory	Type 1 diabetes[c]	Prediabetes[c] (IFG, IGT)	Type 2 diabetes	Refs.
Simple steatosis	+	++	++	1, 70, 76, 78
NASH with high NAS[a]	+/−	++	++	77
NASH with substantial fibrosis (F2-F3)	+/−	++	+++	2, 63, 71, 73, 77, 78, 79
Cirrhosis due to NAFLD (includes F4)[b]	+/−	+++	++++	21, 71, 118

[a] NAS is the sum of standardized scores of steatosis, inflammation, and hepatocyte ballooning at pathology (see *Section IV*).

[b] Overlaps clinically with hepatogenous diabetes.

[c] Based on cross-sectional studies only.

Downloaded from https://academic.oup.com/edrv/article/34/1/84/2354662 by U.S. Department of Justice user on 16 August 2022

bidity and mortality outcomes, both liver related and otherwise (1, 3, 4, 74).

The condition of prediabetes, which includes impaired fasting glucose (IFG) and IGT, is present when hyperglycemia occurs, but by definition, it is not into the blood glucose range of diabetes mellitus (75). Prediabetes reflects relative insulin deficiency, and it is along a continuum from normal plasma glucose to diabetes range levels. As reported in Table 1, in the limited number of series where it has been examined, prediabetes was found to be associated with an increased risk of NAFLD. In a large Korean cohort of 99,969 individuals without clinical evidence of diabetes, the risk of NAFLD on ultrasound increased with an increasing level of glycosylated hemoglobin (HbA1c) (OR, 1.44 at HbA1c 5–5.4%, 2.62 at HbA1c 5.5–5.9%, and 7.18 at HbA1c 6.0–6.4%) when compared with the lowest group by HbA1c category (HbA1c $\leq$ 4.9%; $P <$ 0.001) (76). In that study, prediabetes range blood glucose levels will have cosegregated mainly with the highest HbA1c readings of 6.0–6.4%, where the odds of NAFLD were greatest. A study undertaking liver biopsies on recruited patients (n = 73) found that those with any degree of NAFLD were more likely to have IGT (29%), compared with the normal glucose tolerant controls (14%) ($P <$ 0.0001) (77). In an Australian study of 70 patients not known to have diabetes, with fasting glucose levels below 7.0 mmol/liter, but with NAFLD as determined by ultrasound, 24% had IGT and 10% had diabetes on standard oral glucose tolerance test (OGTT) (78). Over half of the NAFLD patients with a normal fasting glucose had abnormal glucose tolerance as detected by OGTT. In addition, irrespective of the status of glucose tolerance, 2-h hyperinsulinemia during the OGTT occurred in all subjects with NAFLD, and fasting insulin resistance (HOMA-IR) was found in 73%.

Prediabetes in small cross-sectional series examining NAFLD has been associated more so with increased NASH rather than simple steatosis. In one publication (n = 73), prediabetes (IFG or IGT) occurred in 20% of patients with simple steatosis and in 55% of NASH patients ($P = 0.036$), and in that study, a higher 2-h plasma glucose reading on the OGTT correlated, albeit weakly, with higher fibrosis stage ($r = 0.25$; $P = 0.046$); stage 3–4 liver fibrosis occurred in none of the controls with normal glucose regulation, in 4% with prediabetes, and in 17% of those with newly diagnosed type 2 diabetes (77). By univariate analysis, prediabetes presence (IFG or IGT) as well as hyperinsulinemia and insulin resistance each correlated with NAFLD, although relative relationships with NASH were not reported (77). Interestingly, in a larger series (n = 173) in patients without a history of diabetes, hyperinsulinemia 2 h after oral glucose loading, rather than hyperglycemia, was independently associated with the severity of NASH and fibrosis ($P = 0.0001$; OR, 3.56; 95% CI, 1.61–7.86), suggesting that circulating insulin, rather than glucose, is a better marker of histological severity in NAFLD (79).

In contrast to findings in type 2 diabetes, prediabetes has not yet been studied in longitudinal series to determine whether it is associated with fibrosis progression in NASH and also the strength of such association compared with that present in type 2 diabetes and NASH with fibrosis. Larger, prospective studies are needed to better define the histological findings of NASH, including fibrosis progression with the continuous variables of hyperinsulinemia, insulin resistance, and hyperglycemia caused by relative insulin deficiency.

B. NAFLD predicts diabetes development

In approaching the relationship between NAFLD and diabetes from another perspective, multiple studies supported by a recent meta-analysis have shown that NAFLD is associated with insulin resistance and diabetes and that NAFLD presence predicts the development of diabetes (1, 78–80). In a Korean study of 11,091 individuals without diabetes at baseline, where fasting insulin levels were also measured and divided into quartiles, the 5-yr (crude) OR for developing type 2 diabetes in the presence of ultrasound evidence of fatty liver at baseline was 5.05 (95% CI, 2.08–12.29) for the lowest insulin quartile and 6.34 (95% CI, 3.58–11.21) for the highest insulin quartile. After multivariate adjustment, including for baseline glucose level, the OR for the highest insulin quartile remained significant at 2.42 (95% CI, 1.23–4.75) (81). Bae *et al.* (82), also from Korea, using data from 2049 individuals without diabetes, showed that the multivariate adjusted hazard ratio (HR) for developing diabetes in subjects who had both NAFLD on ultrasound and IFG at baseline was 8.95 (95% CI, 6.49–12.35) at 4 yr. Musso *et al.* (1) reported a 3.51 multiple-adjusted OR for incident diabetes in patients with evidence of ultrasonographic/histological NAFLD in their recent meta-analysis of three large community-based cohorts with a range of follow-up from 4 to 10 yr.

VII. Diabetes Complications Are Linked to NAFLD

A. Macrovascular disease

NAFLD has been associated with an increased prevalence of both micro- and macrovascular complications in patients with type 1 or 2 diabetes (4). The most studied association is the increased risk of macrovascular,

Downloaded from https://academic.oup.com/edrv/article/34/1/84/2354662 by U.S. Department of Justice user on 16 August 2022

and in particular cardiovascular, disease in patients with NAFLD, with or without diabetes. Although large, long-term, prospective studies are needed to address current limitations of available data, there is a growing body of evidence to suggest that cardiovascular disease is the leading cause of death in patients with advanced NAFLD and that NAFLD is associated with an increased risk of incident cardiovascular disease that is independent of the risk conferred by traditional risk factors and components of the metabolic syndrome (4, 83). In addition, NASH presence appears to afford a greater risk than simple steatosis (84, 85). From studies of NAFLD with histopathological grading, cardiovascular disease was the most frequent cause of death in 173 patients with biopsy-proven NAFLD who were followed for 13 yr (57). Ekstedt *et al.* (60) found that the 14-yr risk of death from cardiovascular disease was higher by a factor of two among 129 patients with NASH than in the general population. Furthermore, Söderberg *et al.* (85) recently confirmed that NASH, not simple steatosis, was associated with increased mortality from all causes, and from cardiovascular disease and liver-related causes among patients with NAFLD who were followed for a mean period of 21 yr (4, 85). Subclinical detection methods using surrogate markers such as carotid intima-media thickness and pulse wave velocity support these clinical findings (84, 86).

Data exploring any association between NAFLD and cardiovascular disease in patients with diabetes is more limited. In a large study involving approximately 3000 unselected patients with type 2 diabetes (87) and in a smaller series of type 1 diabetes (70), the prevalence of coronary, cerebrovascular, and peripheral vascular disease was remarkably higher among patients with NAFLD. This finding was independent of traditional macrovascular risk factors; duration of diabetes; extent of glycemic control; use of lipid-lowering, antidiabetic, antihypertensive, or antiplatelet medications; and components of the metabolic syndrome. Moreover, NAFLD, as assessed by magnetic resonance (MR) spectroscopy (MRS), was associated with reduced myocardial perfusion in patients with type 2 diabetes who were known to have coronary artery disease. This was found to be independent of traditional risk factors, visceral fat mass, and insulin sensitivity (assessed by euglycemic hyperinsulinemic clamp) (4, 88). In addition, in a cohort of 247 Child-Pugh class A patients with biopsy-confirmed NAFLD with advanced fibrosis or cirrhosis, a history of diabetes and hypercholesterolemia was associated with vascular events (*i.e.*, nonfatal myocardial infarction, nonfatal stroke, and vascular death) and vascular-related death. The adjusted OR for vascular event and death combined in the presence of diabetes in this cohort was 10.43 (95% CI, 1.26–82.53; $P = 0.03$) (89). This increase in risk is higher than the risk that diabetes has been estimated to carry for vascular disease in the general population by a factor of approximately 5 (90).

Some counterbalance to the argument that NAFLD is an independent risk factor for cardiovascular disease is demonstrated by two negative studies in type 2 diabetes that found no association with NAFLD and increased carotid intima-media thickness or coronary, aortic, or carotid calcium (91, 92) and studies that have failed to show an association for NAFLD and markers of cardiovascular disease such as carotid intima-media thickness after statistical adjustments were made for traditional risk factors (83, 93).

B. NAFLD and the microvascular complications of diabetes

Although data are limited, being sourced from observational studies and largely from one group, an association between NAFLD and CKD has been suggested, including in populations with diabetes. While this association must be confirmed in larger, controlled studies and across broader ethnic groups, in patients with either type 1 or 2 diabetes, the presence of NAFLD as determined by ultrasound has been associated with an increased prevalence of microalbuminuria and chronic kidney disease (CKD) (3, 94, 95). Prospective studies have also demonstrated an increased incidence of CKD in patients with type 2 diabetes and NAFLD. As with cardiovascular disease risk, the degree of liver inflammation also appears to be important. A cross-sectional study looking at NASH severity by histopathology demonstrated an association between the NASH/fibrosis stage and decreasing mean values of estimated glomerular filtration rate (eGFR), independently of traditional macrovascular risk factors, insulin resistance, and metabolic syndrome components (3, 96).

Clarifying the association of NAFLD with CKD is particularly important in those with significant liver disease requiring liver transplant because CKD is a well-recognized determinant of long-term morbidity and mortality in liver transplant recipients. In a recent retrospective case review on patients undergoing liver transplantation at a single center in the United Kingdom between January 2000 and December 2008, where NASH patients and patients with cryptogenic cirrhosis and three components of the metabolic syndrome were compared with a group matched for age, sex, model end-stage liver disease score, and eGFR by the modified diet in renal disease equation (68.7 *vs.* 69.3 ml/min/1.73 m^2) at baseline, the eGFR was 8.46 ml/min/m^2 lower ($P < 0.001$) in the NASH/cryptogenic cirrhosis group 3 months after transplant after ad-

Downloaded from https://academic.oup.com/edrv/article/34/1/84/2354662 by U.S. Department of Justice user on 16 August 2022

justing for effects of BMI, tacrolimus levels, diabetes mellitus, hypertension, and HCC. In addition, 31.2% of patients in the NASH/cryptogenic cirrhosis group progressed to stage IIIb CKD by 2 yr, compared with 8.3% in the control group ($P = 0.009$). This finding supports the concept that less renal toxic immunosuppressive regimens should be considered in NASH patients after liver transplant (97).

In addition to the association with CKD, Targher *et al.* (98) have also observed a higher prevalence of diabetic retinopathy in patients with diabetes and NAFLD diagnosed by ultrasound in a cohort of 202 patients with type 1 diabetes. The age- and sex-adjusted prevalence of retinopathy was 53.2 *vs.* 19.8% ($P < 0.0001$) in this cohort, and NAFLD was associated with prevalent retinopathy with an OR of 3.3 (95% CI, 1.4–7.6; $P < 0.005$) on multivariate analysis. This finding was independent of age, sex, diabetes duration, medication use, HbA1c, and the presence of the metabolic syndrome (98).

Interestingly, de Lédinghen *et al.* (99), when looking at liver fibrosis assessed noninvasively by Fibrotest and FibroScan (see *Section IX.B* pertaining to assessment of liver fibrosis) in 277 hospitalized patients with type 1 or 2 diabetes (52% with type 1 diabetes), found that the absence of retinopathy (OR, 5.4; 95% CI, 1.44–20.57; $P = 0.01$) but a past history of foot ulcer (OR, 5.2; CI, 1.50–18.03; $P = 0.009$) was associated with a higher prevalence of liver fibrosis by multivariate analysis in type 2 diabetes. In type 1 diabetes, no factor was associated with liver fibrosis (99). Clearly, more studies are needed to make conclusions about the impact of NAFLD on the micro- and macrovascular complications of diabetes.

C. Cancer risk in NAFLD and diabetes

Apart from the traditional vascular complications associated with diabetes, more recent evidence has suggested links between diabetes and various cancers, particularly HCC (100), breast cancer, and colorectal cancer (101). Several systemic reviews and one retrospective cohort study demonstrated an increased adjusted HR for HCC in patients with diabetes from 1.73- to 3.64-fold. This risk appears to be independent of alcohol and viral hepatitis but to vary with diabetes duration and other comorbidities (102–104). In particular, a large meta-analysis of participant data from 97 prospective studies, including 123,205 deaths in 820,900 people, found that the HR for HCC in people with diabetes was 2.16 (95% CI, 1.62–2.88) when compared with people without diabetes after adjustment for age, smoking status, and BMI (105). Because NAFLD is clearly linked to an increased risk of HCC, by assumption, the presence of diabetes in NAFLD may confer additional risk. Indeed, diabetes was present in 59% of a recent cohort of 87 patients with NASH who developed HCC (40).

Several studies have also linked NAFLD to adenomatous polyps of the bowel, and in particular high-grade right-sided lesions (74, 106). Whether the effect of diabetes and NAFLD on cancer risk is synergistic is not yet clear, but the pathogenic mechanisms behind these shared associations with neoplasm are likely to have a common basis.

D. Other associations with NAFLD relevant to diabetes

NAFLD and diabetes are each associated with cognitive impairment (107–109) and bacterial sepsis (110, 111). In a small population of obese individuals who underwent liver biopsy during bariatric surgery, mild cognitive impairment, as defined by extensive psychometric testing, was found in five of 11 patients with NASH in the absence of cirrhosis. This finding was associated both with markers of inflammation and with hyperammonemia. In this same study, 29 patients with simple steatosis did not have mild cognitive impairment or markers of inflammation or hyperammonemia (109). In addition, in a study of 247 hospitalized patients with evidence of NAFLD on ultrasound compared with 100 gender- and age-matched controls, the adjusted OR of recurrent (at least two) bacterial infections per year for a 3-yr period was 3.0 (95% CI, 2.6–4.2; $P < 0.001$) (111).

Mild cardiac dysfunction has also been noted in populations with NAFLD and type 2 diabetes. In a small population (n = 50) of patients with well-controlled type 2 diabetes without a history of ischemic heart disease, patients with NAFLD detected by ultrasound (n = 32) had early features of left ventricular (LV) diastolic dysfunction, independent of hypertension and several other potential confounders (OR, 30.9; 95% CI, 2.45–79.2; $P = 0.008$ on multivariate analysis) (112). Previously, NAFLD presence in a nondiabetic, normotensive population has been linked to increased risk of early LV diastolic dysfunction and impaired LV metabolism (113). Further studies are needed to clarify any association of NAFLD with LV diastolic dysfunction, including in diabetes.

VIII. Pathogenic Mechanisms in the Relationship of NAFLD and Diabetes

A. Evidence derived from animal models

As described earlier in this review, diabetes does appear to be an independent risk factor for NAFLD progression in humans, especially the fibrosis in NASH. Animal models of diabetes and NAFLD help to further characterize the pathogenic mechanisms potentially involved in progres-

Downloaded from https://academic.oup.com/edrv/article/34/1/84/2354662 by U.S. Department of Justice user on 16 August 2022

sion of NAFLD in humans, but in a more controlled manner than in human studies.

NAFLD as a model in animals can be achieved by various means, and the challenge is to achieve the pathology of human NAFLD, especially NASH, in the correct metabolic context (114). The literature contains many different mouse models that exhibit histological evidence of hepatic steatosis and more variably, steatohepatitis, and although none to date fully replicate the human phenotype (115, 116), NASH animal models are receiving increased attention, and their relevance to human disease is increasingly being realized (117). NASH animal models include diet-induced, methionine-choline-deficient approaches, which induce only transient insulin resistance and without weight gain (118, 119). Genetic models of leptin gene or receptor knockout in diabetic ob/ob and db/db mice, respectively (118, 120), with the added metabolic insult of a methacholine deficient diet, will create all the histological features of NASH (121), and exacerbate hepatic fatty acid oxidation through functional leptin deficiency (122).

Models of NAFLD that include diabetes mellitus are summarized in Table 2, including their relative strengths and weaknesses. One particularly attractive genetic model of the metabolic syndrome and NASH involves a homozygous truncating mutation in the Alms1 gene (*foz/foz*). This leads to overfeeding, metabolic syndrome, type 2 diabetes, and on exposure to a high-fat and/or cholesterol diet, NASH with fibrosis (123, 124).

Although there are multiple genetic models of diabetes in animals, few have systematically developed NAFLD models and then studied whether diabetes exacerbates the NAFLD or its progression. This is partly because in the genetic models of NAFLD, diabetes occurs usually as part of the overall syndrome and gene defect (121, 123, 124), or it develops late in the process after NAFLD or NASH is well established (125). Therefore, these models are unable to facilitate systematic examination of the effect of timed onset of diabetes added to a high-fat diet or to a metabolic syndrome phenotype; however, interventions targeting hyperglycemia in diabetes, for example, can be trialled in them. To date, these interventions are relatively few and include hepatic antisteatotic and systemic antiinflammatory effects of a selective dipeptidyl peptidase-IV (DPP-IV) inhibitor in a murine diabetes model (126), statin therapy with either metformin (127) or insulin (128) in db/db fat-fed mice, and peroxisome proliferator-activated receptor (PPAR)-α agonist treatment in the *foz/foz* cholesterol-fed model (129). This limited series of studies is collated in Table 2 and collectively implicates dyslipidemia, insulin resistance, and possibly hyperglycemia in effects of type 2 diabetes on NAFLD and its progression.

In contrast to the genetic models of obesity with diabetes (118, 120, 121, 123) or the effects of modified diet

TABLE 2. Animal models of NAFLD that include diabetes

Model basis	Model subtype	Advantages of the model	Disadvantages of the model	Role implicated for diabetes in the model	Refs.
Genetically manipulated ± nutritional modification	ob/ob mice-leptin deficient	Reliable development of diabetes, extreme obesity, and metabolic syndrome	Role of diabetes in NASH development is difficult to examine. Model needs further hits to progress to NASH	Not systematically studied using diabetes therapy	120, 121
	db/db mice-leptin resistant	Reliable development of diabetes, metabolic syndrome	Role of diabetes in NASH development is difficult to examine. Model needs further hits to progress to NASH	Not systematically studied using diabetes therapy	118, 121
	Goto-Kakizaki inbred rat	Reliable 2 diabetes onset after 18 wk of age	Model develops NASH, but without significant fibrosis	After 4 months high-fat feeding, 1 month metformin or insulin, + atorvastatin, decreased oxidative stress and inflammation	125
	Alms1 homozygous (*foz/foz*) mutant mice; some fed high fat and/or cholesterol	Reliable development of diabetes, metabolic syndrome, and all features of NASH including progressive fibrosis	Role of diabetes added to metabolic syndrome in NAFLD development is difficult to examine	PPAR-α agonist therapy improves blood glucose and steatosis but not liver inflammation	123, 124, 129
Wild-type with chemical or nutritional modification	ICR neonatal mice injected up to 5 consecutive days with monosodium glutamate	Reliable induction of severe obesity at 24 wk, then diabetes, progressive NAFLD with all NASH features by 54 wk	Mechanism of effect of MSG on diabetes development has not been fully determined. Not physiological in the pathological insult used	Not systematically studied	132
	Outbred Sprague-Dawley rats (male), fed 2% (wt/wt) cholesterol	All features of metabolic syndrome and NASH develop including progressive fibrosis, consistently after 24 wk	Diabetes development occurs late, after wk 36–48 and after NASH is well-developed. Diet very high in cholesterol	Role of preexisting diabetes in NASH pathogenesis cannot be studied	226
	Wild-type c57BL/6J mice with high-fat feeding then timed induced type 2 diabetes	Reflects human disease process of NAFLD progression to NASH with fibrosis. Role of diabetes can be interrogated	Needs prolonged 15 wk of high-fat feeding to induce insulin resistance before diabetes induced by low-dose streptozotocin	Diabetes added to high-fat feeding accelerates NASH fibrosis progression, which is prevented by insulin	6

Reliable development of diabetes refers to the onset of diabetes that occurs in the majority of animals.

ICR, Imprinting control region.

Downloaded from https://academic.oup.com/edrv/article/34/1/84/2354662 by U.S. Department of Justice user on 16 August 2022

alone without diabetes (130, 131) or chemically induced obesity resulting later in diabetes (132), some recent animal models of NAFLD have focused on studying effects of timed induction of diabetes on NAFLD progression, in settings arguably more relevant to human diabetes (6). As an example of this, in the authors' published mouse model of NASH, the C57BL/6 mouse is rendered insulin resistant by high-fat feeding with a subsequent increase in total body fat mass, and then partial failure of the pancreatic β-cell is induced chemically by low-dose streptozotocin (6), thus simulating the clinical scenario of development of type 2 diabetes (133). In this model, the induction of diabetes was found to cause more severe NASH, and in particular, progression of fibrosis in NASH (Fig. 1) (6). This effect was attenuated by insulin therapy, indicating that poorly controlled diabetes is required to cause the NASH worsening. This animal model thus reflects what is apparent clinically, where insulin resistance and hyperinsulinemia present from high-fat feeding causes NASH, whereas the addition of type 2 diabetes with relative insulin deficiency causes an additional accelerator for fibrosis progression in NASH.

In the diabetes and high-fat-fed rodent in combination with increased liver fibrosis in NASH, there was up-regulation of the profibrotic connective tissue growth factor (CTGF), the fibroblast-activating protein mRNA expression as a marker of the activation of the hepatic stellate cell (HSC) responsible for deposition of extracellular matrix in the liver, and also increased TIMP-1 mRNA expression as an inhibitor of the matrix metalloproteinase enzymes that degrade extracellular matrix. Indeed, TIMP-1 mRNA in the high-fat-fed rodents with diabetes was increased 6-fold when compared with chow-fed mice and 3-fold when compared with high-fat-fed mice without diabetes (6), and CTGF mRNA correlated with collagen-I, collagen-III, and TIMP-1 mRNA. Intrahepatic CTGF (61), and TIMP-1 (134) are increased in human NASH, and both molecules are potential hepatic molecular targets to prevent progressive NASH fibrosis in this model. Extension of the mouse model (6) to 37 wk of diabetes results in development of cirrhosis in approximately half of the mice on the *ad libitum* high-fat diet (7). In summary, this mouse model indicates that on a background of high-fat feeding and insulin resistance, type 2 diabetes causes progression of liver fibrosis in NASH. Recently, chemical diabetes induction preceding high-fat feeding resulting in NASH and HCC

Figure 1.

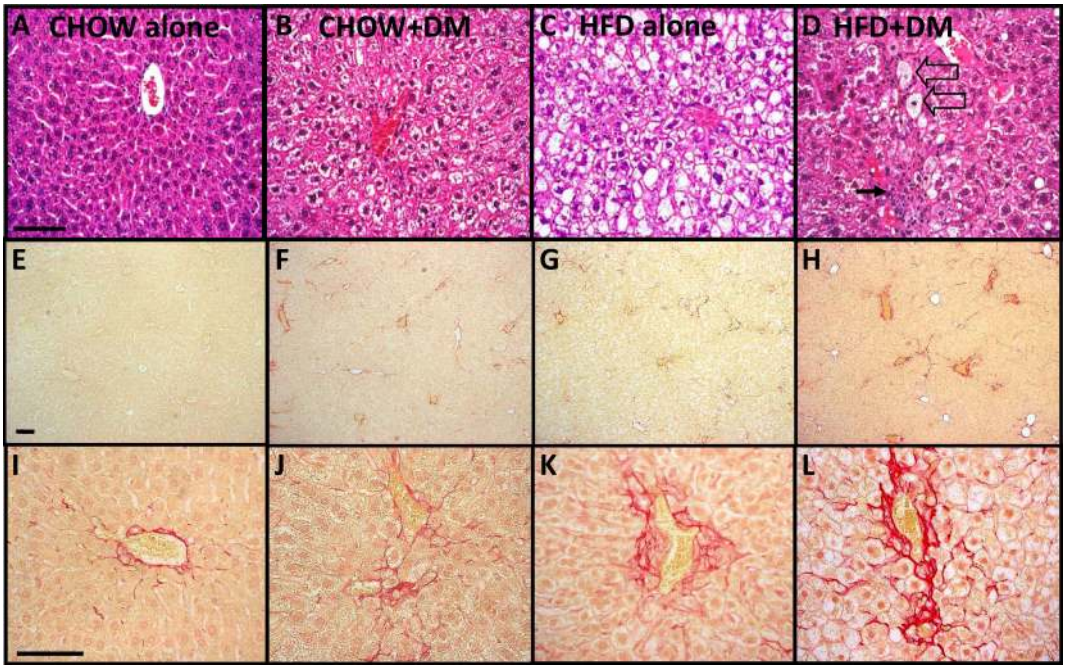

Figure 1. Representative liver images of a wild-type mouse model where a high-fat diet fed *ad libitum* for 15 wk is then combined with induced type 2 diabetes for subsequent 5 wk in C57BL/6 animals (6). Hematoxylin and eosin-stained sections (A–D) show hepatocyte ballooning and lobular inflammatory change in HFD+DM (D, *arrows*). E–L, Picrosirius red staining of liver sections indicates fibrosis, including in the perisinusoid site, which is most marked in the HFD+DM mice (H and L). Images are magnified at ×400 (A–D; I–L) and ×100 (E–H), respectively. Images in I–L are of the portal tract. The published model shows all histological features of NASH in the HFD+DM group, particularly fibrosis progression (6). D, *Open and closed arrows* indicate hepatocyte ballooning and inflammatory change, respectively. In each image, scale bar = 200 μM. CHOW alone, Normal diet (A, E, I); DM, diabetes mellitus (B, F, J); HFD, high-fat diet.

Downloaded from https://academic.oup.com/edrv/article/34/1/84/2354662 by U.S. Department of Justice user on 16 August 2022

has been reported in a C57BL/6 model by other groups in abstract form (135), and it is expected that to help dissect the contribution of diabetes to NASH, further models of timed induction of diabetes will be developed along similar lines.

B. Preclinical and clinical studies examining pathogenic relationships of diabetes and NAFLD

Describing the pathogenesis of NAFLD and NASH in the human with diabetes is complex, and it has been challenging to separate the relative contributions from obesity and other components of the metabolic syndrome. Factors causing NAFLD progression in diabetes are, as yet, not well defined, but they are likely to be an interplay of dysfunctional lipid metabolism and disordered glucose regulation related to insulin resistance with hyperinsulinemia and a relative insulin deficiency, increased oxidative stress, and local and systemic inflammation. Both genetic and environmental conditions are likely to interact to cause NAFLD and NASH and also influence the strong relationship that exists with type 2 diabetes (136, 137).

1. Genetic factors—"common soil"

Genetic susceptibility appears to explain to a large degree familial clustering and ethnic differences in the prevalence of NAFLD/NASH and also why these conditions associate with diabetes and the metabolic syndrome (138). Like diabetes, NAFLD appears to be a heritable disease (139). In addition, familial aggregation of insulin resistance and diabetes in first-degree relatives of patients with NAFLD has been demonstrated (140, 141). A large number of gene variations have been associated with NAFLD (142, 143), but, as is the case with type 2 diabetes, their predictive value is likely to be quite small. In type 2 diabetes, the statistically more important genetic determinants of disease found on genome-wide scanning, such as variation at the TCF7L2 gene, relate mainly to β-cell function rather than to insulin resistance (142, 144). These data reflect that there is a low likelihood of finding a single clear genetic link between type 2 diabetes and NAFLD.

Susceptibility to NAFLD/NASH has been associated with genes influencing insulin sensitivity, in addition to those regulating fatty acid metabolism, oxidative stress, immune regulation, and fibrosis development. Through several genome-wide association studies, the most consistent predictive "NAFLD gene" described involves variations in the adiponutrin/patatin-like phospholipase domain-containing 3 (PNPLA3) gene, with a gene product that is likely to be involved in energy mobilization and storage of lipid droplets (143, 145, 146). Polymorphisms in this gene, the most well-described being rs738409, are thought to alter liver fat content and also to sensitize the liver to environmental stressors, such as obesity or diabetes. The frequency of the rs738409 mutation in Europeans and American Europeans is estimated at 0.23–0.26 (147). Variations in this gene are likely to contribute to racial differences seen in NAFLD prevalence, with people of Hispanic origin demonstrating the highest rates of fatty liver and also polymorphic variation in PNPLA3, followed by individuals of European descent and then African-Americans (143, 148). Specific variations in this domain have also been associated with severe steatosis and progressive fibrosis in adult and pediatric populations. For example, in a small population of 234 patients with type 2 diabetes, the PNPLA3 rs738409 polymorphism was associated with liver fibrosis, assessed noninvasively by FibroTest (a panel of serum markers including bilirubin, γ-glutamyl peptidase, α2 macroglobulin, apolipoprotein A1, and haptoglobulin). This finding was independent of BMI or liver fat content as measured by MRS (149). This functional polymorphism has also been associated with an increased risk of HCC in the setting of cirrhosis (150). The mutation does not appear to be associated with insulin resistance (147) and has not been shown to be increased in type 2 diabetes independent of NAFLD presence (151).

Other single-nucleotide polymorphisms of interest for NAFLD that have been identified in smaller studies include mutations associated with microsomal triglyceride transfer protein (MTP), phosphatidylethanolamine methyltransferase, apolipoprotein C3 (ApoC3), the pregnane X receptor, the superoxide dismutase 2 (SOD2) mitochondrial targeting sequence, and variations in TNF-α, IL-6, and the angiotensin II receptor (143, 152–160). The genetic links between diabetes and NAFLD can be explored by looking at some of these mutations of interest more closely. Further studies examining ApoC3 polymorphisms (T-455C and C-482T) have failed to demonstrate associations with NAFLD in populations other than the original Indian cohort (161, 162), and no relationship of ApoC3 polymorphisms with liver enzymes or insulin resistance was demonstrable by another group (163). In contrast, ApoC3 polymorphisms have been associated with the risk of type 1 and 2 diabetes, with the −482T allele conferring higher risk in lean individuals and the −455C allele being protective for type 2 diabetes in obese individuals (164). Polymorphisms in the SOD2 gene (particularly C47T: rs 4880), thought to play an important role in protection from superoxide radicals, are of ongoing interest in both NAFLD and diabetes. The C allele, when compared with the T allele, results in more efficient transport of SOD2 into the mitochondrial matrix, which can increase the ability of the cell to neutralize superoxide radicals. The T allele has been associated with fibrosis progression in NAFLD by both case-control and intrafamilial association meth-

Downloaded from https://academic.oup.com/edrv/article/34/1/84/2354662 by U.S. Department of Justice user on 16 August 2022

odologies (165). The T allele has also been associated with nephropathy in type 1 diabetes (166) and retinopathy in type 2 diabetes (167). In addition, meta-analysis demonstrated gene protective effects on the risk of diabetes, diabetic nephropathy, and diabetic retinopathy from the C allele of the C47T polymorphism in the SOD2 gene (168). Functional MTP polymorphisms associated with NAFLD have been associated with incident type 2 diabetes (169). More recently, the MTP −493G/T polymorphism has been shown to be associated with pancreatic β-cell dysfunction in NASH patients without diabetes and also in healthy controls (170). Recent meta-analyses have suggested a link between TNF-α mutation −238 but not −308 and an increased risk for NAFLD (65), but data for −238 and −308 are generally negative for an increased risk of type 2 diabetes (171, 172). TNF-α −308 polymorphism has been associated with type 1 diabetes (173).

Intrauterine growth retardation has also been shown to be associated with NAFLD in pediatric populations and may be related to similar epigenetic changes that have been linked to increased type 2 diabetes risk and development of the metabolic syndrome (174).

2. A seeming paradox: insulin resistance with hyperinsulinemia and a simultaneous relative insulin deficiency in diabetes may cause the progression of NAFLD

Insulin resistance and NAFLD can be seen to exist in a self-perpetuating pathogenic cycle, and insulin resistance is recognized as a fundamental orchestrator of NAFLD/NASH (138). The progression of NAFLD in the setting of insulin resistance is likely to be driven by the pathogenic effects of the subsequent hyperinsulinemia and also by varying degrees of relative insulin deficiency, particularly in diabetes. Insulin resistance is almost invariable in type 2 diabetes, and it is also increasingly recognized as an ongoing issue in many patients with type 1 diabetes, especially those of increasing age (175, 176). By definition, prediabetes and diabetes are states of relative insulin deficiency that, when combined with excess caloric intake and obesity, will commonly contribute to increased portal fatty acids and ectopic fat deposition, including in the liver. Conversely, the subsequent pathogenic changes in fatty liver, especially in the setting of NASH, can lead to exacerbation of insulin resistance and, by assumption, diabetes. The specific factors behind the pathogenic association between NAFLD and diabetes will be addressed below (see *Sections VIII.B.2.a and b*).

a. Disordered lipid metabolism in NAFLD pathogenesis: insulin resistance, diabetes, and hepatic steatosis. The steatosis seen in NAFLD is the result of disordered lipid metabolism, where the balance between lipid accumulation and lipid export is disrupted. This is largely the result of the effects of insulin resistance and the subsequent states of hyperinsulinemia and varying degrees of relative insulin deficiency. Because it is difficult to examine turnover of fatty acids in the liver, including their origin and fate in animals, gene expression (steady-state mRNA) and protein levels of key enzymes involved in fatty acid uptake, *de novo* lipogenesis, transport from the liver, and lipid oxidation are often measured as surrogate markers of lipid metabolism (177). These methods show that in insulin-resistant states, both uptake of exogenously derived fatty acid and *de novo* hepatic synthesis of fatty acid, exacerbated by decreased lipid export, lead to an increase in lipid synthesis and hepatic lipid content in NAFLD (177).

The mechanisms by which type 2 diabetes mellitus may exacerbate and cause NAFLD progression remain to be fully defined. However, there is evidence to implicate a number of metabolic and cellular mechanisms. First, diabetes presence indicates some degree of relative insulin deficiency. Any degree of insulin lack, especially in the presence of increased body weight or obesity, will cause increased lipase activity and consequent lipolysis in adipose tissues. This will lead to elevated circulating and portal free fatty acids (FFAs) and subsequently to their increased hepatic delivery and uptake (178). Human data demonstrate that insulin resistance at the level of adipose tissue is the critical location for the development of lipotoxic diseases, including NASH (179), and that liver fibrosis worsens as quartiles of adipose insulin tissue resistance worsen (180). Indeed, insulin resistance at the level of the adipocyte may be the primary defect in NAFLD, leading to increased lipolysis and FFA delivery to skeletal muscle and the liver and subsequent secondary increases in insulin resistance in these tissues (83, 180). A recent study including 118 patients with NAFLD and 20 controls without NAFLD found that muscle and liver insulin sensitivity were impaired in patients with NAFLD to a similar degree, whether they had prediabetes or type 2 diabetes, but that adipose tissue insulin resistance worsened in type 2 diabetes (80). The relative insulin deficiency present in type 2 diabetes will thus fail to properly suppress fatty acid flux to the liver.

The second main mechanism by which diabetes may contribute to NAFLD is via the hyperinsulinemia commonly present in type 2 diabetes, especially in the early years after type 2 diabetes diagnosis. Hyperinsulinemia may exacerbate the liver insult caused by the increased portal delivery of fatty acids due to the relative insulin deficiency. Selective resistance to insulin action can occur in insulin-resistant states, leading to potential adverse effects of endogenous hyperinsulinemia present (181, 182). Specifically, as demonstrated in rodent models, insulin re-

Downloaded from https://academic.oup.com/edrv/article/34/1/84/2354662 by U.S. Department of Justice user on 16 August 2022

sistance may develop at the level of pathways that regulate glucose, including those in the liver, and yet insulin signaling regulating *de novo* lipogenesis can remain sensitively intact. To illustrate this point, differential sensitivity in insulin-mediated lipogenesis compared with gluconeogenesis pathways was recently reported in rat liver in the intracellular signaling system mammalian target of rapamycin complex 1 and its downstream factor S6 kinase (182). Particularly important to NAFLD, this differential signaling is likely to be involved in the up-regulation of gene expression and activated cleavage of the key enzyme regulating lipid synthesis, sterol regulatory element-binding protein (SREBP)-1, which is induced by both insulin and high-fat diets (136, 183). Up-regulation of SREBP-1 will lead to subsequent increases in *de novo* lipogenesis compounded by increased delivery of carbohydrate substrate to the liver due to peripheral insulin resistance and reduced capacity to store carbohydrate in muscle as glycogen (184).

Multiple lines of evidence suggest that the stimulatory effect of insulin on fatty acid synthesis is mediated by an increase in SREBP-1c expression, activation, and nuclear localization (185, 186), including through chromatin remodeling (187). Studies in Goto-Kakizaki rats as a model of type 2 diabetes showed that SREBP1c induction by insulin is dependent more on the intracellular insulin receptor docking protein IRS-2 in postreceptor signaling, involving atypical protein kinase C (PKC)-mediated pathways, rather than through IRS-1 (188). In this model, nuclear factor κB (NF-κB) was also induced through this insulin-sensitive IRS-2 dependent pathway. In contrast to intact insulin-mediated IRS-2 signaling, impaired hepatic protein kinase B (PKB/Akt) activation is well documented in diabetes and has been linked mainly to reduced IRS-1 activation by insulin (188). Furthermore, mouse models overexpressing SREBP1c showed that NAFLD develops (189), whereas animal models of type 2 diabetes show increased SREBP1c expression (186). Finally, there are data in obese rodents exhibiting hepatic steatosis reporting that endoplasmic reticulum (ER) stress may activate SREBP1c protein in insulin-resistant states (190).

Increased lipid delivery to the liver in NAFLD is exacerbated by decreased lipid disposal. This is likely to be the result of many factors. SREBP-1 has been shown to reduce the disposal of FFAs by inhibiting their uptake and oxidation by mitochondria (191). Reduced disposal of FFA is also the result of inadequate extrahepatic transport of triglyceride due to an insulin-dependent reduction in production of apolipoprotein B, leading to a relative reduction in very low-density lipoprotein (LDL) production (137, 191, 192).

The increased storage and decreased disposal of intrahepatic lipids are collectively thought to lead to hepatic FFA storage and oxidative capacity being exceeded and accumulation of lipids that can exert toxic effects on the liver. These toxicities are thought to occur via inefficient-mitochondrial (due to mitochondrial uncoupling) and peroxisomal β-oxidation. This process can contribute to the pathogenic cycle of NAFLD and insulin resistance by resulting in further increases in insulin resistance directly, and also through the induction of inflammation, oxidative stress signaling, and ER stress (137, 138, 179, 193, 194). Inflammatory pathways may be another mechanism by which lipids exert toxic effects. Indeed, inflammatory pathways have been reported to be activated by lipids and are also likely to be induced by ER stress (195). Liver X receptors, part of the superfamily of metabolic nuclear receptors involved in many processes correlating with lipid metabolism and also capable of regulating inflammation, are an important link between lipids and inflammation (196).

Notably, in cases where simple steatosis progresses to NASH, evidence suggests that *de novo* lipogenesis appears to become less prominent, and lobular inflammatory change becomes predominant (49, 197). Although this may reflect that a switch from progressive liver lipid synthesis and accumulation to a more severe phenotype occurs in NASH, it is recognized that the source of most fatty acids for triglyceride synthesis in the liver comes from adipose tissue rather than *de novo* lipogenesis (198), and that toxic lipid metabolites cause inflammation with parallel continued accumulation of lipid, including triglycerides (199). Thus, collectively, current data suggest that some lipid accumulation continues to occur as hepatic inflammation emerges in NASH.

b. Lipid toxicity may progress simple steatosis to NASH. The lipids that develop in hepatic steatosis may themselves be toxic to the liver and contribute to NAFLD progression. Specifically, there is evidence that cholesterol, diacylglycerol (DAG), and glycosphingolipids may mediate this toxicity (179, 194). Analysis of circulating lipids utilizing lipidomic screening has shown that increased DAG and triglyceride, impaired peroxisomal polyunsaturated fatty acid metabolism, and also nonenzymatic oxidation of lipid are associated with progression to NASH (200), as are lower levels of phosphatidyl choline and a higher ratio of free cholesterol to phosphatidyl choline (201).

Cholesterol has been implicated as a pathogenic factor for NAFLD progression in the *foz/foz* mouse with type 2 diabetes fed a high-cholesterol diet. In this model, hyperinsulinemia was identified as the factor leading to hepatic cholesterol accumulation, which then resulted in NASH

Downloaded from https://academic.oup.com/edrv/article/34/1/84/2354662 by U.S. Department of Justice user on 16 August 2022

with fibrosis (202). In addition, insulin applied *in vitro* to hepatocyte primary cultures in concentrations present in type 2 diabetes *in vivo* was shown to recapitulate the accumulation of cholesterol, up-regulation of LDL receptor via activation of SREBP-2, and reduced cholesterol biotransformation to bile acids for cholesterol and bile acid excretion (202). A high-fat diet with a high cholesterol level has also been implicated in hepatic immune Kupffer cell activation (50).

Hepatic lipid present in a fatty liver may cause NAFLD progression from simple steatosis to NASH. Although data have shown that hepatic triglyceride (triacylglycerol) is thought to be protective of NASH progression, DAG is a lipid product implicated through downstream atypical PKCε signaling, in exacerbating both systemic insulin resistance (203) and hepatocyte toxicity (204). Lysophosphatidyl choline, derived from phosphatidyl choline, a component of the lipid membrane and also of very LDL, may also contribute to lipotoxicity with increased levels of this phospholipid found to be proportional to disease severity in a small liver biopsy series. Direct lipotoxic effects of lysophosphatidyl choline have also been observed in human liver cell lines, mouse hepatocytes, and in mice (179).

As an additional potential method by which lipid may be toxic in NAFLD, saturated fatty acid may lead to increased hepatic glycosphingolipids, including ceramide (205), which may then contribute to apoptosis of native liver cells as well as inflammatory change (206). Although ceramides have recently also been proposed to cause NAFLD progression through the effects of TNF-α (207), lipidomic screening has not implicated circulating ceramides in NAFLD and NASH pathogenesis, and further studies are required to determine whether they have a role.

c. Hepatic inflammation and oxidative stress. The main inflammatory factors and cellular pathways implicated in causing NASH, compared with profiles in simple steatosis, have recently been extensively reviewed (208, 209). These are: NF-κB and its downstream pathway; macrophage chemoattractant protein-1 and its receptor, C-C chemokine receptor 2; TNF-α and its signaling; and the interleukins IL-1β, IL-18, and IL-33 (208, 210, 211). c-Jun N-terminal kinase is an important second messenger system activated in inflammation in NASH (212). Hepatocytes are thought to have major proinflammatory cell roles in NASH, as are other cells such as Kupffer cells and, potentially, natural killer cells, natural killer T cells, T cells, sinusoidal endothelial cells, and HSCs (213). The inflammation seen in NAFLD may contribute to defects in insulin signaling. This is supported by data showing that inflammatory signals such as TNF-α and IL-6 affect cellular pathways that intersect with insulin action and appear to activate serine and threonine kinases that have been implicated in increased serine phosphorylation of IRS-1 and subsequent decreased insulin signaling (184). Although it has not been clarified to date whether increasing severity or a certain cytokine profile of hepatic inflammation will cause NASH progression, some data support the hypothesis that the presence of inflammation predicts fibrosis in NASH (214, 215).

Oxidative stress generated from ROS may augment inflammation and up-regulate key factors and pathways of NF-κB activation via inhibitor of κ B kinase, c-Jun N-terminal kinase activation, and toll-like receptor (TLR) signaling (178, 208, 216). Many sources of oxidative stress have been identified in NASH and may be derived from mitochondria, possibly ER, cytochromes P450 2E1 and 4A, and also cytosolic peroxisomes and NADPH oxidase as well as infiltrating and resident inflammatory cells. In a study using both case-control and intrafamilial association methodologies, a consistent association between a functional single-nucleotide polymorphism in the mitochondrial targeting sequence of *SOD2*, a gene thought to be important in protecting against superoxide radicals, and fibrosis severity in NAFLD provides persuasive genetic evidence that mitochondria-derived oxidative stress is important in the pathogenesis of advanced NAFLD (165). Innate immunity, in particular in the resident macrophage, the Kupffer cell, has also been implicated in inflammation in NASH, possibly secondary to responses after hepatocyte apoptosis (217, 218). The "inflammasome," which is a larger multimeric structure that regulates caspase 1 activation, is linked to mitochondrial production of ROS and innate immunity (219). Although oxidative stress and related cytokines may not be the primary initiators of liver inflammation in NASH, their roles in insulin resistance, perpetuation of liver necroinflammatory change, and progressive fibrogenesis are thought to be likely (208, 220). Oxidative stress has been linked with altered mitochondrial function, as evidenced by diminished respiratory chain activity and reduced ATP production in steatohepatitis (221).

To date, diabetes has not been reported to magnify inflammatory changes present in NASH either at the level of the hepatic cytokine profile, in the degree of hepatic oxidate stress induced, or in liver histopathological studies of inflammation in NASH (6). In contrast, the circulating level of the adipocyte-derived cytokine, adiponectin, does fall in prediabetes and type 2 diabetes (222), and its reduced levels will in an endocrine manner remove protection against proapoptotic effects of TNF-α on hepatocytes (223). Serum adiponectin levels can distinguish simple steatosis from established NASH (224). Thus, reduced adiponectin could be one

Downloaded from https://academic.oup.com/edrv/article/34/1/84/2354662 by U.S. Department of Justice user on 16 August 2022

link between adipocytes and NASH in type 2 diabetes, including through loss of its direct antiinflammatory effects on the liver.

d. Disordered insulin signaling and hepatic fibrosis. It can be speculated, but remains to be substantiated, that hyperinsulinemia in type 2 diabetes, possibly in combination with the elevated blood glucose in diabetes, contributes to the fibrosis seen in progressive NASH (77, 79). In the animal model referred to earlier, where diabetes is induced in a high-fat-fed murine model, the diabetic high-fat-fed rodents that showed less hyperglycemia, and yet hyperinsulinemia, demonstrated the most liver fibrosis on histology. This finding suggests that some insulin action in diabetes is necessary for progression of fibrosis and also that hyperglycemia may be a permissive factor for liver fibrosis rather than the most important mediator causing fibrosis in this model of NASH (6). Furthermore, selective hepatic insulin resistance may also play a role in the development of liver fibrosis in NASH. Although insulin resistance in phosphatidylinositol-3 kinase signaling occurs in some tissues, in parallel, selective retention of insulin-mediated signaling via MAPK (ERK1/2) pathways occurs (225). There are limited, recent data in Sprague-Dawley rats to indicate that similar selective postreceptor insulin signaling may occur in the liver in insulin-resistant states, mediated in part by C-reactive protein (226). This may be important because phosphatidylinositol-3 kinase mainly mediates glucose cellular metabolism, and MAPK regulates mainly other cellular effects including mitogenesis and laying down of extracellular matrix. The ERK MAPK pathway has been described *in vitro* to induce certain profibrotic factors, including CTGF and TGF-β in activated HSCs (227, 228) and to cause proliferation of rat HSCs in primary culture (229). Decreased adiponectin levels seen in diabetes may also contribute to fibrosis progression; adiponectin has antifibrogenic properties, associated with decreased expression of markers of activation of HSCs and increased apoptosis of activated HSCs. Inhibition of leptin signaling, a profibrotic adipokine, via inhibition of JAK2 and specific up-regulation of suppressor of cytokine signaling 3 expression by adiponectin are likely to contribute (230).

3. NAFLD can contribute to development of diabetes by disordered lipid metabolism

One of the primary mechanisms by which NAFLD contributes to type 2 diabetes is by increasing insulin resistance. Patients with NAFLD have significantly increased levels of hepatic and peripheral insulin resistance (skeletal muscle and adipose tissue), and diabetes presence is associated with an apparent further increase in insulin resistance at the level of the adipocyte (80). In NAFLD, insulin resistance is thought to be promoted by the accumulation of fatty-acid metabolites (primarily DAG) within insulin-sensitive tissues, and particularly in the liver (184). Although whole body insulin resistance typically cosegregates with generalized obesity (184), it has been reported that insulin-resistant compared with insulin-sensitive individuals have increased lipid accumulation in their skeletal muscle and liver, but no demonstrable difference in sc or visceral adiposity (231), thus implicating liver more than visceral fat as a marker and possible mediator of insulin resistance. In addition, in people with lipodystrophies, which are an extreme example of insulin resistance but with an overall lack of adiposity, there is an increased prevalence of type 2 diabetes.

Lipodystrophies, as a discrete collection of genetic and acquired disorders involving a lack of adipocytes and hypoleptinemia, are associated with profound hepatic and peripheral insulin resistance. People with lipodystrophy typically have hypertriglyceridemia and ectopic fat deposition, including hepatic steatosis. In a study of a genetic form of this condition, severe congenital generalized lipodystrophy, patients were found to have increased basal rates of glucose production, inability to suppress hepatic glucose production, and inability to stimulate peripheral glucose uptake during hyperinsulinemic-normoglycemic conditions. When given leptin, these patients had a 90% reduction in liver triglyceride content and improved hepatic insulin sensitivity, in addition to a 30% reduction in muscle triglyceride content and an approximate 2-fold increase in insulin-stimulated whole body glucose disposal (184, 232).

The effect of the accumulation of fatty acid metabolites (mainly DAG) within insulin-responsive tissues has been most extensively studied in skeletal muscle via experiments in first-degree, lean, relatives of people with type 2 diabetes, by lipid infusions in normal controls, and by genetic murine models (83). Hepatic steatosis also develops in murine models of peripheral insulin resistance [*e.g.*, models involving muscle-specific deletions of the glucose transporter (GLUT) 4] and is also associated with hepatic insulin resistance in its own right (184). Wild-type mice and rats develop hepatic steatosis after a few days of high-fat feeding that is associated with hepatic insulin resistance, without much change in muscle lipid content or peripheral insulin action. The effect of hepatic steatosis on insulin resistance, as demonstrated in this rodent model, seems linked to decreased signaling of IRS-1 and -2 by the insulin receptor, leading to an impaired ability of insulin to activate hepatic glycogen synthesis and suppress hepatic glucose production (184, 233). A similar defect has been shown in patients with type 2 diabetes. As in skeletal mus-

Downloaded from https://academic.oup.com/edrv/article/34/1/84/2354662 by U.S. Department of Justice user on 16 August 2022

cle, an isoform of PKC is the likely link. PKCε is expressed in increased concentration and is activated in fatty liver. The activation of PKCε is prevented in mouse models if hepatic steatosis is prevented from developing (184).

Apart from insulin resistance, there is some evidence that insulin secretion may also be defective in people with NAFLD. It is proposed that a sustained elevation in FFAs, as can occur in NAFLD, can lead to pancreatic β-cell lipotoxicity (83). In humans, a sustained elevation of plasma FFA levels has been shown to impair insulin secretion in lean, nondiabetic subjects genetically predisposed to develop type 2 diabetes (234), and it was shown that FFA-induced pancreatic β-cell dysfunction can be rapidly reversed in these subjects by decreasing the release of FFAs from adipose tissue by experimentally inhibiting hormone-sensitive lipase with the nicotinic acid derivative acipimox (83, 235).

4. Chronic hyperglycemia in diabetes may contribute to NAFLD progression

Advanced glycosylation end-products (AGE), the result of nonenzymatic reactions between sugars and proteins, lipids or nucleic acid, and up-regulation of their receptors, are a recognized metabolic outcome of chronic hyperglycemia, as occurs in diabetes. Many of the micro- and macrovascular complications described in diabetes have been attributed to AGEs, due to direct effects of AGEs on the structure and function of proteins and also their indirect effects via intracellular signaling. One of the main intracellular signaling pathways involves activation of NF-κB signaling with subsequent up-regulation of the transcription of intracellular adhesion molecule-1, E-selectin, endothelin 1, tissue factor, vascular epithelial growth factor, and proinflammatory cytokines including TNF-α and IL-6. This pathway also leads to increased expression of the receptor for AGEs (236). Increased levels of AGEs have been observed in NAFLD. It has also been shown *in vitro* that AGEs promote triglyceride deposition in hepatocytes and fibrogenesis through induction of ROS in HSCs (237, 238).

Hyperglycemia *per se* can also directly contribute to hepatic steatosis and lipotoxicity by increasing hepatic fat synthesis and reducing fat oxidation by activation of the hepatic transcription factor carbohydrate response element-binding protein (136, 239). Subsequent FFA and DAG accumulation in the liver will reduce the inhibitory effect of insulin on gluconeogenesis, contributing further to hyperglycemia (191, 240).

Preclinical *in vitro* studies show that hyperglycemia in the presence of hyperinsulinemia can also promote fibrogenesis by causing up-regulation of CTGF and inducing oxidative stress in HSCs, thus promoting fibrogenesis (137, 241). *In vitro* experiments using rodent HSCs have shown that hyperglycemia can lead to proliferation and activation of these cells in a dose-dependent manner, associated with a biphasic increase in intracellular glucose (242). These increases in intracellular glucose in the HSC are caused by increased cellular synthesis of GLUT2 and by up-regulation of plasma membrane translocation of the glucose transporter GLUT2 (242). Activated rodent HSCs *in vitro* in the setting of hyperglycemia were shown to have increased abundance of α1 procollagen and α-smooth muscle actin (markers of activation), increased promitogenic factors including platelet-derived growth factor-β receptor, cyclin D1, and antiapoptotic Bcl-2, whereas having reduced abundance of the pro-apoptotic Bax. They also had increased expression of the profibrogenic proteins type-I and type-II TGF-β receptors and CTGF. There were increased levels of intracellular ROS and lipid peroxides, with a decreased level of the antioxidant glutathione, presumed secondary to reduced activity of the rate-limiting enzyme for *de novo* synthesis of glutathione, glutamate cysteine ligase (242).

5. Endotoxemia may contribute to NAFLD progression

The gut/liver axis is increasingly being recognized as a significant contributor to NAFLD pathogenesis. It is thought that the increased absorption of lipopolysaccharides (LPSs) resulting from a "leaky" small intestinal mucosa may cause activation of the innate immune system, leading to inflammation and increased expression of inflammatory cytokines. Thus, LPSs can indirectly increase insulin resistance via these mechanisms but may also have a direct effect on insulin sensitivity itself (243, 244) and may also impair pancreatic β-cell function (245). Conversely, the accumulation of FFAs in the liver that is exacerbated by insulin resistance can in turn facilitate activation of the innate immune system by direct stimulation of TLR-signaling (243, 246–249), including hepatic TLR-4 (208).

Patients with type 2 diabetes have mean plasma values of LPS 76% higher than controls, and levels in these patients can be significantly reduced with the use of the PPAR-γ agonist, rosiglitazone (250). The increased LPS levels in patients with diabetes are likely to be due in part to their decreased clearance as a result of the detrimental effects of chronic hyperinsulinemia on the hepatic resident phagocytic cell, the Kupffer cell (248, 251). Hyperinsulinemia, in combination with hyperglycemia, may also decrease jejunal motility and increase gastrointestinal transit time (248, 252). These changes to gut motility can lead to small intestinal bacterial overgrowth and a subsequent increased leakiness of the intestinal mucosa (253). The

Downloaded from https://academic.oup.com/edrv/article/34/1/84/2354662 by U.S. Department of Justice user on 16 August 2022

absorption of LPS can also be enhanced by a diet high in saturated fat (248, 254).

6. Environmental common soil of NAFLD and type 2 diabetes

Visceral adiposity is a common phenotype of, and an established risk factor for, type 2 diabetes and also NAFLD (255, 256), and it may contribute to the unfavorable cytokine/adipokine balance and fatty acid metabolism that exists in both of these conditions. In an Australian study of 38 individuals who underwent liver biopsy and MRS to assess for visceral fat content, every 1% increase in visceral fat had an OR of 2.4 for increasing inflammation and an OR of 3.5 for fibrosis on liver histopathology (255). The literature in this area is conflicting, however, because in a study of 2589 individuals from the Framingham Heart Study cohort, where the presence of NAFLD was assessed by abdominal computed tomography (CT), the association between NAFLD and diabetes and IFG was found to be independent of visceral adipose tissue and other obesity measures (257). Similarly, an Italian group used retrospective data on 431 patients with NAFLD confirmed on liver biopsy, split into three groups by waist circumference as a widely accepted indirect measure of visceral adiposity, and found that whereas the prevalence of metabolic derangement and severe steatosis increased with a higher waist circumference, the prevalence of NASH or severe fibrosis ($F \geq 2$) was not significantly associated on multivariate analysis. The authors proposed that once NAFLD is present, visceral fat may no longer be a major determinant of the severity of liver damage (258, 259).

Associated with increased adiposity is the tendency for patients with both NAFLD and/or type 2 diabetes to consume a diet higher in saturated fat and fructose, to perform less exercise (138, 260–262), and to smoke (5, 263). Conversely, higher caffeine intake and intense exercise for more than 150 min/wk were associated with less fibrosis on liver biopsy (264, 265). To support the contribution of diet to NAFLD, in a mouse model involving the feeding of a "fast-food" diet (AIN-76 Western Diet, Test Diet; relatively rich in saturated fats, cholesterol and fructose) to genetically unaltered C57BL/6 mice for 25 wk, researchers found that these animals developed similar levels of obesity and insulin resistance to mice fed a high-fat diet, but that they had significantly more hepatomegaly, higher serum cholesterol, higher AST, and significantly more hepatic inflammation, hepatocellular ballooning, and fibrosis (266).

The link between NASH, smoking, and diabetes appears complex. A recent analysis of data collected on 1091 adult subjects from the U.S. multicenter NASH Clinical Research Network found in patients without diabetes the OR of advanced fibrosis was 2.48-fold in patients that had at least a 10-yr smoking history compared with those that had never smoked on multivariate analysis. A dose-response relationship was observed between packet years and fibrosis stage. However, whereas patients with diabetes were at a similar increased risk for advanced fibrosis as those who did not have diabetes who had a significant smoking history, the addition of smoking did not further increase their risk. The authors proposed that smoking may cause hepatic injury by increasing insulin resistance. Therefore, the effect of smoking in diabetes may be obscured by confounding related to the intermediary variable effect of insulin resistance (5).

Biochemical and hormonal links between diabetes and NAFLD include associations with low vitamin D (267–269) and elevated ferritin levels (270, 271), in addition to abnormalities in GH and IGF-I (272–276). Abnormalities in sympathetic outflow and the renin-angiotensin system (RAS) are also shared between these two conditions (191, 277–279). Obstructive sleep apnea may also be important in both NAFLD and diabetes (280–282).

C. Pathogenesis of NAFLD progression summarized

Although the concept of a "two-hit hypothesis" was popular some years ago, where NASH was thought to develop as the result of a pathogenic "second hit" occurring after the initial insult of steatosis (283), it is now recognized that an integrated response to parallel and interacting hepatic insults may be a more correct model to consider in NAFLD progression (50, 138). Current preclinical and clinical data suggest that when added to a high-fat diet with obesity, diabetes can cause increased NASH. As described, the common soil of both genetic predisposition and environmental factors is likely to interact with the milieu of metabolic derangements present in type 2 diabetes to cause NAFLD progression. Diabetes, acting through some degree of relative insulin deficiency causing increased lipolysis and thus increased fatty acid delivery to the liver, combined with hyperinsulinemia and selective hepatic sensitivity to insulin leading to preferential stimulation of certain hepatic lipid synthesis and cholesterol pathways, is likely to be key to the progression of NAFLD in this setting. Selective hepatic response to hyperinsulinemia, combined with ongoing liver inflammation, may then induce profibrotic factors in the liver. In addition, as described above, the hyperglycemia and advanced glycation of diabetes may contribute to increased cellular oxidant stress and induction of proinflammatory and especially profibrotic factors, activating HSCs to cause accumulation of liver extracellular matrix (241). Metabolic and cellular mechanisms by which diabetes

Downloaded from https://academic.oup.com/edrv/article/34/1/84/2354662 by U.S. Department of Justice user on 16 August 2022

Figure 2.

Figure 2. Schematic diagram indicating proposed pathogenic process by which type 2 diabetes exacerbates NAFLD and how NAFLD may lead to type 2 diabetes and suboptimal glycemic control. Sequentially, 1) in obese individuals, diabetes onset causes increased lipolysis in adipose tissue and increased fatty acid delivery to the liver; 2) hyperinsulinemia persisting in diabetes and selective hepatic insulin resistance induces *de novo* lipogenesis early, and later, hepatic fibrosis; 3) hyperglycemia and AGE contribute to hepatocyte death, activation of HSCs, and induction of profibrotic factors, such as CTGF. In turn, hepatic insulin resistance secondary to NAFLD may lead to compensatory hyperinsulinemia and secondary pancreatic β-cell failure with progressive hyperglycemia and type 2 diabetes onset. *Asterisk* indicates where genetic susceptibility to type 2 diabetes development is thought to play a particularly important role for most diabetes susceptibility genes, for example TCF7L2.

may exacerbate NASH, including evidence from animal models, are summarized in the schematic diagrams in Fig. 2 as an overview and Fig. 3 in the context of the liver.

D. Type 1 diabetes and liver pathology

Type 1 diabetes is characterized by autoimmune destruction of pancreatic β-cells and absolute insulin deficiency, requiring insulin therapy to sustain life. Classically, a person with type 1 diabetes will not have excess body weight or major features of the metabolic syndrome. In contrast to type 2 diabetes series, clinical and preclinical studies in type 1 diabetes and potential effects in the liver are relatively few. Although in a long-term 10-yr diabetes duration nonhuman primate (baboon) model of type 1 diabetes overt NASH did not occur, at the electron microscopic level, liver sinusoidal epithelium thickness was increased and fenestrations were markedly reduced, potentially affecting clearance of lipoproteins by the liver and producing dyslipidemia (284). Studies of type 1 diabetes in rodents (285) and in humans (286) have previously led to similar findings, with the term "diabetic hepatosclerosis" being used to describe these microangiopathic liver changes that reflect microangiopathy in small vessels in other organs affected by diabetes (287). Therefore, in long-term type 1 diabetes without the metabolic syndrome, it is possible that ultrastructural pathological changes occur in the hepatic sinusoidal spaces causing hepatosclerosis, which is distinct from any subtype of NAFLD. Some people who have type 1 diabetes also display metabolic syndrome features seen in type 2 diabetes (288), and it is possible, but not yet reported, that NAFLD and diabetic hepatosclerosis coexist in people with type 1 diabetes who have the metabolic syndrome phenotype.

IX. Assessing NAFLD in Patients with Diabetes

Current data suggest that adverse outcomes related to NAFLD appear closely linked to the degree of necroinflammatory activity and/or fibrosis in the liver, rather than the presence of steatosis (1). This makes the identification and grading of NASH an essential component to a comprehensive assessment of NAFLD. Indeed, defining the degree of NASH will become even more important as effective treatment options become available. Unfortunately, whereas many reasonable tests exist for detecting fatty liver *per se*, few established techniques exist for grading the degree of NASH, apart from the invasive method of liver biopsy (68).

A. Screening for NAFLD

1. Imaging

NAFLD may be detected by a variety of imaging techniques including ultrasound, CT scan, and nuclear MR imaging (MRI) and MRS (136, 289–291). Ultrasound uses a combination of hepatorenal echo contrast, liver brightness, deep attenuation, and vascular blurring to make a diagnosis. Due to its low cost, safety profile, and availability, ultrasound is currently the study of choice (68, 69). Ultrasound has a sensitivity of 60–94% and specificity of 84–95% to detect fatty liver (78, 136). Unfortunately, it becomes less sensitive with a decrease in the liver fat content below 33% (68) or with an increase in abdominal obesity (69, 136). Therefore, ultrasound may be missing significant burden of disease, such as in the case of a patient with a highly fibrosed but lipid-poor liver (292), or the fatty liver of an obese patient at higher risk of NASH and its complications (136). Various grading systems have been developed to

Downloaded from https://academic.oup.com/edrv/article/34/1/84/2354662 by U.S. Department of Justice user on 16 August 2022

Figure 3.

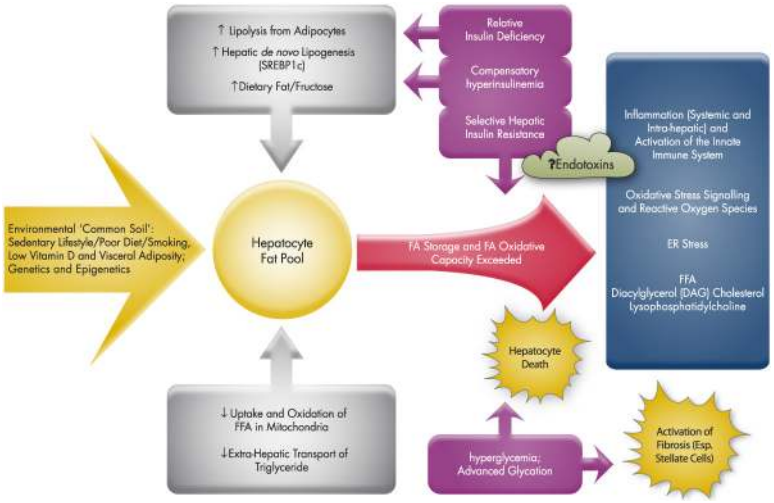

Figure 3. Pathogenesis of progressive NAFLD and the proposed mechanism(s) of type 2 diabetes (shown in *central purple boxes*), in increased lipolysis, hyperinsulinemia, selective insulin resistance, and hyperglycaemia. NAFLD processes of proinflammatory transcription factor up-regulation and Kupffer cell activation are not shown in the schematic; to date, diabetes has not been shown to impact on these pathogenic factors.

assess for severity of steatosis in NAFLD with ultrasound, in addition to complementary measures of visceral fat; however, none of them are externally validated and they do not appear to provide any further useful prognostic information about inflammation or fibrosis (293, 294).

2. Liver enzymes

Liver enzymes are not a sensitive marker of NAFLD. In patients with type 2 diabetes and histologically proven NASH, abnormal liver enzymes may be seen in less than 20% of patients, depending on the reference ranges applied (295). Indeed, the most severe forms of liver disease can occur in patients with completely normal liver enzymes (136, 296, 297). One factor contributing to the low rate of liver enzyme abnormality in NAFLD/NASH is likely to be the falsely high levels of traditional reference ranges. These ranges were developed from populations that included individuals with undiagnosed HCV and NAFLD. For this reason, various groups have suggested lowering the upper limit of normal of liver enzyme reference ranges (298). For example, new normal levels for ALT specify serum levels of 19 IU/liter or less for women and 30 IU/liter or less for men (299). In a study of 233 women with class II or III obesity using these reference ranges, only 36% of patients had normal liver enzymes compared with 72.5% using their traditional reference range for ALT of 30 IU/liter or less for women. When comparing these results to liver biopsy in this cohort, the researchers found that the new, lower reference ranges were able to increase the detection of patients with fatty liver and portal fibrosis, but were unhelpful in identifying NASH of any severity (300).

B. Determining the degree of inflammation and fibrosis in the liver

1. Liver biopsy

Liver biopsy remains the "gold standard" method to diagnose NASH, enabling a confirmation of the diagnosis and also a grading of fibrosis and other histological features such as the degree of steatosis, iron deposition, necroinflammatory activity, and associated architectural distortion (66). When reporting studies looking at liver histopathology in type 2 diabetes, some investigators suggest that liver biopsy should be considered in all patients diagnosed with diabetes and hepatic steatosis on ultrasound because of the high prevalence of NASH and advanced fibrosis in these cohorts (71). However, liver biopsy is an invasive procedure with attendant risk of pain, bleeding, or perforation (1/1,000) and, rarely, death (1/10,000). Liver biopsy is also costly, an important factor when considering a condition as common as NAFLD, and is inconvenient to both the patient and physician (66).

Liver biopsy is an imperfect gold standard due to inherent problems that limit its accuracy. Sampling error may occur because the biopsy itself represents only 1/50,000th of the total liver. Paired biopsy studies of NAFLD have demonstrated a rate of discordance for fibrosis stage as high as 22–37% (66, 301, 302). In fact, substantial agreement on paired biopsy samples in NAFLD has only been shown for steatosis (301). This problem is accentuated if the biopsy sample does not contain enough liver tissue (303). Consistency of histological staging is also hampered by relatively high rates of intra- and interobserver variability among histopathologists. When grading fibrosis in NAFLD, intraobserver variability has been estimated at 0.65–0.85 and interobserver variability at 0.84. Like the findings on paired biopsies, intra- and interobserver variabilities are also significant for necroinflammation and ballooning, which in consequence has the potential to affect the diagnosis of NASH or "not NASH" (66, 302).

2. Imaging

As previously mentioned, few imaging studies are available for assessing NAFLD severity. Liver stiffness, as measured by ultrasonographic techniques such as FibroScan or acoustic radiation force impulse (ARFI), or with the less available MR elastography (MRE), have been used to es-

Downloaded from https://academic.oup.com/edrv/article/34/1/84/2354662 by U.S. Department of Justice user on 16 August 2022

timate the presence and severity of hepatic fibrosis. These and other methods are summarized in Table 3.

a. Transient elastography using FibroScan (EchoSens, Paris, France). The FibroScan device is being used increasingly as a noninvasive tool to assess for liver fibrosis. Vibrations of mild amplitude (1 mm) and low frequency (50 Hz) are transmitted by a transducer, inducing an elastic shear wave that propagates through the underlying tissues. Pulse-echo ultrasound acquisitions are used to follow propagation of the shear wave and to measure its velocity, which is directly related to tissue stiffness (the elastic modulus): the stiffer the tissue, the faster the shear wave propagates. The device measures a volume that approximates a cylinder 1 cm wide and 4 cm long, 25–65 mm below the skin surface. This volume is at least 100 times larger than that of a needle biopsy sample.

The diagnostic accuracy for measurement of significant (stage ≥ F2) or advanced (stage ≥ F3) fibrosis by FibroScan has been assessed in multiple studies in NAFLD. The diagnostic accuracy and reproducibility of FibroScan for advanced liver fibrosis was good and was unaffected by the severity of steatosis or inflammation in most studies (1). In one study, Wong *et al.* (304) found the area under the receiver operator characteristic curve (AUROC) for significant fibrosis (perisinusoidal and portal/periportal, F2), advanced fibrosis (septal or bridging fibrosis, F3) and cirrhosis (F4) to be 0.84, 0.93, and 0.95 respectively, in a series of 246 individuals from France and Hong Kong who underwent liver biopsy and FibroScan.

Although FibroScan is of value at the extreme ends of the spectrum to rule out significant pathology and also to identify patients who are likely to have advanced fibrosis, it does result in a large number of individuals having an "intermediate" value requiring further assessment and counseling (51). In addition, in all studies to date using the standard probe, the presence of obesity has been an independent predictor of failure to measure liver fibrosis (1, 305). In the study by Wong *et al.* (304), over 25% of patients with a BMI of 30 kg/m^2 or greater had an unsuccessful measurement due to attenuation of shear waves into the liver by sc fat. Moreover, even when FibroScan succeeds in producing a measurement, sc fat tends to lead to an overestimation of liver stiffness (306). These factors are of particular relevance in individuals with diabetes who collectively comprise a population recognized to have a higher BMI than the general population (305), and they have been the driving force behind the development of an extra large (XL) probe for obese patients.

The XL FibroScan probe is useful in obesity because it transmits at a lower frequency (3.5 *vs.* 2.5 Hz) and has a more sensitive ultrasonic transducer, a deeper focal length, a larger vibration amplitude (3 *vs.* 2 mm), and a greater depth of measurement below the skin surface (35–75 *vs.* 25–65 mm), allowing stiffness measurement at a greater depth. In an "in-house" clinical trial, the maximum BMI of a patient with a successful measurement was 61 kg/m^2 (307). The XL probe has now been validated in at least three studies in NAFLD (308–310). In a study of 276 patients with chronic liver disease and a BMI greater than 28 kg/m^2, 46% of whom had NAFLD, the failure rate of measurement with the XL probe was significantly lower than with the standard medium (M) probe at 1.1% compared with 16%, respectively. The measurements obtained were also more reliable. The XL probe readings correlated well with the M probe values, with similar AUROCs for significant fibrosis (0.83 *vs.* 0.86; $P = 0.19$) and cirrhosis (0.94 *vs.* 0.91; $P = 0.28$). Median stiffness readings for fibrosis levels were lower for the XL probe

TABLE 3. Summary of noninvasive imaging investigations for NAFLD and its progression, with a focus on studies including diabetes

Test	Dx NAFLD	Dx NASH	Assess fibrosis	External validation	Experimental *vs.* clinical use	Cost	Availability	Factors affecting result	Refs.
Liver ultrasound (i)	√	x	x	√	C/E	+	++++	Liver fat < 30%, obesity, operator experience, and machine quality	68, 69, 136, 290
Unenhanced liver CT (ii)	√	x	x	√	C/E	++	+++	Liver fat < 30%, protocol used, operator experience, other liver pathology (glycogen/siderosis)	136, 289
Liver MRI/MRS (iii, iv)	√	x	x	√	C/E	++++	++	Operator experience	136, 291
Transient elastography (FibroScan) (iii)	x	x	√	√	E	++	++	Higher BMI/obesity, ascites, operator experience, probe used (M *vs.* XL), ?diabetes	1, 51, 304, 305, 306, 308, 309, 310, 311
Acoustic force radiation-pulse imaging (iii, vi)	x	x	√	√	E	++	++	Higher BMI, motion artifact, operator experience	312, 313
MRE (iii, v, vi)	x	√	√	x	E	++++	+		314

Dx, Diagnosis; E, experimental; C, clinical; ?, possibly; √, yes; x, no.

i) Qualitative; ii) semiquantitative; iii) quantitative; iv) MRS is noninvasive reference standard for steatosis assessment; v) limited data suggest that MRE can detect steatohepatitis before fibrosis development with sensitivity 94% and specificity 73% for NASH compared with simple steatosis; vi) limited validation data available.

Downloaded from https://academic.oup.com/edrv/article/34/1/84/2354662 by U.S. Department of Justice user on 16 August 2022

than the M probe (310), perhaps demanding a reassessment of the recommended cutoff values used for defining degrees of fibrosis in individual patients (310). Similar to the M probe in obesity, the XL probe may be less accurate at levels of severe obesity (BMI > 40 kg/m^2) due to the attenuation of signal from sc fat (308).

FibroScan appears particularly suitable for screening at-risk populations, such as a large diabetes cohort, given its convenient size, acceptability to patients, and ability to produce an immediate estimate of liver stiffness. In a study using FibroScan as a screening tool for significant liver disease involving 1190 patients over the age of 45 and presenting for a general medical check-up, elevated liver stiffness measurements (>8 kPa) were found in 7.5% of the population (311). Although liver enzymes were found to be normal in 43% of the patients with elevated liver stiffness measurements, a cause for liver disease was determined in all cases by clinical assessment, with or without liver biopsy. NAFLD was detected in 58% (52 from a total of 89) of these patients, being the most common diagnosis. Liver biopsy was obtained in 27 patients, including nine patients with liver stiffness measurement greater than 13 kPa. All nine patients with liver stiffness measurement greater than 13 kPa had cirrhosis on liver biopsy (five secondary to alcoholic liver disease, three due to HCV, and one due to chronic hepatitis B virus). All except one (simple steatosis) of the remaining 17 liver biopsies demonstrated fibrosis, and eight of these 17 liver biopsies were consistent with NAFLD (311).

b. Acoustic radiation force-based shear stiffness (ARFI). ARFI involves the generation of shear waves in the liver by inducing transient tissue deformations of several microns by acoustic radiation force (312). Advantages of ARFI over FibroScan include the ability to obtain measurement in the presence of ascites and the capacity to use some commercial ultrasound scanners for measurement. The success of this technique also appears to be affected by higher BMI, although reconstructions have been achieved up to a BMI of 66 kg/m^2 (312). It is also probable that a higher level of ultrasound technical expertise may be required in comparison to the FibroScan, although accuracy of both modalities is likely to be affected by operator experience (305, 312). From limited studies, this technique does appear to produce comparable results to FibroScan (312, 313). More multicenter prospective studies are needed to rigorously evaluate the clinical utility of ARFI in comparison to equivalent techniques and also to establish reliable cutoff values for diagnosis, as a function of controlled patient population variables including gender, age, BMI, and liver disease etiology (312).

c. Magnetic resonance elastography. MRE involves a drum-like acoustic passive driver positioned against the body wall and secured with an elastic belt. This passive driver is then connected to an active acoustic driver system located outside of the MR scanner room via a polyvinylchloride tube. The active driver generates acoustic vibrations in the body, producing shear wave motion within the liver. This technique has minimal validation data but does appear to be more accurate than ultrasound techniques, particularly at lower levels of fibrosis. In particular, from limited data, MRE appeared to have the capacity to identify NASH before the onset of fibrosis. Therefore, a potential clinical use for this tool may be to differentiate patients with NASH from those with simple steatosis. Financial cost and a lack of access to this technology currently limit its use outside of the research setting (314).

3. Biomarkers and clinical algorithms

Recently, as summarized in Table 4, much attention has been paid to developing and validating biomarkers and clinical algorithms in an attempt to overcome the problems associated with liver biopsy (1). In particular, these techniques could lead to a simple, noninvasive method of screening for and assessing response to treatment in patients with NAFLD/NASH. Unfortunately, this process has been hampered by small and biased sample populations. In addition, because validation must be done by liver biopsy with its inherent inaccuracy, even the perfect noninvasive diagnostic test would not replicate liver biopsy results. It has been estimated that even if biopsy was 90% sensitive and specific, a perfect biomarker could only obtain a maximum AUROC of 0.90 (66).

Despite these limitations, several biomarkers and clinical algorithms are currently being used in the research setting and may well in the future offer benefit to the clinician for the diagnosis and monitoring of NAFLD. Biomarker panels that have been used in a research setting and have been internally validated in a NAFLD population with encouraging results include: the NAFLD fibrosis score (NFS) [$-1.675 + 0.037 \times$ age in years $+ 0.094 \times$ BMI (kg/m^2) $+ 1.13 \times$ (yes = 1/no = 0) IFG or diabetes $+ 0.99 \times$ AST/ALT ratio $- 0.013 \times$ platelets ($\times 10^9$ liters) $- 0.66 \times$ albumin (g/dl)] (62); the European liver fibrosis panel (ELF) combined clinical algorithm [combination of simple markers panel + ELF; ELF: $-7.412 +$ (ln(hyaluronic acid) $\times 0.0681) \times$ (ln(PIIINP) $\times 0.775) +$ (ln(TIMP-1) $\times 0.494)$] (315); and cytokeratin 18 (316, 317). Cytokeratin 18 and the NFS have also been externally validated, including in populations with diabetes (318). However, among studies there is no consensus regarding a cutoff value for cytokeratin 18. In a recent review by Musso *et al.* (1), various biomarkers were examined, and

Downloaded from https://academic.oup.com/edrv/article/34/1/84/2354662 by U.S. Department of Justice user on 16 August 2022

TABLE 4. Summary of clinical and blood test investigations for NAFLD and its progression, with a focus on studies including diabetes

Test	Dx NAFLD	Dx NASH	Assess fibrosis	External validation	Validation in diabetes	Constituents/algorithm	Refs.
Liver enzymes (i)	√/x	√/x	x	√	√	ALT/AST/GGT and ALP	4, 60, 71, 290, 296, 297, 298, 299
NFS (ii)	x	x	√	√	√	$-1.675 + 0.037 \times$ age (yr) $+ 0.094 \times$ BMI (kg/m^2) $+ 1.13 \times$ IFG/diabetes (yes = 1, no = 0) $+ 0.99 \times$ AST/ALT ratio $- 0.013 \times$ platelet ($\times 10^9$/liter) $- 0.66 \times$ albumin (g/dl) Rule in disease: > 0.676; rule out disease: < -1.455 (see www.nafldscore.com)	1
ELF combined clinical algorithm (iii, iv, v, viii)	x	x	√	x	√	ELF discriminant score (DS) $= -7.412 +$ (ln(HA) $*0.681$) + (ln(PIIINP) $*0.775$) + (ln(TIMP1) $*0.494$) Panel for detecting severe fibrosis $= -20.870 + 5.506 *$ELF (DS) $+ 4.513 *$diabetes/IFG (yes = 1, no = 0) $- 3.144$ AST/ALT ratio $- 0.058 *$BMI (kg/m^2) $- 0.026 *$platelets ($\times 10^9$ /liter) $+ 0.639 *$albumin (g/liter)	1, 315
Cytokeratin-18 (iv, vi)	x	√	x	√	√	Cytokeratin-18	1, 317, 318
FibroTest (iv, vii, viii)	x	x	√	x	√	Formula comprised to weighted sum of macroglobulin, haptoglobin, GGT, age, bilirubin, ApoA1, gender. (see http://www.biopredictive.com)	1, 99, 301

Dx, Diagnosis; ApoA1, apolipoprotein A1; GGT, γ-glutamyl peptidase; HA, hyaluronic acid; √, yes; x, no.

i) Liver enzyme abnormalities are associated with liver and cardiovascular complications.

ii) On meta-analysis, for F ≥ 3: sensitivity, 64%; specificity, 97%. For *not* F ≥ 3: sensitivity, 90%; specificity, 60%. AUROC for detection of F ≥ 3 was 0.85 (0.81–0.90).

iii) In a study of 91 patients for fibrosis ≥ F3 (cutoff value, −0.2826): AUROC, 0.98 (95% CI, 0.96–1); sensitivity, 91%; and specificity, 96%.

iv) Includes markers that are used in research capacity only with poor standardization across some assays.

v) ELF has separate panels for "no," "moderate," and "severe" fibrosis.

vi) Cytokeratin-18 is a marker of hepatocyte apoptosis with AUROC for detecting NASH 0.82 (0.78–0.88); sensitivity, 78%; specificity, 87% on meta-analysis.

vii) For Fibrotest, by meta-analysis, to rule out disease for F≥2: cutoff, <0.3; sensitivity, 76%; specificity, 74%; rule in disease for F≥2: cutoff > 0.7; sensitivity, 32%; specificity, 87%. Bilirubin can affect result.

viii) Lack of high-quality external validation data.

the AUROC for the NFS was found to be 0.85 (0.81–0.90) for the detection of a fibrosis score of 3 or greater from 13 studies in 3064 patients, whereas the AUROC for the ELF combined clinical algorithm for fibrosis score of 3 or greater was 0.98 (0.96–1) based on a small study using 91 patients, and the AUROC for cytokeratin 18 to detect NASH was found to be 0.82 (0.78–0.88), based on data from nine studies with a total of 812 patients.

The use of several algorithms combining biomarker panels, such as the NFS and cytokeratin 18, with the FibroScan has been proposed in high-risk individuals, such as those with diabetes, to help identify the patients that may need liver biopsy (1). Although this approach appears useful, further data are needed to validate these biomarker panels and algorithms for use in serial follow-up and also in assessing prognosis.

X. Assessing for Diabetes in the Patient with NAFLD

Given the high prevalence of diabetes in patients with NAFLD, it is mandatory to screen patients with NAFLD for hyperglycemia. An early diagnosis of diabetes in patients with NAFLD would provide an opportunity to direct therapy toward insulin resistance and optimal glycemic control to create a favorable "metabolic memory" that would reduce the subsequent risk of diabetes complications specifically and also perhaps liver complications (319). Traditionally, screening has been done by formal laboratory-based fasting blood glucose levels. However, whereas the cost and resource demands involved in screening the large number of patients with NAFLD needs to be considered, the OGTT has been proposed as a better screening tool for diabetes in NAFLD. The OGTT improves the sensitivity of diabetes detection and also detects patients with IGT, a prediabetic state with established links to cardiovascular risk and early retinopathy (320).

Completely separate from the diagnosis of diabetes, the OGTT may also be useful for assessment of liver fibrosis risk by quantifying insulin and glucose levels after a fixed carbohydrate load (78, 79). Thus, whereas insulin levels are not necessary nor are they used in the diagnosis of diabetes, as described in earlier sections, detecting hyperinsulinemia and insulin resistance parameters after a glucose load may help to assess risk of fibrosis and its progression in NAFLD in those with, and without, glucose dysregulation (78, 79).

Downloaded from https://academic.oup.com/edrv/article/34/1/84/2354662 by U.S. Department of Justice user on 16 August 2022

By identifying patients with IGT, the opportunity exists for early intervention to prevent or delay type 2 diabetes. Lifestyle intervention projects such as the Diabetes Prevention Program have shown that type 2 diabetes can be delayed or prevented in the majority (58%) of cases. The benefits demonstrated in this program were sustained beyond the initial intervention period; compared with controls, there was a 34% reduction in the relative risk of developing diabetes at follow-up, 10 yr after randomization (321).

Data supporting the use of the OGTT compared with fasting glucose in patients with NAFLD are increasing (77–79, 320). A recent alternative to identifying diabetes using HbA1c estimation (HbA1c $\geq$ 6.5%), as has recently been endorsed by the World Health Organization and the American Diabetes Association (322, 323), does not appear to offer benefit over fasting glucose in NAFLD (78). NAFLD has been associated with postprandial hyperglycemia more than fasting glucose abnormalities (324), and whereas there are no specific publications addressing the sensitivity of HbA1c measures in diabetes screening in people with NAFLD, it is recognized that HbA1c may be less effective at detecting isolated postprandial hyperglycemia that is into the range defined as diabetes, compared with people who also have fasting hyperglycemia (325). Of further note, HbA1c levels are lower in the setting of cirrhosis (326), largely due to decreased red cell life span. Given that people with NAFLD are at particularly high risk of glucose abnormalities, while awaiting published series to address the topic, there are arguments to caution against routine use of HbA1c to screen for diabetes in patients with NAFLD, and such screening should not be used in the setting of cirrhosis.

XI. Targeting Improved Liver Outcomes in the Patient with Diabetes and NASH

Despite the increasing need for an effective treatment for NASH, there is no specific single intervention that has been shown to be convincingly effective. The approaches are summarized in Table 5. The search for treatments has been hampered by small clinical trials, a lack of longitudinal data, and the need for serial liver biopsy to define improvement. Given the importance of preventing future complications, the main goal of therapy should be to prevent or improve liver fibrosis (1, 59). However, largely due to the limitations already described, no treatment to date has been proven to prevent fibrosis in NASH in a large-scale randomized controlled trial (RCT). To examine this issue, in a systematic literature search of published reports, Hojo and Watanabe (327) selected 38 articles with 47 intervention arms, including 11 high-quality, double-blinded RCTs. Histological features improved in greater than 75% of the intervention arms; however, fibrosis improved in less than 30%. In addition, treatment is likely to be long-term because cessation of any intervention to date has resulted in a reversal of any histological improvement seen (327).

A. Tailoring traditional diabetes management to the patient with NASH

1. Lifestyle intervention: weight loss

As with type 2 diabetes, the most effective treatment, and therefore the mainstay of management in patients with NASH who are overweight, is directed at weight loss and exercise. Effective and sustained weight loss has been associated with marked improvement in liver enzymes and stable or improved liver histology over time (328–330). A recent systematic review and meta-analysis of randomized trials by Musso *et al.* (331) reported improvement of hepatic steatosis at 5% or greater weight loss by diet and/or weight-reducing pharmacological therapy, but improvement in NAS only when weight loss was at least 7%. There was no improvement in fibrosis found. In the setting of type 2 diabetes, intensive lifestyle intervention over 12 months with the combination of diet and exercise resulted in a significant reduction in hepatic steatosis, as measured by MRS, in 96 individuals (332). In addition, a recent study of 11 patients (diabetes duration $<$ 4 yr) who were placed on a very low-calorie diet (600 kcal/d) for 8 wk had a 70 $\pm$ 5% reduction of hepatic triacylglycerol content as assessed by MR. Associated with this change were significant improvements in glycemic control, hepatic insulin sensitivity, and β-cell function. Pancreatic fat content was also noted to decrease (333).

2. Bariatric surgery

Despite good evidence that sustained weight loss is associated with histological improvement in NAFLD populations, a target of at least 7% is achieved by less than 50% of individuals in clinical trials alone (331). Bariatric surgery is a more definitive technique to achieve significant and sustained weight loss and is currently recommended in patients with diabetes or other obesity-related complications at a BMI above 35 kg/m^2, who have not achieved recommended weight-loss targets with medical therapies. Data are increasing that bariatric surgery can achieve significant levels of remission of diabetes of up to 95% in populations with obesity, at least in the medium term. This is associated with an apparent immediate reduction in hepatic insulin resistance, followed by a reduction in skeletal muscle intramyocellular fat and insulin resistance (72, 334). A recent position statement by the

Downloaded from https://academic.oup.com/edrv/article/34/1/84/2354662 by U.S. Department of Justice user on 16 August 2022

TABLE 5. Summary of studies targeting NAFLD and its subgroups, with a focus on those including subjects with diabetes

Intervention	No. of studies	Level of evidence	Participants per study range (n)	% with diabetes, range in studies	Improved	Outcomes possibly improved	Unchanged or worsened	Refs.
Lifestyle intervention and diabetes medicines								
Weight loss by lifestyle ± pharmacological intervention	4	I, II, III	31–373	8–100	Liver enzymes steatosis[c,d], NAS[d], inflammation[d]		Fibrosis[d]	191, 328, 332
Weight loss by bariatric surgery	3	III, IV	7–381	25–42[a]	Liver enzymes, NAS[d], steatosis[d], inflammation[d], hepatocyte ballooning[d]	?Fibrosis[d]	?Fibrosis[d,e]	337, 338, 339
Exercise	6	I, II, III	19–813	25–100[a]	Liver enzymes, steatosis[c]	NASH[d], fibrosis[d]		1, 19, 265, 347, 348, 349
Metformin	4	I, II, III	51–671	0–100	?Hepatocyte-ballooning[d,f]	Liver enzymes, ?steatosis[d], ?hepatocyte-ballooning[d]	Steatosis[c], ?steatosis[d], inflammation[d], fibrosis[d], NAS[d]	1, 358, 361, 387
Thiazolidinediones	4	I, II	55–862	0–100[k]	Liver enzymes, steatosis[c,d], inflammation[d], hepatocellular ballooning[d]	?Fibrosis[d]	?Fibrosis[d]	1, 366, 367, 368
Incretins[b]	2	I, IV	8 and 3900	100 and NR		Liver enzymes fibrosis[d], NAS[d]		379, 380
Nonhypoglycemic pharmacotherapies								
Statins[b]	2	II	16 and 455	44 and 7	Steatosis[c,g]		Liver enzymes, steatosis[d,h], inflammation[d,g], hepatocyte-ballooning[d,g], fibrosis[d,g]	391, 394
Ezetimibe[b]	2	IV	10 and 45	50 and 22	Liver enzymes, steatosis[d], NAS[d], hepatocellular ballooning[d], inflammation[d]	?Fibrosis[d], serological markers of fibrosis	?Fibrosis[d]	400, 401
PPAR-α agonists[b]	2	II, IV	16 and 46	38 and NR	Liver enzymes		Steatosis[d], inflammation[d], hepatocellular ballooning[d], fibrosis[d]	403, 405
Angiotensin receptor blockers[b]	2	II	54 and 137	NR and 17	Liver enzymes, steatosis[d], hepatocellular ballooning[d,i], inflammation[d,i], fibrosis[d,i]			358, 407
Vitamin E	2	II	247 and 173	0	Liver enzymes[j], steatosis[d,j], inflammation[d,j], NASH diagnosis[d]		Fibrosis[d]	361, 367

NR, Not reported; ?, possibly.

[a] Data not available for all studies.

[b] Histological evidence is very limited, and findings should be interpreted with caution.

[c] Measured by noninvasive assessment such as by ultrasound, CT, MRI, or MRS.

[d] On histology.

[e] Mild worsening of fibrosis was seen in the first 12 months of the study by Mathurin *et al.* (340), with stable disease at 5 yr (although > 95% of study participants had a fibrosis score of <1 at 5 yr).

[f] Pediatric study.

[g] When atorvastatin 20 mg was combined with vitamin E 1000 IU and vitamin C 1 g/d for an average of 3.6 yr.

[h] More patients with diabetes were allocated to the treatment group in this study with the potential to bias results to the negative.

[i] Findings with telmisartan only.

[j] Findings in adults only.

[k] Belfort *et al.* (368) report that all patients in the study had either diabetes or IGT, but exact numbers of each group were not specified by authors.

International Diabetes Federation has recommended that bariatric surgery should also be considered as a treatment option in patients with type 2 diabetes and a BMI of 30–35 kg/m^2, especially in the presence of other major cardiovascular disease risk factors (335), and there is increasing evidence for benefit in this setting (336). NAFLD could therefore be considered as an indication for bariatric surgery (330) and certainly should influence the decision-making process in a patient with type 2 diabetes, particularly at lower cutoff levels of BMI between 30 and 35 kg/m^2. There are no RCTs examining bariatric surgery as a treatment for NASH, and therefore no formal recom-

Downloaded from https://academic.oup.com/edrv/article/34/1/84/2354662 by U.S. Department of Justice user on 16 August 2022

mendations for treatment have been made to date (337). Many small studies of bariatric surgery in patients with NAFLD and obesity have shown an improvement in histology, with a regression in fibrosis and inflammation in some over time (338–340). In addition, data demonstrating a decrease in the hepatic factors involved in regulating liver inflammation and fibrosis support the concept of an expected regression of NASH over a longer period of time (341–343). Interestingly, a significant decrease in fibrosis (and by definition reversal of cirrhosis) and inflammation was seen on serial liver biopsy in a small series of 11 patients with cryptogenic cirrhosis presumed secondary to NASH who underwent biliopancreatic diversion (344). Because cirrhosis may be a relative contraindication to surgery, a liver biopsy and, if appropriate, assessment for complications of cirrhosis such as portal hypertension, should be considered before performing any bariatric procedure (345). NASH *per se* was not associated with an increased rate of complications after bariatric surgery in a prospective cohort of 437 patients from the United States (346).

3. Lifestyle intervention: exercise

Exercise is a recommended treatment in NAFLD and in type 2 diabetes (347, 348). However, the exact nature, intensity, and duration of exercise required to be effective to ameliorate NAFLD is not yet clear. From recent studies, it appears that intense exercise and resistance training may provide the most benefit, especially when distributed over the week. In a retrospective analysis of data from 813 individuals with biopsy-proven NAFLD who enrolled in the NASH Clinical Research Network, using a self-reported time spent in physical activity, it was found that meeting vigorous [≥75 min/wk at metabolic equivalent (MET) of ≥6] but not moderate (≥150 min/wk of MET 3–5.9) intensity exercise, as per recommendations by the U.S. Department of Health and Human Services and the U.S. Department of Agriculture, was associated with a decreased adjusted odds of having NASH (OR, 0.65; 95% CI, 0.43–0.98) (265). Doubling the recommended time spent in vigorous exercise (*i.e.*, ≥150 min/wk at MET of ≥6) was associated with a decreased adjusted odds of advanced fibrosis (OR, 0.53; 95% CI, 0.29–0.97). Total duration of exercise per week was not associated with NASH stage or fibrosis (265).

In an attempt to find more palatable options for exercise that will be beneficial in NAFLD, a small prospective study of 21 sedentary adults who had clinically defined NAFLD were assigned to 8 wk of resistance training exercise (n = 11) or standard care and demonstrated a 13% relative reduction in liver lipid as measured by MRS in the resistance training group compared with controls (349). Despite a sizeable relative difference in liver fat reduction between the groups, the absolute change of intrahepatic lipid was only 2%. The authors conceded that this reduction in liver fat was quite modest when compared with studies that have demonstrated an approximate 10% reduction after 8-kg weight loss from caloric restriction (349). A further prospective study involving 35 patients with NAFLD (as defined on ultrasound and by elevated serum ALT levels), who were resistant to standard lifestyle intervention, reported favorable results from a 12-wk intervention involving hybrid training, or co-contraction of agonist and antagonist muscles, for 19 min, twice per week. They found significant changes in steatosis grade on ultrasound examination and serum ALT levels in the intervention group when compared with controls; however, this finding was likely to have been affected by a significant reduction in body weight in the intervention group (-0.56 ± 0.60 compared with 2.39 ± 0.56 kg). There were also significant changes in abdominal circumference and body fat in the hybrid training group (350).

To examine the mechanism by which exercise may exert its effects on NAFLD, a group from Yale University studied a small (n = 12) group of healthy, young, lean, insulin-resistant individuals in a crossover trial. They looked at the response to a carbohydrate-rich meal, before and 45 min after exercise on a cross trainer, and found that exercise resulted in a greater than 3-fold increase in muscle glycogen synthesis and an approximate 40% reduction in net hepatic triglyceride synthesis. These changes were also associated with an approximate 30% decrease in hepatic *de novo* lipogenesis. The authors proposed that exercise may exert its effects on NAFLD by affecting the distribution and fate of ingested carbohydrate and, specifically, by diverting ingested carbohydrate away from the liver and into the muscle by reducing skeletal muscle insulin resistance. Presumably, by increasing carbohydrate uptake into muscle, there would be a reduction in hepatic *de novo* lipogenesis and hepatic triglyceride synthesis (351).

4. Targeting hyperglycemia with antidiabetic medications

a. Insulin. Although there is little direct evidence that improving glycemic control in patients with diabetes will improve liver histology, some preclinical data are supportive of this concept (6). In addition, a small clinical study from Japan in which 39 patients with NAFLD and diabetes underwent paired liver biopsy after a median follow-up time of 2.4 yr found that a decrease in HbA1c and the use of insulin were associated with improvement of liver fibrosis independent of age, sex, and BMI. HbA1c was more strongly associated with the improvement in liver fibrosis than the use of insulin after adjustment for each other (χ^2; 7.97 *vs.* 4.58, respectively) (352), suggesting that im-

Downloaded from https://academic.oup.com/edrv/article/34/1/84/2354662 by U.S. Department of Justice user on 16 August 2022

proved blood glucose may be at least a marker, if not a mechanism, by which the fibrosis in NASH is lessened.

Although some data have supported an association between insulin use in type 2 diabetes and increased risk of HCC and other cancers (353, 354), the studies have significant methodological limitations such that the many confounders, including treatment duration, medication history, and indication for therapy, are inadequately addressed (355, 356).

b. Insulin sensitizers. Diabetes-specific treatments that have the most data for efficacy in NASH are the insulin sensitizers, including metformin and the PPAR-γ agonists, the thiazolidinediones (TZDs).

Metformin. Available data indicate improvement in insulin resistance and perhaps liver enzymes with metformin use, but improvement in histology is inconsistent (345, 357–360). The TONIC trial, a randomized, double-blind, double-dummy, placebo-controlled clinical trial with paired liver biopsy over 96 wk that was designed to evaluate whether metformin (1 g/d) or vitamin E (800 IU/d) is an effective treatment in biopsy-proven NAFLD in 173 children (age, 8–17 yr) without diabetes, demonstrated significant improvement in hepatocellular ballooning only in the metformin group compared with placebo (−0.3 *vs.* 0.1; $P = 0.04$). There were no significant differences between metformin and placebo when examining other histological features or the primary outcome of sustained improvement in ALT over time. A possible factor affecting this outcome was the relatively low dose of metformin (361).

Despite disappointing results on NAFLD itself, metformin use may be beneficial in minimizing the increased risk of HCC. Metformin has been shown to inhibit cancer cell growth *in vitro* and *in vivo* (362). Furthermore, it has been associated with a reduced risk of HCC in patients with type 2 diabetes. Case-control studies have shown that the OR of developing HCC in patients with diabetes treated with metformin reduces to 0.3 when compared with those without this therapy (363, 364). In a retrospective cohort study previously mentioned, using population-based representative claims data from the Taiwan National Health Insurance Database, including 19,349 patients with newly diagnosed type 2 diabetes and 77,396 control patients without diabetes enrolled between 2000 and 2005, Lai *et al.* (102) found that the use of metformin for a median duration of 2.1 yr was associated with a 51% risk reduction for the development of HCC. A longer duration of metformin therapy appeared to provide more risk reduction in this study (102).

Thiazolidinediones. Pioglitazone is the most extensively studied and most clinically relevant PPAR-γ agonist in RCT in NASH (365). From available data, histological improvement is significant, with clear reductions in steatosis and inflammation, but only minor effects on fibrosis apparent on paired biopsy. The current findings in regard to fibrosis are hampered by inadequately sized cohorts, including individuals with relatively mild fibrosis, studied for inadequate periods of time, such that the effects of pioglitazone still need further study in NAFLD.

In patients without diabetes, whereas a small study by Aithal *et al.* (366) did show an improvement in fibrosis when compared with placebo ($P = 0.05$) after 12 months of pioglitazone 30 mg/d, the largest RCT using pioglitazone, the PIVENS study, failed to show a statistically significant difference in fibrosis grading when compared with placebo (367). A meta-analysis by Musso *et al.* (331) found no effect of TZDs on fibrosis stage; however, the authors did find that there was a reduced risk of fibrosis progression in those with worsening fibrosis stage. In the setting of diabetes, Belfort *et al.* (368) recruited 55 patients with IGT or diabetes and performed a small randomized, placebo-controlled trial looking at the effects of pioglitazone 45 mg/d and a hypocaloric diet *vs.* hypocaloric diet alone over 6 months. They found significant decreases in liver enzymes and liver steatosis and inflammation when compared with placebo. The difference in fibrosis stage was not statistically significant compared with placebo in this study ($P = 0.08$); however, there was a significant reduction in fibrosis stage in the pioglitazone group when compared with baseline ($P = 0.002$) (368).

It is apparent from current literature that liver histology has tended to return to pretreatment levels after cessation of TZDs (369, 370), mandating long-term commitment to therapy. As with metformin, the use of pioglitazone in patients with diabetes and NAFLD may have uses outside of its effects on liver histology. For example, pioglitazone use was associated with lower risk of death, myocardial infarction, and stroke in a meta-analysis of studies involving patients with type 2 diabetes (371). Weight gain is a common problem in TZD use, with a mean gain of 2% occurring in up to 75% of patients, and a minority will experience much greater gain (331). However, notably, whereas overall fat increases with TZD use, visceral fat does not change or it decreases, leading to a redistribution of fat stores (372) and an associated improvement in the metabolic profile. Peripheral edema and cardiac failure are well recognized adverse effects of TZD therapy in some patients (371, 373), and long-term therapy with TZDs may also have other significant negative effects on health because increased cardiovascular disease risk has been associated with rosiglitazone (374, 375) and increased blad-

Downloaded from https://academic.oup.com/edrv/article/34/1/84/2354662 by U.S. Department of Justice user on 16 August 2022

der cancer risk has been associated with pioglitazone use (376). Problems with osteoporosis and bone fragility and exacerbation of any macular edema present are also of concern (370, 377).

c. Incretin-based therapy. Newer antidiabetic agents, the incretin mimetics and DPP-IV inhibitors, by acting to increase gut-derived proteins including glucagon-like peptide-1 (GLP-1), have been shown to improve diabetes control by potentiating meal-induced insulin production and by slowing gastric emptying and inhibiting glucagon secretion. Of particular note, these agents have been able to maintain weight neutrality in the case of DPP-IV inhibitors or weight loss in the case of incretin mimetics (378). There is increasing evidence that GLP-1 action is also likely to have direct metabolically desirable effects on the liver (126, 330, 379–382), including suppression of hepatic lipogenesis (383) and stimulation of lipid oxidation (384) and, albeit limited, evidence that it may improve hepatic insulin sensitivity (385, 386). At a cellular level, a G protein-coupled receptor for GLP-1 has been discovered on human hepatocytes. In addition, *in vitro* treatment with exendin-4, a GLP-1 agonist, directly reduced liver steatosis in human hepatocytes (384, 387). Improvements in hepatocyte insulin sensitivity have also been observed with GLP-1 agonist therapy in this setting (384). In an open-label, uncontrolled trial to assess exenatide (a synthetic analog of exendin-4) safety in subjects with diabetes over 3.5 yr, the treatment was associated with improved liver enzymes, independent of body weight changes and insulin sensitivity (330, 381). A recent meta-analysis of GLP-1 agonists in populations with and without diabetes, including data on liver enzyme tests from 12 of the 25 trials included, found that ALT concentrations decreased after treatment with liraglutide (−2.2 U/liter; −3.6 to −0.9) but not with exenatide (0.7 U/liter; −1.1 to 2.4) on subgroup analysis (379). Evidence that DPP-IV inhibitors confer a liver protective effect is emerging in preclinical studies, and further liver studies with incretin mimetics are reporting similar findings (388). Human trials with DPP-IV inhibitors are under way (330). Although data are promising, enthusiasm for these therapies should be tempered by a lack of long-term data and, in particular, details of long-term side effects (390).

5. Lipid-lowering therapy

a. Statins. Given the increased risk of macrovascular disease in patients with NASH or diabetes, it is paramount to address any modifiable traditional macrovascular risk factors such as smoking, hyperlipidemia, and hypertension in a cohort of patients with coexisting diabetes and NASH. Lipid lowering of LDL-cholesterol by statin (hydroxymethylglutaryl coenzyme A reductase inhibitor) therapy has obvious therapeutic benefits in addressing cardiovascular risk but also has theoretical benefits in NASH through antiinflammatory, immunomodulatory, and antioxidant properties. Small studies of hyperlipidemic patients with NAFLD demonstrate improvement in liver enzymes and a decrease in liver steatosis on histology (391–393). However, in a small double-blind RCT (n = 16) looking at effects of simvastatin on a small group of biopsy-proven NASH over 12 months, there was no statistically significant change in liver enzymes, hepatic steatosis, necroinflammatory activity, or stage of fibrosis despite a 26% reduction in LDL in the intervention group (394). Thus, current data support the use of statins for risk factor modification rather than primary treatment of NASH *per se*. Adverse effects of statins on the liver are exceedingly rare and should not be a reason to avoid this therapy in a statin-naive patient with NAFLD (395, 396).

b. Ezetimibe. Ezetimibe is another lipid-lowering therapy that works by inhibiting Niemann-Pick C1 like 1-dependent cholesterol transport at the brush border of the small intestine. It has been shown to improve liver steatosis and insulin resistance in an obese rat model of metabolic syndrome (397, 398). In humans, Niemann-Pick C1 like 1 mRNA expression is present in both the small intestine and the liver. Ezetimibe may therefore be an effective treatment in NASH by traditional mechanisms and also via specific pathways, such as by the inhibition of biliary cholesterol absorption (399). In two small clinical trials, ezetimibe at a dose of 10 mg/d has been shown to reduce liver enzymes, high-sensitivity C-reactive protein, and type IV collagen (as a surrogate marker of fibrosis), and to improve the NAS on liver biopsy (largely attributable to a decrease in steatosis). In the study by Yoneda *et al.* (400), there was a nonsignificant decrease in fibrosis stage, but no participants in the study had advanced fibrosis (stage 3–4) at baseline (400, 401).

c. PPAR-α agonists. Lipid modulation through the use of PPAR-α agonists also has theoretical benefit in the treatment of NAFLD/NASH through their influence on the mitochondrial and peroxisomal fatty acid β-oxidation pathways. PPAR-α has also been implicated in the negative regulation of inflammatory responses (137). Supplementation of η-3 polyunsaturated fatty acid, a PPAR-α ligand, to rodent models of NAFLD has led to decreases in liver steatosis, transaminases, and liver inflammation and fibrosis (402). In humans, reduced transaminases and steatosis have been observed with this treatment (137, 403). Limited small studies in humans with NAFLD using fenofibrate, gemfibrozil, or benzofibrate have shown improve-

Downloaded from https://academic.oup.com/edrv/article/34/1/84/2354662 by U.S. Department of Justice user on 16 August 2022

ments in biochemistry and imaging (404). However, a study with clofibrate failed to show histological improvement after 12 months of therapy (404, 405).

6. Addressing hypertension

As with lipid-lowering therapy, tailoring medications to treat hypertension has theoretical advantages in patients with NASH and diabetes. In particular, modulation of the RAS has received particular attention in recent NAFLD literature. It has previously been shown that chronic liver injury up-regulates the local tissue RAS, contributing to the recruitment of inflammatory cells and the development of fibrosis (406, 407). Angiotensin II affects insulin signaling and promotes inflammation, endothelial dysfunction, and fibrosis, with direct activation of the HSC (327, 389, 407). In studies of NASH in rats, use of angiotensin-converting enzyme inhibitors and angiotensin receptor blockers, and in particular telmisartan with the theoretical benefit of concomitant PPAR-γ activity, has led to improved liver histology and markers of fibrosis (406, 408). Human studies of angiotensin receptor blockers and NASH are few and are limited by small numbers. In a small pilot study involving 54 patients with biopsy-proven NASH and systemic hypertension, telmisartan was compared with valsartan. Improvements were greater in plasma lipids, insulin sensitivity and liver steatosis, necroinflammation, and fibrosis in the telmisartan group at 20 months (327, 330, 409). A recent randomized, open-labeled, prospective clinical trial involving 137 patients with biopsy-proven NASH found no benefit of combination therapy with rosiglitazone (4 mg twice daily) plus losartan (50 mg once daily) or rosiglitazone (4 mg twice daily) plus metformin (500 mg twice daily) over rosiglitazone (4 mg twice daily) alone, although doses for metformin and losartan may have been inadequate, as may have been the duration of observation (358).

Modulation of the renin angiotensin aldosterone system may also be achieved with mineralocorticoid receptor antagonists, such as spironolactone. The beneficial effects of an 8-wk treatment of spironolactone in a mouse model with diet-induced diabetes and NAFLD have been reported (410). Early data from a small single-center RCT, including 10 patients treated with vitamin E (400 IU) *vs.* 10 patients treated with spironolactone (25 mg) plus vitamin E (400 IU) in biopsy-proven NAFLD, have shown a favorable effect on HOMA-IR with the addition of spironolactone in patients with NAFLD. Larger clinical trials are needed to provide definitive data (279).

B. Other agents with potential benefit in the patient with NASH and diabetes

1. Antioxidants

Antioxidants, including vitamins E and C, silymarin (milk thistle), and betaine, have been studied in NAFLD with evidence of limited benefit. Vitamin E has the most significant data supporting its use and is currently the only accepted treatment in pediatric NAFLD. The PIVENS and TONIC trials have been the most recent RCTs to show benefit with vitamin E. The PIVENS study involved 247 individuals with NASH, but without diabetes or cirrhosis, and treatment with pioglitazone 30 mg/d, vitamin E 800 IU/d, or placebo for 96 wk. The primary outcome was histological improvement in the features of NASH, with a P value < 0.025 considered statistically significant given the two planned primary comparisons. Vitamin E therapy, as compared with placebo, was associated with a significantly higher rate of improvement in the composite NAS score (43% compared with 19%; $P = 0.001$), whereas on the basis of the statistical significance preestablished, pioglitazone was not (34% compared with 19%, respectively; $P = 0.04$). As compared with placebo, both vitamin E and pioglitazone treatment improved the scores of steatosis, inflammation, ballooning, and serum ALT to a similar extent, but fibrosis did not significantly improve in either group (367). Combination of these two agents may be useful, with a small pilot study suggesting that pioglitazone and vitamin E may be better than pioglitazone alone (345). The TONIC study demonstrated significant improvement in resolution of NASH, hepatocellular ballooning, and NAS in the vitamin E-treated children when compared with placebo but, unlike PIVENS, was unable to demonstrate improvements in liver steatosis or lobular inflammation (361).

Despite the positive findings associated with the use of vitamin E, because treatment is likely to be needed long term, some caution must be applied given the results of several meta-analyses suggesting a small but significant increase in risk of all-cause mortality with supplementation, particularly at doses greater than 400 IU/d (343, 411–413). Given current evidence, vitamin E (α-tocopherol) has been recommended at a dose of 800 IU/d in patients with active NASH (NAS $\geq$ 4), without diabetes. There is a need for validation of vitamin E as an effective treatment of NASH in patients with diabetes, with close attention paid to long-term safety data (331, 345).

2. Ursodeoxycholic acid (UDCA)

UDCA has theoretical benefits in NASH through its antiapoptotic properties, by decreasing TNF-α levels and perhaps by reducing ER stress and increasing insulin sensitivity. Disappointingly, three placebo-controlled RCTs with UDCA have demonstrated mixed but largely negative results (345, 414–416). A subsequent review has suggested the possible benefit of UDCA plus vitamin E and also the examination of non-UDCA bile acids that may regulate metabolism via the farnesoid X receptor (FXR),

Downloaded from https://academic.oup.com/edrv/article/34/1/84/2354662 by U.S. Department of Justice user on 16 August 2022

a nuclear receptor involved in sensing bile acids. FXR is thought to be a key regulator of lipid and carbohydrate metabolism (137, 417, 418).

3. Novel agents

There have been limited trials of other potential agents in NAFLD. Data in the rat and also small studies in the human suggest that modification of the intestinal flora with probiotics may be a useful adjunct in patients with NAFLD (330). Modulation of the endocannabinoid receptor has been found to affect *de novo* lipogenesis in the liver and promote steatosis (138). Rimonabant, a cannabinoid 1 receptor antagonist, received attention after it was shown to induce weight loss and improve insulin resistance in clinical trials, but it has recently been withdrawn from the market due to psychiatric complications, primarily depression (138). Studies in animal models and early human studies are proceeding with agents that can modify insulin sensitivity and lipid metabolism via PKC isoform modulation, including via FXR regulation (137, 345). Attention is also being given to agents that can modify cytokines, adipokines, or growth factors such as pentoxifylline, a TNF-α antagonist, and antifibrotic agents that target the hedgehog pathway, thought to mediate hepatocyte apoptosis and regeneration (330, 331, 345, 419). In addition, PPAR-δ agonists, perhaps by increased fatty acid metabolism or by decreased inflammation (mediated via alternative activation of tissue macrophages), have been shown to be protective against diet-induced steatohepatitis in mice fed a methacholine-deficient diet and have limited data in humans suggesting that they are safe (420).

Recent *in vitro* work examining the benefits of curcumin (the yellow pigment from turmeric) on HSCs in the setting of hyperglycemia is notable. *In vitro*, curcumin appears to reduce the biphasic intracellular increase in glucose that occurs in HSCs as a result of hyperglycemia, first by blocking membrane translocation of GLUT2 by interrupting p38 MAPK signaling, and then by suppressing gene expression of GLUT2 by stimulating PPAR-γ activation and by attenuating oxidative stress. These changes have led to a reduction in HSC activation and proliferation *in vitro* that is otherwise seen with the induction of hyperglycemia. The challenge for *in vivo* studies will be achieving adequate concentrations of curcumin because it has very poor bioavailability (242).

Other agents of interest that have not yet been studied in NAFLD trials but may be of interest due to their effects in diabetes and/or on lipid metabolism include sodium-glucose-linked transport inhibitors as glycosurics, advanced glycation end-product inhibitors, cholesterol ester transfer protein inhibitors, and 11β-hydroxysteroid dehydrogenase type 1 inhibitors (421–424).

XII. Monitoring for Complications of Liver Disease in Patients with NASH and Type 2 Diabetes

NAFLD appears to have lower rates of liver-related complications but similar overall mortality compared with HCV, even after adjustment for potential confounders (89). In particular, HCC rates in cirrhosis related to NASH appear to be less than in cirrhosis related to other causes. From transplant data in the United States, the frequency of HCC among liver transplant recipients with NASH as a primary or secondary indication was 12%, compared with 19% for other indications ($P < 0.001$). Despite this, patients with cirrhosis secondary to NASH are clearly at risk for development of HCC (56), and diabetes may exacerbate this risk (102). HCC is twice as likely to develop in a patient with type 2 diabetes as in a person with no diabetes (425).

Liver disease is the third leading cause of death in NASH, and HCC represents the main cause of death in this group. In addition, HCC can occur in NASH without cirrhosis. In a cross-sectional Japanese study of 87 patients with HCC secondary to NASH, 59% of whom had diabetes, 11% had stage 1 fibrosis, 17% had stage 2 fibrosis, and 21% had stage 3 fibrosis. Only 51% of the patients with HCC had cirrhosis, or stage 4 fibrosis (40). These findings were replicated in a European study of HCC and chronic liver disease, with 41.7% of HCC secondary to NASH occurring in patients without cirrhosis. This was in contrast to alcoholic liver disease, where 95% of HCC occurred in the setting of cirrhosis in this study (426). Without specific recommendations for NASH, patients found to have advanced fibrosis or cirrhosis should have an ultrasound and an α-fetoprotein measurement every 6–12 months (425). Follow-up for patients with lesser degrees of NASH, with or without fibrosis is less clear, although it may involve regular noninvasive assessment for fibrosis progression.

XIII. Final Comments

End-stage NAFLD, including NASH-related cirrhosis and HCC, is currently the third leading indication for liver transplant worldwide and will likely become the leading indication in the next one to two decades (56, 330). In addition, the sheer and increasing magnitude of the combined problem of NAFLD and diabetes mandates that it

Downloaded from https://academic.oup.com/edrv/article/34/1/84/2354662 by U.S. Department of Justice user on 16 August 2022

should be better recognized by clinicians and also given a high priority for study. Multiple contemporary studies have been able to shed light on possible risk factors for NAFLD/NASH and particularly why diabetes may lead to increased rates and progression of this condition. Despite this, the exact pathogenic mechanisms that link the metabolic milieu that exists in diabetes with NAFLD are far from clear, but substantial improvements in our research tools and in animal models for both NAFLD/NASH and diabetes are helping to advance understanding.

Given the high level of clinical success of bariatric surgery in achieving sustained weight loss and in inducing remission in obese people with type 2 diabetes, RCTs are required to substantiate the degree of change in NASH after particular types of bariatric surgery and to aid in the setting of guidelines for bariatric surgery in people with diabetes and NASH. From a lifestyle perspective, increased focus on exercise type, duration, and intensity is also required to define regimens that will best improve NAFLD and, specifically, NASH.

From available data, it is also clear that the coexistence of NAFLD and diabetes is likely to require a customized pharmacological management, above that used in either condition alone. The identification of effective strategies for both of these conditions is expected to occur as new diabetes-targeted therapies such as the glucosuric agents, AGE inhibitors, and incretin mimetics and DPP-IV inhibitors, in addition to other potentially effective agents, are studied in animal trials with both NASH and diabetes. This knowledge will help pave the way toward much needed RCTs in humans. Clinical trials must be conducted with ample power and continued for adequate duration to allow the demonstration of significant differences in treatment and placebo arms, particularly in relation to fibrosis. This will be increasingly facilitated by the development of accurate noninvasive assessment for initial staging of disease burden and also for monitoring disease progression. It is likely that noninvasive assessment will consist of well-validated algorithms that involve blood-specific markers and also measurements of liver stiffness by imaging technologies. It is also envisioned that, over the course of time, such noninvasive assessments will form part of routine clinical practice, helping to exclude those who require liver-specific investigation and to identify those requiring diagnostic liver biopsy and intensified therapy.

Acknowledgments

We thank Professor James Kench, Head of Tissue Pathology and Diagnostic Oncology Department, Royal Prince Alfred Hospital, for his review of some of the pathology images (shown in Fig. 1).

Address all correspondence and requests for reprints to: Stephen M. Twigg, Professor in Medicine, Sydney Medical School, and Department of Endocrinology, The University of Sydney and Royal Prince Alfred Hospital, Sydney, New South Wales 2006, Australia. E-mail: stephen.twigg@sydney.edu.au.

This work was supported by National Health and Medical Research Council (NHMRC) of Australia Project Grant 632822 on diabetes and NAFLD and a NHMRC Postgraduate Research Scholarship (to K.H.W.).

Disclosure Summary: All authors have nothing to declare.

References

1. **Musso G, Gambino R, Cassader M, Pagano G** 2011 Meta-analysis: natural history of non-alcoholic fatty liver disease (NAFLD) and diagnostic accuracy of non-invasive tests for liver disease severity. Ann Med 43:617–649
2. **Hossain N, Afendy A, Stepanova M, Nader F, Srishord M, Rafiq N, Goodman Z, Younossi Z** 2009 Independent predictors of fibrosis in patients with nonalcoholic fatty liver disease. Clin Gastroenterol Hepatol 7:1224–1229, 1229.e1–1229e2
3. **Targher G, Chonchol M, Zoppini G, Abaterusso C, Bonora E** 2011 Risk of chronic kidney disease in patients with non-alcoholic fatty liver disease: is there a link? J Hepatol 54:1020–1029
4. **Targher G, Day CP, Bonora E** 2010 Risk of cardiovascular disease in patients with nonalcoholic fatty liver disease. N Engl J Med 363:1341–1350
5. **Zein CO, Unalp A, Colvin R, Liu YC, McCullough AJ** 2011 Smoking and severity of hepatic fibrosis in nonalcoholic fatty liver disease. J Hepatol 54:753–759
6. **Lo L, McLennan SV, Williams PF, Bonner J, Chowdhury S, McCaughan GW, Gorrell MD, Yue DK, Twigg SM** 2011 Diabetes is a progression factor for hepatic fibrosis in a high fat fed mouse obesity model of non-alcoholic steatohepatitis. J Hepatol 55:435–444
7. **Lo L, McLennan SV, Kench JG, McCaughan GW, Bonner J, Williams PF, Gorrell MD, Twigg SM,** Diabetes is a progression factor for liver fibrosis and induces cirrhosis in a mouse diet-induced obesity model of NASH. Proc 71st Annual Scientific Sessions of the American Diabetes Association, San Diego, CA, 2011 (Abstract 1682)
8. **Defronzo RA** 2009 Banting Lecture. From the triumvirate to the ominous octet: a new paradigm for the treatment of type 2 diabetes mellitus. Diabetes 58:773–795
9. **Reaven GM** 2011 Insulin resistance: the link between obesity and cardiovascular disease. Med Clin North Am 95: 875–892
10. **de Alwis NM, Day CP** 2008 Non-alcoholic fatty liver disease: the mist gradually clears. J Hepatol 48:S104–S112
11. **Starley BQ, Calcagno CJ, Harrison SA** 2010 Nonalcoholic fatty liver disease and hepatocellular carcinoma: a weighty connection. Hepatology 51:1820–1832
12. **Milner KL, Chisholm D, George J** 2009 The many faces of hepatitis C: liver disease and type 2 diabetes. Hepatology 50:668–670
13. **Virkamäki A, Ueki K, Kahn CR** 1999 Protein-protein interaction in insulin signaling and the molecular mechanisms of insulin resistance. J Clin Invest 103:931–943

Downloaded from https://academic.oup.com/edrv/article/34/1/84/2354662 by U.S. Department of Justice user on 16 August 2022

14. **White MF** 1998 The IRS-signalling system: a network of docking proteins that mediate insulin action. Mol Cell Biochem 182:3–11
15. **Kahn CR** 2003 Knockout mice challenge our concepts of glucose homeostasis and the pathogenesis of diabetes. Exp Diabesity Res 4:169–182
16. **Reaven GM, Hollenbeck CB, Chen YD** 1989 Relationship between glucose tolerance, insulin secretion, and insulin action in non-obese individuals with varying degrees of glucose tolerance. Diabetologia 32:52–55
17. **Groop LC, Bonadonna RC, DelPrato S, Ratheiser K, Zyck K, Ferrannini E, DeFronzo RA** 1989 Glucose and free fatty acid metabolism in non-insulin-dependent diabetes mellitus: evidence for multiple sites of insulin resistance. J Clin Invest 84:205–213
18. **Pocai A, Obici S, Schwartz GJ, Rossetti L** 2005 A brain-liver circuit regulates glucose homeostasis. Cell Metab 1:53–61
19. **Zsombok A, Smith BN** 2009 Plasticity of central autonomic neural circuits in diabetes. Biochim Biophys Acta 1792:423–431
20. **Kawaguchi T, Taniguchi E, Itou M, Sakata M, Sumie S, Sata M** 2011 Insulin resistance and chronic liver disease. World J Hepatol 3:99–107
21. **García-Compean D, Jaquez-Quintana JO, Maldonado-Garza H** 2009 Hepatogenous diabetes. Current view of an ancient problem. Ann Hepatol 8:13–20
22. **Holstein A, Hinze S, Thiessen E, Plaschke A, Egberts EH** 2002 Clinical implications of hepatogenous diabetes in liver cirrhosis. J Gastroenterol Hepatol 17:677–681
23. **Mehta SH, Brancati FL, Strathdee SA, Pankow JS, Netski D, Coresh J, Szklo M, Thomas DL** 2003 Hepatitis C virus infection and incident type 2 diabetes. Hepatology 38:50–56
24. **Mehta SH, Brancati FL, Sulkowski MS, Strathdee SA, Szklo M, Thomas DL** 2000 Prevalence of type 2 diabetes mellitus among persons with hepatitis C infection in the United States. Ann Intern Med 133:592–599
25. **Mason AL, Lau JY, Hoang N, Qian K, Alexander GJ, Xu L, Guo L, Jacob S, Regenstein FG, Zimmerman R, Everhart JE, Wasserfall C, Maclaren NK, Perrillo RP** 1999 Association of diabetes mellitus and chronic hepatitis C infection. Hepatology 29:328–333
26. **Stepanova M, Lam B, Younossi Y, Srishord MK, Younossi ZM** 2012 Association of hepatitis C with insulin resistance and type 2 diabetes in US general population: the impact of the epidemic of obesity. J Viral Hepat 19:341–345
27. **Milner KL, van der Poorten D, Trenell M, Jenkins AB, Xu A, Smythe G, Dore GJ, Zekry A, Weltman M, Fragomeli V, George J, Chisholm DJ** 2010 Chronic hepatitis C is associated with peripheral rather than hepatic insulin resistance. Gastroenterology 138:932–941.e1–3
28. **Yazicioglu G, Isitan F, Altunbas H, Suleymanlar I, Ozdogan M, Balci MK, Karayalcin U** 2004 Insulin resistance in chronic hepatitis C. Int J Clin Pract 58:1020–1022
29. **Vanni E, Abate ML, Gentilcore E, Hickman I, Gambino R, Cassader M, Smedile A, Ferrannini E, Rizzetto M, Marchesini G, Gastaldelli A, Bugianesi E** 2009 Sites and mechanisms of insulin resistance in nonobese, nondiabetic patients with chronic hepatitis C. Hepatology 50:697–706
30. **Bose SK, Shrivastava S, Meyer K, Ray RB, Ray R** 2012 Hepatitis C virus activates the mTOR/S6K1 signaling pathway in inhibiting IRS-1 function for insulin resistance. J Virol 86:6315–6322
31. **Kawaguchi T, Yoshida T, Harada M, Hisamoto T, Nagao Y, Ide T, Taniguchi E, Kumemura H, Hanada S, Maeyama M, Baba S, Koga H, Kumashiro R, Ueno T, Ogata H, Yoshimura A, Sata M** 2004 Hepatitis C virus down-regulates insulin receptor substrates 1 and 2 through up-regulation of suppressor of cytokine signalling 3. Am J Pathol 165:1499–1508
32. **Lecube A, Hernández C, Genescà J, Simó R** 2006 Proinflammatory cytokines, insulin resistance, and insulin secretion in chronic hepatitis C patients: a case-control study. Diabetes Care 29:1096–1101
33. **Deng L, Shoji I, Ogawa W, Kaneda S, Soga T, Jiang DP, Ide YH, Hotta H** 2011 Hepatitis C virus infection promotes hepatic glucogenogenesis through an NS5A-mediated, FoxO1-dependent pathway. J Virol 85:8556–8568
34. **Younossi ZM, McCullough AJ** 2009 Metabolic syndrome, non-alcoholic fatty liver disease and hepatitis C virus: impact on disease progression and treatment response. Liver Int 29:3–12
35. **Masini M, Campani D, Boggi U, Menicagli M, Funel N, Pollera M, Lupi R, Del Guerra S, Bugliani M, Torri S, Del Prato S, Mosca F, Filipponi F, Marchetti P** 2005 Hepatitis C virus infection and human pancreatic β-cell dysfunction. Diabetes Care 28:940–941
36. **Brandman D, Pingitore A, Lai JC, Roberts JP, Ferrell L, Bass NM, Terrault NA** 2011 Hepatic steatosis at 1 year is an additional predictor of subsequent fibrosis severity in liver transplant recipients with recurrent hepatitis C virus. Liver Transpl 17:1380–1386
37. **Eslam M, Kawaguchi T, Del Campo JA, Sata M, Khattab MA, Romero-Gomez M** 2011 Use of HOMA-IR in hepatitis C. J Viral Hepat 18:675–684
38. **Younossi ZM, McCullough AJ, Ong JP, Barnes DS, Post A, Tavill A, Bringman D, Martin LM, Assmann J, Gramlich T, Mullen KD, O'Shea R, Carey WD, Ferguson R** 2004 Obesity and non-alcoholic fatty liver disease in chronic hepatitis C. J Clin Gastroenterol 38:705–709
39. **Hübscher SG** 2011 Steatosis and fibrosis progression in patients with recurrent hepatitis C infection: complex interactions providing diagnostic and therapeutic challenges. Liver Transpl 17:1374–1379
40. **Yasui K, Hashimoto E, Komorizono Y, Koike K, Arii S, Imai Y, Shima T, Kanbara Y, Saibara T, Mori T, Kawata S, Uto H, Takami S, Sumida Y, Takamura T, Kawanaka M, Okanoue T** 2011 Characteristics of patients with non-alcoholic steatohepatitis who develop hepatocellular carcinoma. Clin Gastroenterol Hepatol 9:428–433; quiz e50
41. **Petta S, Cammà C, Di Marco V, Alessi N, Cabibi D, Caldarella R, Licata A, Massenti F, Tarantino G, Marchesini G, Craxì A** 2008 Insulin resistance and diabetes increase fibrosis in the liver of patients with genotype 1 HCV infection. Am J Gastroenterol 103:1136–1144
42. **Powell EE, Jonsson JR, Clouston AD** 2010 Metabolic factors and non-alcoholic fatty liver disease as co-factors in other liver diseases. Dig Dis 28:186–191
43. **Ludwig J, Viggiano TR, McGill DB, Oh BJ** 1980 Nonalcoholic steatohepatitis: Mayo Clinic experiences with a hitherto unnamed disease. Mayo Clin Proc 55:434–438

Downloaded from https://academic.oup.com/edrv/article/34/1/84/2354662 by U.S. Department of Justice user on 16 August 2022

44. **Matteoni CA, Younossi ZM, Gramlich T, Boparai N, Liu YC, McCullough AJ** 1999 Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. Gastroenterology 116:1413–1419
45. **Brunt EM, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR** 1999 Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. Am J Gastroenterol 94:2467–2474
46. **Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, Ferrell LD, Liu YC, Torbenson MS, Unalp-Arida A, Yeh M, McCullough AJ, Sanyal AJ** 2005 Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 41:1313–1321
47. **Younossi ZM, Stepanova M, Rafiq N, Makhlouf H, Younoszai Z, Agrawal R, Goodman Z** 2011 Pathologic criteria for nonalcoholic steatohepatitis: interprotocol agreement and ability to predict liver-related mortality. Hepatology 53:1874–1882
48. **Brunt EM, Kleiner DE, Wilson LA, Belt P, Neuschwander-Tetri BA, Network NCR** 2011 Nonalcoholic fatty liver disease (NAFLD) activity score and the histopathologic diagnosis in NAFLD: distinct clinicopathologic meanings. Hepatology 53:810–820
49. **Dorn C, Riener MO, Kirovski G, Saugspier M, Steib K, Weiss TS, Gäbele E, Kristiansen G, Hartmann A, Hellerbrand C** 2010 Expression of fatty acid synthase in nonalcoholic fatty liver disease. Int J Clin Exp Pathol 3:505–514
50. **Hebbard L, George J** 2011 Animal models of nonalcoholic fatty liver disease. Nat Rev Gastroenterol Hepatol 8:35–44
51. **Adams L** 2010 Transient elastography in nonalcoholic fatty liver disease: making sense of echoes. Hepatology 51:370–372
52. **Browning JD, Szczepaniak LS, Dobbins R, Nuremberg P, Horton JD, Cohen JC, Grundy SM, Hobbs HH** 2004 Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. Hepatology 40:1387–1395
53. **Rinella ME** 2011 Will the increased prevalence of nonalcoholic steatohepatitis (NASH) in the age of better hepatitis C virus therapy make NASH the deadlier disease? Hepatology 54:1118–1120
54. **Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, Charlton M, Sanyal AJ** 2012 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. Am J Gastroenterol 107:811–826
55. **Williams CD, Stengel J, Asike MI, Torres DM, Shaw J, Contreras M, Landt CL, Harrison SA** 2011 Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: prospective study. Gastroenterology 140:124–131
56. **Charlton MR, Burns JM, Pedersen RA, Watt KD, Heimbach JK, Dierkhising RA** 2011 Frequency and outcomes of liver transplantation for nonalcoholic steatohepatitis in the United States. Gastroenterology 141:1249–1253
57. **Rafiq N, Bai C, Fang Y, Srishord M, McCullough A, Gramlich T, Younossi ZM** 2009 Long-term follow-up of patients with nonalcoholic fatty liver. Clin Gastroenterol Hepatol 7:234–238
58. **Wong VW, Wong GL, Choi PC, Chan AW, Li MK, Chan HY, Chim AM, Yu J, Sung JJ, Chan HL** 2010 Disease progression of non-alcoholic fatty liver disease: a prospective study with paired liver biopsies at 3 years. Gut 59:969–974
59. **Angulo P** 2010 Long-term mortality in nonalcoholic fatty liver disease: is liver histology of any prognostic significance? Hepatology 51:373–375
60. **Ekstedt M, Franzén LE, Mathiesen UL, Thorelius L, Holmqvist M, Bodemar G, Kechagias S** 2006 Long-term follow-up of patients with NAFLD and elevated liver enzymes. Hepatology 44:865–873
61. **Dixon JB, Bhathal PS, O'Brien PE** 2001 Nonalcoholic fatty liver disease: predictors of nonalcoholic steatohepatitis and liver fibrosis in the severely obese. Gastroenterology 121:91–100
62. **Angulo P, Hui JM, Marchesini G, Bugianesi E, George J, Farrell GC, Enders F, Saksena S, Burt AD, Bida JP, Lindor K, Sanderson SO, Lenzi M, Adams LA, Kench J, Therneau TM, Day CP** 2007 The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. Hepatology 45:846–854
63. **Adams LA, Sanderson S, Lindor KD, Angulo P** 2005 The histological course of nonalcoholic fatty liver disease: a longitudinal study of 103 patients with sequential liver biopsies. J Hepatol 42:132–138
64. **Polyzos SA, Toulis KA, Goulis DG, Zavos C, Kountouras J** 2011 Serum total adiponectin in nonalcoholic fatty liver disease: a systematic review and meta-analysis. Metabolism 60:313–326
65. **Wang JK, Feng ZW, Li YC, Li QY, Tao XY** 2012 Association of tumour necrosis factor-α gene promoter polymorphism at sites -308 and -238 with non-alcoholic fatty liver disease: a meta-analysis. J Gastroenterol Hepatol 27:670–676
66. **Adams LA** 2011 Biomarkers of liver fibrosis. J Gastroenterol Hepatol 26:802–809
67. **Younossi ZM, Page S, Rafiq N, Birerdinc A, Stepanova M, Hossain N, Afendy A, Younoszai Z, Goodman Z, Baranova A** 2011 A biomarker panel for non-alcoholic steatohepatitis (NASH) and NASH-related fibrosis. Obes Surg 21:431–439
68. **Saadeh S, Younossi ZM, Remer EM, Gramlich T, Ong JP, Hurley M, Mullen KD, Cooper JN, Sheridan MJ** 2002 The utility of radiological imaging in nonalcoholic fatty liver disease. Gastroenterology 123:745–750
69. **Williamson RM, Perry E, Glancy S, Marshall I, Gray C, Nee LD, Hayes PC, Forbes S, Frier BM, Johnston GI, Lee AJ, Reynolds RM, Price JF, Strachan MWJ** 2011 The use of ultrasound to diagnose hepatic steatosis in type 2 diabetes: intra- and interobserver variability and comparison with magnetic resonance spectroscopy. Clin Radiol 66:434–439
70. **Targher G, Bertolini L, Padovani R, Rodella S, Zoppini G, Pichiri I, Sorgato C, Zenari L, Bonora E** 2010 Prevalence of non-alcoholic fatty liver disease and its association with cardiovascular disease in patients with type 1 diabetes. J Hepatol 53:713–718

Downloaded from https://academic.oup.com/edrv/article/34/1/84/2354662 by U.S. Department of Justice user on 16 August 2022

71. **Leite NC, Villela-Nogueira CA, Pannain VL, Bottino AC, Rezende GF, Cardoso CR, Salles GF** 2011 Histopathological stages of nonalcoholic fatty liver disease in type 2 diabetes: prevalences and correlated factors. Liver Int 31: 700–706
72. **Schauer PR, Kashyap SR, Wolski K, Brethauer SA, Kirwan JP, Pothier CE, Thomas S, Abood B, Nissen SE, Bhatt DL** 2012 Bariatric surgery versus intensive medical therapy in obese patients with diabetes. N Engl J Med 366:1567–1576
73. **Angulo P, Keach JC, Batts KP, Lindor KD** 1999 Independent predictors of liver fibrosis in patients with nonalcoholic steatohepatitis. Hepatology 30:1356–1362
74. **Wong VW, Wong GL, Tsang SW, Fan T, Chu WC, Woo J, Chan AW, Choi PC, Chim AM, Lau JY, Chan FK, Sung JJ, Chan HL** 2011 High prevalence of colorectal neoplasm in patients with non-alcoholic steatohepatitis. Gut 60: 829–836
75. **Twigg SM, Kamp MC, Davis TM, Neylon EK, Flack JR, Australian Diabetes Society, Australian Diabetes Educators Association** 2007 Prediabetes: a position statement from the Australian Diabetes Society and Australian Diabetes Educators Association. Med J Aust 186:461–465
76. **Bae JC, Cho YK, Lee WY, Seo HI, Rhee EJ, Park SE, Park CY, Oh KW, Sung KC, Kim BI** 2010 Impact of nonalcoholic fatty liver disease on insulin resistance in relation to HbA1c levels in nondiabetic subjects. Am J Gastroenterol 105:2389–2395
77. **Wong VW, Hui AY, Tsang SW, Chan JL, Wong GL, Chan AW, So WY, Cheng AY, Tong PC, Chan FK, Sung JJ, Chan HL** 2006 Prevalence of undiagnosed diabetes and postchallenge hyperglycaemia in Chinese patients with nonalcoholic fatty liver disease. Aliment Pharmacol Ther 24: 1215–1222
78. **Manchanayake J, Chitturi S, Nolan C, Farrell GC** 2011 Postprandial hyperinsulinemia is universal in non-diabetic patients with nonalcoholic fatty liver disease. J Gastroenterol Hepatol 26:510–516
79. **Kimura Y, Hyogo H, Ishitobi T, Nabeshima Y, Arihiro K, Chayama K** 2011 Postprandial insulin secretion pattern is associated with histological severity in non-alcoholic fatty liver disease patients without prior known diabetes mellitus. J Gastroenterol Hepatol 26:517–522
80. **Ortiz-Lopez C, Lomonaco R, Orsak B, Finch J, Chang Z, Kochunov VG, Hardies J, Cusi K** 2012 Prevalence of prediabetes and diabetes and metabolic profile of patients with nonalcoholic fatty liver disease (NAFLD). Diabetes Care 35:873–878
81. **Sung KC, Kim SH** 2011 Interrelationship between fatty liver and insulin resistance in the development of type 2 diabetes. J Clin Endocrinol Metab 96:1093–1097
82. **Bae JC, Rhee EJ, Lee WY, Park SE, Park CY, Oh KW, Park SW, Kim SW** 2011 Combined effect of nonalcoholic fatty liver disease and impaired fasting glucose on the development of type 2 diabetes: a 4-year retrospective longitudinal study. Diabetes Care 34:727–729
83. **Cusi K** 2012 Role of obesity and lipotoxicity in the development of nonalcoholic steatohepatitis: pathophysiology and clinical implications. Gastroenterology 142:711–725.e6
84. **Targher G, Bertolini L, Padovani R, Rodella S, Zoppini G, Zenari L, Cigolini M, Falezza G, Arcaro G** 2006 Relations between carotid artery wall thickness and liver histology in subjects with nonalcoholic fatty liver disease. Diabetes Care 29:1325–1330
85. **Söderberg C, Stål P, Askling J, Glaumann H, Lindberg G, Marmur J, Hultcrantz R** 2010 Decreased survival of subjects with elevated liver function tests during a 28-year follow-up. Hepatology 51:595–602
86. **Lee YJ, Shim JY, Moon BS, Shin YH, Jung DH, Lee JH, Lee HR** 2012 The relationship between arterial stiffness and nonalcoholic fatty liver disease. Dig Dis Sci 57:196–203
87. **Targher G, Bertolini L, Padovani R, Rodella S, Tessari R, Zenari L, Day C, Arcaro G** 2007 Prevalence of nonalcoholic fatty liver disease and its assocation with cardiovascular disease among type 2 diabetic patients. Diabetes Care 30:1212–1218
88. **Lautamäki R, Borra R, Iozzo P, Komu M, Lehtimäki T, Salmi M, Jalkanen S, Airaksinen KE, Knuuti J, Parkkola R, Nuutila P** 2006 Liver steatosis coexists with myocardial insulin resistance and coronary dysfunction in patients with type 2 diabetes. Am J Physiol Endocrinol Metab 291: E282–E290
89. **Bhala N, Angulo P, van der Poorten D, Lee E, Hui JM, Saracco G, Adams LA, Charatcharoenwitthaya P, Topping JH, Bugianesi E, Day CP, George J** 2011 The natural history of nonalcoholic fatty liver disease with advanced fibrosis or cirrhosis: an international collaborative study. Hepatology 54:1208–1216
90. **The Emerging Risk Factors Collaboration, Sarwar N, Gao P, Seshasai SR, Gobin R, Kaptoge S, Di Angelantonio E, Ingelsson E, Lawlor DA, Selvin E, Stampfer M, Stehouwer CD, Lewington S, Pennells L, Thompson A, Sattar N, White IR, Ray KK, Danesh J** 2010 Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: a collaborative meta-analysis of 102 prospective studies. Lancet 375:2215–2222
91. **McKimmie RL, Daniel KR, Carr JJ, Bowden DW, Freedman BI, Register TC, Hsu FC, Lohman KK, Weinberg RB, Wagenknecht LE** 2008 Hepatic steatosis and subclinical cardiovascular disease in a cohort enriched for type 2 diabetes: the Diabetes Heart Study. Am J Gastroenterol 103: 3029–3035
92. **Poanta LI, Albu A, Fodor D** 2011 Association between fatty liver disease and carotid atherosclerosis in patients with uncomplicated type 2 diabetes mellitus. Med Ultrason 13:215–219
93. **Volzke H, Robinson DM, Kleine V, Deutscher R, Hoffmann W, Ludemann J, Schminke U, Kessler C, John U** 2005 Hepatic steatosis is associated with an increased risk of carotid atherosclerosis. World J Gastroenterol 11: 1848–1853
94. **Catalano D, Trovato GM, Martines GF, Pirri C, Trovato FM** 2011 Renal function and severity of bright liver. Relationship with insulin resistance, intrarenal resistive index, and glomerular filtration rate. Hepatol Int 5:822–829
95. **Targher G, Pichiri I, Zoppini G, Trombetta M, Bonora E** 2012 Increased prevalence of chronic kidney disease in patients with type 1 diabetes and non-alcoholic fatty liver. Diabet Med 29:220–226
96. **Targher G, Bertolini L, Rodella S, Lippi G, Zoppini G, Chonchol M** 2010 Relationship between kidney function

Downloaded from https://academic.oup.com/edrv/article/34/1/84/2354662 by U.S. Department of Justice user on 16 August 2022

and liver histology in subjects with nonalcoholic steatohepatitis. Clin J Am Soc Nephrol 5:2166–2171
97. **Houlihan DD, Armstrong MJ, Davidov Y, Hodson J, Nightingale P, Rowe IA, Paris S, Gunson BK, Bramhall SB, Mutimer DJ, Neuberger JM, Newsome PN** 2011 Renal function in patients undergoing transplantation for non-alcoholic steatohepatitis cirrhosis: time to reconsider immunosuppression regimens? Liver Transpl 17:1292–1298
98. **Targher G, Bertolini L, Chonchol M, Rodella S, Zoppini G, Lippi G, Zenari L, Bonora E** 2010 Non-alcoholic fatty liver disease is independently associated with an increased prevalence of chronic kidney disease and retinopathy in type 1 diabetic patients. Diabetologia 53:1341–1348
99. **de Lédinghen V, Vergniol J, Gonzalez C, Foucher J, Maury E, Chemineau L, Villars S, Gin H, Rigalleau V** 2012 Screening for liver fibrosis by using FibroScan and FibroTest in patients with diabetes. Dig Liver Dis 44:413–418
100. **Donadon V, Balbi M, Valent F, Avogaro A** 2010 Glycated hemoglobin and antidiabetic strategies as risk factors for hepatocellular carcinoma. World J Gastroenterol 16: 3025–3032
101. **Barone BB, Yeh HC, Snyder CF, Peairs KS, Stein KB, Derr RL, Wolff AC, Brancati FL** 2008 Long-term all-cause mortality in cancer patients with preexisting diabetes mellitus: a systemic review and meta-analysis. JAMA 300:2754–2764
102. **Lai SW, Chen PC, Liao KF, Muo CH, Lin CC, Sung FC** 2012 Risk of hepatocellular carcinoma in diabetic patients and risk reduction associated with anti-diabetic therapy: a population-based cohort study. Am J Gastroenterol 107: 46–52
103. **El-Serag HB, Hampel H, Javadi F** 2006 The association between diabetes and hepatocellular carcinoma: a systemic review of epidemiological evidence. Clin Gastroenterol Hepatol 4:369–380
104. **Noto H, Osame K, Sasazuki T, Noda M** 2010 Substantially increased risk of cancer in patients with diabetes mellitus: a systemic review and meta-analysis of epidemiological evidence in Japan. J Diabetes Complications 24: 345–353
105. **Seshasai SR, Kaptoge S, Thompson A, Di Angelantonio E, Gao P, Sarwar N, Whincup PH, Mukamal KJ, Gillum RF, Holme I, Njølstad I, Fletcher A, Nilsson P, Lewington S, Collins R, Gudnason V, Thompson SG, Sattar N, Selvin E, Hu FB, Danesh J** 2011 Diabetes mellitus, fasting glucose, and risk of cause-specific death. N Engl J Med 364: 829–841
106. **Tilg H, Diehl AM** 2011 NAFLD and extrahepatic cancers: have a look at the colon. Gut 60:745–746
107. **Sahathevan R, Brodtmann A, Donnan GA** 2012 Dementia, stroke, and vascular risk factors: a review. Int J Stroke 7:61–73
108. **Li L, Wang Y, Yan J, Chen Y, Zhou R, Yi X, Shi Q, Zhou H, The Chongqing Aging Study Group** 2012 Clinical predictors of cognitive decline in patients with mild cognitive impairment: the Chongqing Aging Study. J Neurol 259: 1303–1311
109. **Felipo V, Urios A, Montesinos E, Molina I, Garcia-Torres ML, Civera M, Olmo JA, Ortega J, Martinez-Valls J, Serra MA, Cassinello N, Wassel A, Jordá E, Montoliu C** 2012 Contribution of hyperammonemia and inflammatory factors to cognitive impairment in minimal hepatic encephalopathy. Metab Brain Dis 27:51–58
110. **Schuetz P, Castro P, Shapiro NI** 2011 Diabetes and sepsis: preclinical findings and clinical relevance. Diabetes Care 34:771–778
111. **Nseir W, Taha H, Khateeb J, Grosovski M, Assy N** 2011 fatty liver is associated with recurrent bacterial infections independent of metabolic syndrome. Dig Dis Sci 56:3328–3334
112. **Bonapace S, Perseghin G, Molon G, Canali G, Bertolini L, Zoppini G, Barbieri E, Targher G** 2012 Nonalcoholic fatty liver disease is associated with left ventricular diastolic dysfunction in patients with type 2 diabetes. Diabetes Care 35:389–395
113. **Goland S, Shimoni S, Zornitzki T, Knobler H, Azoulai O, Lutaty G, Melzer E, Orr A, Caspi A, Malnick S** 2006 Cardiac abnormalities as a new manifestation of nonalcoholic fatty liver disease: echocardiographic and tissue Doppler imaging assessment. J Clin Gastroenterol 40:949–955
114. **Larter CZ, Yeh MM** 2008 Animal models of NASH: getting both pathology and metabolic context right. J Gastroenterol Hepatol 23:1635–1648
115. **Anstee QM, Goldin RD** 2006 Mouse models in non-alcoholic fatty liver disease and steatohepatitis research. Int J Exp Pathol 87:1–16
116. **Ariz U, Mato JM, Lu SC, Martínez Chantar ML** 2010 Nonalcoholic steatohepatitis, animal models, and biomarkers: what is new? Methods Mol Biol 593:109–136
117. **Tetri LH, Basaranoglu M, Brunt EM, Yerian LM, Neuschwander-Tetri BA** 2008 Severe NAFLD with hepatic necroinflammatory changes in mice fed trans fats and high-fructose corn syrup equivalent. Am J Physiol Gastrointest Liver Physiol 295:G987–G995
118. **Varela-Rey M, Embade N, Ariz U, Lu SC, Mato JM, Martínez-Chantar ML** 2009 Non-alcoholic steatohepatitis and animal models: understanding the human disease. Int J Biochem Cell Biol 41:969–976
119. **Rinella ME, Green RM** 2004 The methionine-choline deficient dietary model of steatohepatitis does not exhibit insulin resistance. J Hepatol 40:47–51
120. **de Oliveira CP, de Lima VM, Simplicio FI, Soriano FG, de Mello ES, de Souza HP, Alves VA, Laurindo FR, Carrilho FJ, de Oliveira MG** 2008 Prevention and reversion of non-alcoholic steatohepatitis in ob/ob mice by S-nitroso-N-acetylcysteine treatment. J Am Coll Nutr 27:299–305
121. **Sahai A, Malladi P, Pan X, Paul R, Melin-Aldana H, Green RM, Whitington PF** 2004 Obese and diabetic db/db mice develop marked liver fibrosis in a model of non-alcoholic steatohepatitis: role of short-form leptin receptors and osteopontin. Am J Physiol Gastroenterol Liver Physiol 287: G1035–G1043
122. **Minokoshi Y, Kim YB, Peroni OD, Fryer LG, Müller C, Carling D, Kahn BB** 2002 Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. Nature 415:339–343
123. **Arsov T, Larter CZ, Nolan CJ, Petrovsky N, Goodnow CC, Teoh NC, Yeh MM, Farrell GC** 2006 Adaptive failure to high-fat diet characterizes steatohepatitis in Alms1 mutant mice. Biochem Biophys Res Commun 342:1152–1159
124. **Larter CZ, Yeh MM, Van Rooyen DM, Teoh NC, Brooling J, Hou JY, Williams J, Clyne M, Nolan CJ, Farrell GC**

Downloaded from https://academic.oup.com/edrv/article/34/1/84/2354662 by U.S. Department of Justice user on 16 August 2022

2009 Roles of adipose restriction and metabolic factors in progression of steatosis to steatohepatitis in obese, diabetic mice. J Gastroenterol Hepatol 24:1658–1668

125. **Zhou H, Saito S, Piao G, Liu ZP, Wang J, Horimoto K, Chen L** 2011 Network screening of Goto-Kakizaki rat liver microarray data during diabetic progression. BMC Syst Biol 5 Suppl 1:S16
126. **Shirakawa J, Fujii H, Ohnuma K, Sato K, Ito Y, Kaji M, Sakamoto E, Koganei M, Sasaki H, Nagashima Y, Amo K, Aoki K, Morimoto C, Takeda E, Terauchi Y** 2011 Diet-induced adipose tissue inflammation and liver steatosis are prevented by DPP-4 inhibition in diabetic mice. Diabetes 60:1246–1257
127. **Matafome P, Louro T, Rodrigues L, Crisóstomo J, Nunes E, Amaral C, Monteiro P, Cipriano A, Seiça R** 2011 Metformin and atorvastatin combination further protect the liver in type 2 diabetes with hyperlipidaemia. Diabetes Metab Res Rev 27:54–62
128. **Matafome P, Nunes E, Louro T, Amaral C, Crisóstomo J, Rodrigues L, Moedas AR, Monteiro P, Cipriano A, Seiça R** 2009 A role for atorvastatin and insulin combination in protecting from liver injury in a model of type 2 diabetes with hyperlipidemia. Naunyn Schmiedebergs Arch Pharmacol 379:241–251
129. **Larter CZ, Yeh MM, Van Rooyen DM, Brooling J, Ghatora K, Farrell GC** 2012 Peroxisome proliferator-activated receptor-α agonist, 643, improves metabolic indices, steatosis and ballooning in diabetic mice Wy 14 with non-alcoholic steatohepatitis. J Gastroenterol Hepatol 27: 341–350
130. **Duval C, Thissen U, Keshtkar S, Accart B, Stienstra R, Boekschoten MV, Roskams T, Kersten S, Müller M** 2010 Adipose tissue dysfunction signals progression of hepatic steatosis towards nonalcoholic steatohepatitis in C57BL/6 mice. Diabetes 59:3181–3191
131. **Gaemers IC, Stallen JM, Kunne C, Wallner C, van Werven J, Nederveen A, Lamers WH** 2011 Lipotoxicity and steatohepatitis in an overfed mouse model for non-alcoholic fatty liver disease. Biochim Biophys Acta 1812:447–458
132. **Sasaki Y, Suzuki W, Shimada T, Iizuka S, Nakamura S, Nagata M, Fujimoto M, Tsuneyama K, Hokao R, Miyamoto K, Aburada M** 2009 Dose dependent development of diabetes mellitus and non-alcoholic steatohepatitis in monosodium glutamate-induced obese mice. Life Sci 85: 490–498
133. **DeFronzo RA, Ferrannini E** 1991 Insulin resistance. A multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. Diabetes Care 14:173–194
134. **Rosenberg WM, Voelker M, Thiel R, Becka M, Burt A, Schuppan D, Hubscher S, Roskams T, Pinzani M, Arthur MJ, European Liver Fibrosis Group** 2004 Serum markers detect the presence of liver fibrosis: a cohort study. Gastroenterology 127:1704–1713
135. **Klein T,** The dipeptidyl peptidase 4 inhibitor linagliptin is an effective therapeutic for metabolic liver disease in several rodent models of non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH). Proc 62nd Annual Meeting of the American Association for the Study of Liver Diseases, San Francisco, CA, 2011 p 369A (Abstract 20)
136. **Ali R, Cusi K** 2009 New diagnostic and treatment approaches in non-alcoholic fatty liver disease (NAFLD). Ann Med 41:265–278
137. **Vanni E, Bugianesi E, Kotronen A, De Minicis S, Yki-Järvinen H, Svegliati-Baroni G** 2010 From the metabolic syndrome to NAFLD or vice versa? Dig Liver Dis 42:320–330
138. **Larter CZ, Chitturi S, Heydet D, Farrell GC** 2010 A fresh look at NASH pathogenesis. Part 1: the metabolic movers. J Gastroenterol Hepatol 25:672–690
139. **Schwimmer JB, Celedon MA, Lavine JE, Salem R, Campbell N, Schork NJ, Shiehmorteza M, Yokoo T, Chavez A, Middleton MS, Sirlin CB** 2009 Heritability of nonalcoholic fatty liver disease. Gastroenterology 136:1585–1592
140. **Abdelmalek MF, Liu C, Shuster J, Nelson DR, Asal NR** 2006 Familial aggregation of insulin resistance in first-degree relatives of patients with nonalcoholic fatty liver disease. Clin Gastroenterol Hepatol 4:1162–1169
141. **Chitturi S, Abeygunasekera S, Farrell GC, Holmes-Walker J, Hui JM, Fung C, Karim R, Lin R, Samarasinghe D, Liddle C, Weltman M, George J** 2002 NASH and insulin resistance: insulin hypersecretion and specific association with the insulin resistance syndrome. Hepatology 35:373–379
142. **Bonnefond A, Froguel P, Vaxillaire M** 2010 The emerging genetics of type 2 diabetes. Trends Mol Med 16:407–416
143. **Anstee QM, Daly AK, Day CP** 2011 Genetic modifiers of non-alcoholic fatty liver disease progression. Biochim Biophys Acta 1812:1557–1566
144. **Sladek R, Rocheleau G, Rung J, Dina C, Shen L, Serre D, Boutin P, Vincent D, Belisle A, Hadjadj S, Balkau B, Heude B, Charpentier G, Hudson TJ, Montpetit A, Pshezhetsky AV, Prentki M, Posner BI, Balding DJ, Meyre D, Polychronakos C, Froguel P** 2007 A genome-wide association study identifies novel risk loci for type 2 diabetes. Nature 445:881–885
145. **Ono M, Okamoto N, Saibara T** 2010 The latest idea in NAFLD/NASH pathogenesis. Clin J Gastroenterol 3:263–270
146. **Speliotes EK, Yerges-Armstrong LM, Wu J, Hernaez R, Kim LJ, Palmer CD, Gudnason V, Eiriksdottir G, Garcia ME, Launer LJ, Nalls MA, Clark JM, Mitchell BD, Shuldiner AR, Butler JL, Tomas M, Hoffmann U, Hwang SJ, Massaro JM, O'Donnell CJ, Sahani DV, Salomaa V, Schadt EE, Schwartz SM, Siscovick DS, Voight BF, Carr JJ, Feitosa MF, Harris TB, Fox CS, Smith AV, Kao WH, Hirschhorn JN, Borecki IB** 2011 Genome-wide association analysis identifies variants associated with nonalcoholic fatty liver disease that have distinct effects on metabolic traits. PLoS Genet 7:e1001324
147. **Kantartzis K, Peter A, Machicao F, Machann J, Wagner S, Königsrainer I, Königsrainer A, Schick F, Fritsche A, Häring HU, Stefan N** 2009 Dissociation between fatty liver and insulin resistance in humans carrying a variant of the patatin-like phospholipase 3 gene. Diabetes 58:2616–2623
148. **Bambha K, Belt P, Abraham M, Wilson LA, Pabst M, Ferrell L, Unalp-Arida A, Bass N** 2012 Ethnicity and nonalcoholic fatty liver disease. Hepatology 55:769–780
149. **Petit JM, Guiu B, Masson D, Duvillard L, Jooste V, Buffier P, Bouillet B, Brindisi MC, Robin I, Gambert P, Verges B, Cercueil JP, Hillon P** 2011 PNPLA3 polymorphism influ-

Downloaded from https://academic.oup.com/edrv/article/34/1/84/2354662 by U.S. Department of Justice user on 16 August 2022

ences liver fibrosis in unselected patients with type 2 diabetes. Liver Int 31:1332–1336
150. **Falleti E, Fabris C, Cmet S, Cussigh A, Bitetto D, Fontanini E, Fornasiere E, Bignulin S, Fumolo E, Bignulin E, Pirisi M, Toniutto P** 2011 PNPLA3 rs 738409C/G polymorphism in cirrhosis: relationship with the aetiology of liver disease and hepatocellular carcinoma occurrence. Liver Int 31: 1137–1143
151. **Speliotes EK, Butler JL, Palmer CD, Voight BF, Hirschhorn JN** 2010 PNPLA3 variants specifically confer increased risk for histological nonalcoholic fatty liver disease but not metabolic disease. Hepatology 52:904–912
152. **Petersen KF, Dufour S, Hariri A, Nelson-Williams C, Foo JN, Zhang XM, Dziura J, Lifton RP, Shulman GI** 2010 Apolipoprotein C3 gene variants in nonalcoholic fatty liver disease. N Engl J Med 362:1082–1089
153. **Sookoian S, Castaño GO, Burgueño AL, Gianotti TF, Rosselli MS, Pirola CJ** 2010 The nuclear receptor PXR gene variants are associated with liver injury in nonalcoholic fatty liver disease. Pharmacogenet Genomics 20:1–8
154. **Bernard S, Touzet S, Personne I, Lapras V, Bondon PJ, Berthezène F, Moulin P** 2000 Association between microsomal triglyceride transfer protein gene polymorphism and the biological features of liver steatosis in patients with type II diabetes. Diabetologia 43:995–999
155. **Namikawa C, Shu-Ping Z, Vyselaar JR, Nozaki Y, Nemoto Y, Ono M, Akisawa N, Saibara T, Hiroi M, Enzan H, Onishi S** 2004 Polymorphisms of microsomal triglyceride transfer protein gene and manganese superoxide dismutase gene in non-alcoholic steatohepatitis. J Hepatol 40:781–786
156. **Dong H, Wang J, Li C, Hirose A, Nozaki Y, Takahashi M, Ono M, Akisawa N, Iwasaki S, Saibara T, Onishi S** 2007 The phosphatidylethanolamine N-methyltransferase gene V175M single nucleotide polymorphism confers the susceptibility to NASH in Japanese population. J Hepatol 46: 915–920
157. **Song J, da Costa KA, Fischer LM, Kohlmeier M, Kwock L, Wang S, Zeisel SH** 2005 Polymorphism of the PEMT gene and susceptibility to nonalcoholic fatty liver disease (NAFLD). FASEB J 19:1266–1271
158. **Tang H, Wang XX, Zhao SG, Wang JJ, Zhang C, Lu W, Li HY, Wen QS** 2011 Association between the G175a and rs12325817 polymorphisms in the phosphatidylethanolamine N-methyltransferase gene and susceptibility to nonalcoholic fatty liver disease. World Chin J Digestol 19: 3035–3039
159. **Tokushige K, Takakura M, Tsuchiya-Matsushita N, Taniai M, Hashimoto E, Shiratori K** 2007 Influence of TNF gene polymorphisms in Japanese patients with NASH and simple steatosis. J Hepatol 46:1104–1110
160. **Carulli L, Canedi I, Rondinella S, Lombardini S, Ganazzi D, Fargion S, De Palma M, Lonardo A, Ricchi M, Bertolotti M, Carulli N, Loria P** 2009 Genetic polymorphisms in non-alcoholic fatty liver disease: interleukin-6-174G/C polymorphism is associated with non-alcoholic steatohepatitis. Dig Liver Dis 41:823–828
161. **Valenti L, Nobili V, Al-Serri A, Rametta R, Leathart JB, Zappa MA, Dongiovanni P, Fracanzani AL, Alterio A, Roviaro G, Daly AK, Fargion S, Day CP** 2011 The APOC3 T-455C and C-482T promoter region polymorphisms are not associated with the severity of liver damage independently of PNPLA3 I148M genotype in patients with nonalcoholic fatty liver. J Hepatol 55:1409–1414
162. **Kozlitina J, Boerwinkle E, Cohen JC, Hobbs HH** 2011 Dissociation between APOC3 variants, hepatic triglyceride content and insulin resistance. Hepatology 53:467–474
163. **Sentinelli F, Romeo S, Maglio C, Incani M, Burza MA, Scano F, Coccia F, Cossu E, Leonetti F, Baroni MG** 2011 Lack of effect of apolipoprotein C3 polymorphisms on indices of liver steatosis, lipid profile and insulin resistance in obese Southern Europeans. Lipids Health Dis 10:93
164. **van Hoek M, van Herpt TW, Dehghan A, Hofman A, Lieverse AG, van Duijn CM, Witteman JC, Sijbrands EJ** 2011 Association of an APOC3 promoter variant with type 2 diabetes risk and need for insulin treatment in lean persons. Diabetologia 54:1360–1367
165. **Al-Serri A, Anstee QM, Valenti L, Nobili V, Leathart JB, Dongiovanni P, Patch J, Fracanzani A, Fargion S, Day CP, Daly AK** 2012 The SOD2 C47T polymorphism influences NAFLD fibrosis severity: evidence from case-control and intra-familial allele association studies. J Hepatol 56:448–454
166. **Möllsten A, Marklund SL, Wessman M, Svensson M, Forsblom C, Parkkonen M, Brismar K, Groop PH, Dahlquist G** 2007 A functional polymorphism in the manganese superoxide dismutase gene and diabetic nephropathy. Diabetes 56:265–269
167. **Petrovic MG, Cilensek I, Petrovic D** 2008 Manganese superoxide dismutase gene polymorphism (V16A) is associated with diabetic retinopathy in Slovene (Caucasians) type 2 diabetes patients. Dis Markers 24:59–64
168. **Tian C, Fang S, Du X, Jia C** 2011 Association of the C47T polymorphism in SOD2 with diabetes mellitus and diabetic microvascular complications: a meta-analysis. Diabetologia 54:803–811
169. **Rubin D, Helwig U, Pfeuffer M, Schreiber S, Boeing H, Fisher E, Pfeiffer A, Freitag-Wolf S, Foelsch UR, Doering F, Schrezenmeir J** 2006 A common functional exon polymorphism in the microsomal triglyceride transfer protein gene is associated with type 2 diabetes, impaired glucose metabolism and insulin levels. J Hum Genet 51:567–574
170. **Musso G, Gambino R, Cassader M** 2010 Lipoprotein metabolism mediates the association of MTP polymorphism with β-cell dysfunction in healthy subjects and in nondiabetic normolipidemic patients with nonalcoholic steatohepatitis. J Nutr Biochem 21:834–840
171. **Feng R, Li Y, Zhao D, Wang C, Niu Y, Sun C** 2009 Lack of association between TNF 238G/A polymorphism and type 2 diabetes: a meta-analysis. Acta Diabetol 46:339–343
172. **Feng RN, Zhao C, Sun CH, Li Y** 2011 Meta-analysis of TNF 308 G/A polymorphism and type 2 diabetes. PLoS ONE 6:e18480
173. **Feng RN, Li Y, Sun CH** 2009 TNF 308 G/A polymorphism and type 1 diabetes: a meta-analysis. Diabetes Res Clin Pract 85:e4–e7
174. **Alisi A, Cianfarani S, Manco M, Agostoni C, Nobili V** 2012 Non-alcoholic fatty liver disease and metabolic syndrome in adolescents: pathogenetic role of genetic background and intrauterine environment. Ann Med 44:29–40

Downloaded from https://academic.oup.com/edrv/article/34/1/84/2354662 by U.S. Department of Justice user on 16 August 2022

175. **Yazýcý D, Özben B, Yavuz D, Deyneli O, Aydýn H, Tarcin Ö, Akalýn S** 2011 Epicardial adipose tissue thickness in type 1 diabetic patients. Endocrine 40:250–255
176. **Danielson KK, Drum ML, Estrada CL, Lipton RB** 2010 Racial and ethnic differences in an estimated measure of insulin resistance among individuals with type 1 diabetes. Diabetes Care 33:614–619
177. **Jou J, Choi SS, Diehl AM** 2008 Mechanisms of disease progression in nonalcoholic fatty liver disease. Semin Liver Dis 28:370–379
178. **Sheth SG, Gordon FD, Chopra S** 1997 Nonalcoholic steatohepatitis. Ann Intern Med 126:137–145
179. **Neuschwander-Tetri BA** 2010 Hepatic lipotoxicity and the pathogenesis of nonalcoholic steatohepatitis: the central role of nontriglyceride fatty acid metabolites. Hepatology 52:774–788
180. **Lomonaco R, Ortiz-Lopez C, Orsak B, Webb A, Hardies J, Darland C, Finch J, Gastaldelli A, Harrison S, Tio F, Cusi K** 2012 Effect of adipose tissue insulin resistance on metabolic parameters and liver histology in obese patients with nonalcoholic fatty liver disease. Hepatology 55:1389–1397
181. **Brown MS, Goldstein JL** 2008 Selective vs total insulin resistance: a pathogenic paradox. Cell Metab 7:95–96
182. **Li S, Brown MS, Goldstein JL** 2010 Bifurcation of insulin signaling pathway in rat liver: mTORC1 required for stimulation of lipogenesis, but not inhibition of gluconeogenesis. Proc Natl Acad Sci USA 107:3441–3446
183. **Shimomura I, Bashmakov Y, Horton JD** 1999 Increased levels of nuclear SREBP-1c associated with fatty livers in two mouse models of diabetes mellitus. J Biol Chem 274:30028–30032
184. **Samuel VT, Petersen KF, Shulman GI** 2010 Lipid-induced insulin resistance: unravelling the mechanism. Lancet 375:2267–2277
185. **Shimomura I, Bashmakov Y, Ikemoto S, Horton JD, Brown MS, Goldstein JL** 1999 Insulin selectively increases SREBP-1c mRNA in the livers of rats with streptozotocin-induced diabetes. Proc Natl Acad Sci USA 96:13656–13661
186. **Horton JD, Goldstein JL, Brown MS** 2002 SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. J Clin Invest 109:1125–1131
187. **Lee YS, Sohn DH, Han D, Lee HW, Seong RH, Kim JB** 2007 Chromatin remodeling complex interacts with ADD1/SREBP1c to mediate insulin-dependent regulation of gene expression. Mol Cell Biol 27:438–452
188. **Sajan MP, Standaert ML, Rivas J, Miura A, Kanoh Y, Soto J, Taniguchi CM, Kahn CR, Farese RV** 2009 Role of atypical protein kinase C in activation of sterol regulatory element binding protein-1c and nuclear factor κB (NFκB) in liver of rodents used as a model of diabetes, and relationships to hyperlipidaemia and insulin resistance. Diabetologia 52:1197–1207
189. **Shimano H, Horton JD, Shimomura I, Hammer RE, Brown MS, Goldstein JL** 1997 Isoform 1c of sterol regulatory element binding protein is less active than isoform 1a in livers of transgenic mice and in cultured cells. J Clin Invest 99:846–854
190. **Ferré P, Foufelle F** 2010 Hepatic steatosis: a role for de novo lipogenesis and the transcription factor SREBP-1c. Diabetes Obes Metab 12:83–92
191. **Harrison SA, Day CP** 2007 Recent advances in clinical practice: the role of lifestyle modification in NAFLD. Gut 56:1760–1769
192. **Adeli K, Theriault A** 1992 Insulin modulation of human apolipoprotein-B messenger RNA translation—studies in an in vitro cell-free system from HEPG2 cells. Biochem Cell Biol 70:1301–1312
193. **Malaguarnera M, Di Rosa M, Nicoletti F, Malaguarnera L** 2009 Molecular mechanisms involved in NAFLD progression. J Mol Med 87:679–695
194. **Cazanave SC, Gores GJ** 2010 Mechanisms and clinical implications of hepatocyte lipoapoptosis. Clin Lipidol 5:71–85
195. **Wei Y, Wang D, Topczewski F, Pagliassotti MJ** 2006 Saturated fatty acids induce endoplasmic reticulum stress and apoptosis independently of ceramide in liver cells. Am J Physiol Endocrinol Metab 291:E275–E281
196. **Liu Y, Qiu de K, Ma X** 2012 Liver X receptors bridge hepatic lipid metabolism and inflammation. J Dig Dis 13:69–74
197. **Nagaya T, Tanaka N, Suzuki T, Sano K, Horiuchi A, Komatsu M, Nakajima T, Nishizawa T, Joshita S, Umemura T, Ichijo T, Matsumoto A, Yoshizawa K, Nakayama J, Tanaka E, Aoyama T** 2010 Down-regulation of SREBP-1c is associated with the development of burned-out NASH. J Hepatol 53:724–731
198. **Donnelly KL, Smith CI, Schwarzenberg SJ, Jessurun J, Boldt MD, Parks EJ** 2005 Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. J Clin Invest 115:1343–1351
199. **Sunny NE, Parks EJ, Browning JD, Burgess SC** 2011 Excessive hepatic mitochondrial TCA cycle and gluconeogenesis in humans with nonalcoholic fatty liver disease. Cell Metab 14:804–810
200. **Puri P, Wiest MM, Cheung O, Mirshahi F, Sargeant C, Min HK, Contos MJ, Sterling RK, Fuchs M, Zhou H, Watkins SM, Sanyal AJ** 2009 The plasma lipidomic signature of nonalcoholic steatohepatitis. Hepatology 50:1827–1838
201. **Puri P, Baillie RA, Wiest MM, Mirshahi F, Choudhury J, Cheung O, Sargeant C, Contos MJ, Sanyal AJ** 2007 A lipidomic analysis of nonalcoholic fatty liver disease. Hepatology 46:1081–1090
202. **Van Rooyen DM, Larter CZ, Haigh WG, Yeh MM, Ioannou G, Kuver R, Lee SP, Teoh NC, Farrell GC** 2011 Hepatic free cholesterol accumulates in obese, diabetic mice and causes nonalcoholic steatohepatitis. Gastroenterology 141:1393–1403, 1403.e1–e5
203. **Kumashiro N, Erion DM, Zhang D, Kahn M, Beddow SA, Chu X, Still CD, Gerhard GS, Han X, Dziura J, Petersen KF, Samuel VT, Shulman GI** 2011 Cellular mechanism of insulin resistance in nonalcoholic fatty liver disease. Proc Natl Acad Sci USA 108:16381–16385
204. **Gorden DL, Ivanova PT, Myers DS, McIntyre JO, VanSaun MN, Wright JK, Matrisian LM, Brown HA** 2011 Increased diacylglycerols characterize hepatic lipid changes in progression of human nonalcoholic fatty liver disease; comparison to a murine model. PLoS ONE 6:e22775
205. **Lyn-Cook Jr LE, Lawton M, Tong M, Silbermann E, Longato L, Jiao P, Mark P, Wands JR, Xu H, de la Monte SM**

Downloaded from https://academic.oup.com/edrv/article/34/1/84/2354662 by U.S. Department of Justice user on 16 August 2022

2009 Hepatic ceramide may mediate brain insulin resistance and neurodegeneration in type 2 diabetes and nonalcoholic steatohepatitis. J Alzheimers Dis 16:715–729
206. **Garcia-Ruiz C, Marí M, Colell A, Morales A, Fernandez-Checa JC** 2011 Metabolic therapy: lessons from liver diseases. Curr Pharm Des 17:3933–3944
207. **Pagadala M, Kasumov T, McCullough AJ, Zein NN, Kirwan JP** 2012 Role of ceramides in nonalcoholic fatty liver disease. Trends Endocrinol Metab 23:365–371
208. **Farrell GC, van Rooyen D, Gan L, Chitturi S** 2012 NASH is an inflammatory disorder: pathogenic, prognostic and therapeutic implications. Gut Liver 6:149–171
209. **Neuschwander-Tetri BA, Clark JM, Bass NM, Van Natta ML, Unalp-Arida A, Tonascia J, Zein CO, Brunt EM, Kleiner DE, McCullough AJ, Sanyal AJ, Diehl AM, Lavine JE, Chalasani N, Kowdley KV, Nash Clinical Research Network** 2010 Clinical, laboratory and histological associations in adults with nonalcoholic fatty liver disease. Hepatology 52:913–924
210. **Musso G, Gambino R, Cassader M** 2010 Non-alcoholic fatty liver disease from pathogenesis to management: an update. Obes Rev 11:430–445
211. **Leclercq IA, Farrell GC, Sempoux C, dela Peña A, Horsmans Y** 2004 Curcumin inhibits NF-κB activation and reduces the severity of experimental steatohepatitis in mice. J Hepatol 41:926–934
212. **Schattenberg JM, Singh R, Wang Y, Lefkowitch JH, Rigoli RM, Scherer PE, Czaja MJ** 2006 JNK1 but not JNK2 promotes the development of steatohepatitis in mice. Hepatology 43:163–172
213. **Tacke F, Luedde T, Trautwein C** 2009 Inflammatory pathways in liver homeostasis and liver injury. Clin Rev Allergy Immunol 36:4–12
214. **Argo CK, Northup PG, Al-Osaimi AM, Caldwell SH** 2009 Systematic review of risk factors for fibrosis progression in non-alcoholic steatohepatitis. J Hepatol 51:371–379
215. **Brunt EM, Kleiner DE, Wilson LA, Unalp A, Behling CE, Lavine JE, Neuschwander-Tetri BA** 2009 Portal chronic inflammation in nonalcoholic fatty liver disease (NAFLD): a histologic marker of advanced NAFLD-clinicopathologic correlations from the Nonalcoholic Steatohepatitis Clinical Research Network. Hepatology 49:809–820
216. **Brenner DA, Seki E, Taura K, Kisseleva T, Deminicis S, Iwaisako K, Inokuchi S, Schnabl B, Oesterreicher CH, Paik YH, Miura K, Kodama Y** 2011 Non-alcoholic steatohepatitis-induced fibrosis: Toll-like receptors, reactive oxygen species and Jun N-terminal kinase. Hepatol Res 41:683–686
217. **Tomita K, Tamiya G, Ando S, Ohsumi K, Chiyo T, Mizutani A, Kitamura N, Toda K, Kaneko T, Horie Y, Han JY, Kato S, Shimoda M, Oike Y, Tomizawa M, Makino S, Ohkura T, Saito H, Kumagai N, Nagata H, Ishii H, Hibi T** 2006 Tumour necrosis factor α signalling through activation of Kupffer cells plays an essential role in liver fibrosis of non-alcoholic steatohepatitis in mice. Gut 55:415–424
218. **Canbay A, Feldstein AE, Higuchi H, Werneburg N, Grambihler A, Bronk SF, Gores GJ** 2003 Kupffer cell engulfment of apoptotic bodies stimulates death ligand and cytokine expression. Hepatology 38:1188–1198
219. **Nakahira K, Haspel JA, Rathinam VA, Lee SJ, Dolinay T, Lam HC, Englert JA, Rabinovitch M, Cernadas M, Kim HP, Fitzgerald KA, Ryter SW, Choi AM** 2011 Autophagy proteins regulate innate immune responses by inhibiting the release of mitochondrial DNA mediated by the NALP3 inflammasome. Nat Immunol 12:222–230
220. **Neuschwander-Tetri BA** 2010 Nontriglyceride hepatic lipotoxicity: the new paradigm for the pathogenesis of NASH. Curr Gastroenterol Rep 12:49–56
221. **Lockman KA, Baren JP, Pemberton CJ, Baghdadi H, Burgess KE, Plevris-Papaioannou N, Lee P, Howie F, Beckett G, Pryde A, Jaap AJ, Hayes PC, Filippi C, Plevris JN** 2012 Oxidative stress rather than triglyceride accumulation is a determinant of mitochondrial dysfunction in in vitro models of hepatic cellular steatosis. Liver Int 32:1079–1092
222. **Hui JM, Hodge A, Farrell GC, Kench JG, Kriketos A, George J** 2004 Beyond insulin resistance in NASH: TNF-α or adiponectin? Hepatology 40:46–54
223. **Masaki T, Chiba S, Tatsukawa H, Yasuda T, Noguchi H, Seike M, Yoshimatsu H** 2004 Adiponectin protects LPS-induced liver injury through modulation of TNF-α in KK-Ay obese mice. Hepatology 40:177–184
224. **Arvaniti VA, Thomopoulos KC, Tsamandas A, Makri M, Psyrogiannis A, Vafiadis G, Assimakopoulos SF, Asimakopoulos S, Labropoulou-Karatza C** 2008 Serum adiponectin levels in different types of non alcoholic liver disease. Correlation with steatosis, necroinflammation and fibrosis. Acta Gastroenterol Belg 71:355–360
225. **Jiang ZY, Lin YW, Clemont A, Feener EP, Hein KD, Igarashi M, Yamauchi T, White MF, King GL** 1999 Characterization of selective resistance to insulin signaling in the vasculature of obese Zucker (fa/fa) rats. J Clin Invest 104:447–457
226. **Xi L, Xiao C, Bandsma RH, Naples M, Adeli K, Lewis GF** 2011 C-Reactive protein impairs hepatic insulin sensitivity and insulin signaling in rats: role of mitogen-activated protein kinases. Hepatology 53:127–135
227. **Chen A, Zheng S** 2008 Curcumin inhibits connective tissue growth factor gene expression in activated hepatic stellate cells in vitro by blocking NF-κB and ERK signalling. Br J Pharmacol 153:557–567
228. **Jiang MD, Zheng SM, Xu H, Zeng WZ, Zhang Y, Sun HP, Wang YX, Qin JP, Wu XL** 2008 An experimental study of extracellular signal-regulated kinase and its interventional treatments in hepatic fibrosis. Hepatobiliary Pancreat Dis Int 7:51–57
229. **Gao R, Ball DK, Perbal B, Brigstock DR** 2004 Connective tissue growth factor induces c-fos gene activation and cell proliferation through p44/42 MAP kinase in primary rat hepatic stellate cells. J Hepatol 40:431–438
230. **Handy JA, Fu PP, Kumar P, Mells JE, Sharma S, Saxena NK, Anania FA** 2011 Adiponectin inhibits leptin signalling via multiple mechanisms to exert protective effects against hepatic fibrosis. Biochem J 440:385–395
231. **Stefan N, Kantartzis K, Machann J, Schick F, Thamer C, Rittig K, Balletshofer B, Machicao F, Fritsche A, Häring HU** 2008 Identification and characterization of metabolically benign obesity in humans. Arch Intern Med 168:1609–1616
232. **Petersen KF, Oral EA, Dufour S, Befroy D, Ariyan C, Yu C, Cline GW, DePaoli AM, Taylor SI, Gorden P, Shulman GI** 2002 Leptin reverses insulin resistance and hepatic ste-

Downloaded from https://academic.oup.com/edrv/article/34/1/84/2354662 by U.S. Department of Justice user on 16 August 2022

atosis in patients with severe lipodystrophy. J Clin Invest 109:1345–1350
233. **Samuel VT, Liu ZX, Qu X, Elder BD, Bilz S, Befroy D, Romanelli AJ, Shulman GI** 2004 Mechanism of hepatic insulin resistance in non-alcoholic fatty liver disease. J Biol Chem 279:32345–32353
234. **Kashyap S, Belfort R, Gastaldelli A, Pratipanawatr T, Berria R, Pratipanawatr W, Bajaj M, Mandarino L, DeFronzo R, Cusi K** 2003 A sustained increase in plasma free fatty acids impairs insulin secretion in nondiabetic subjects genetically predisposed to develop type 2 diabetes. Diabetes 52:2461–2474
235. **Cusi K, Kashyap S, Gastaldelli A, Bajaj M, Cersosimo E** 2007 Effects on insulin secretion and insulin action of a 48-h reduction of plasma free fatty acids with acipimox in nondiabetic subjects genetically predisposed to type 2 diabetes. Am J Physiol Endocrinol Metab 292:E1775–E1781
236. **Goh SY, Cooper ME** 2008 The role of advanced glycation end products in progression and complications of diabetes. J Clin Endocrinol Metab 93:1143–1152
237. **Kimura Y, Hyogo H, Yamagishi S, Takeuchi M, Ishitobi T, Nabeshima Y, Arihiro K, Chayama K** 2010 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 45:750–757
238. **Guimarães EL, Empsen C, Geerts A, van Grunsven LA** 2010 Advanced glycation end products induce production of reactive oxygen species via the activation of NADPH oxidase in murine hepatic stellate cells. J Hepatol 52:389–397
239. **Iizuka K, Bruick RK, Liang G, Horton JD, Uyeda K** 2004 Deficiency of carbohydrate response element-binding protein (ChREBP) reduces lipogenesis as well as glycolysis. Proc Natl Acad Sci USA 101:7281–7286
240. **Dentin R, Benhamed F, Hainault I, Fauveau V, Foufelle F, Dyck JR, Girard J, Postic C** 2006 Liver-specific inhibition of ChREBP improves hepatic steatosis and insulin resistance in ob/ob mice. Diabetes 55:2159–2170
241. **Paradis V, Perlemuter G, Bonvoust F, Dargere D, Parfait B, Vidaud M, Conti M, Huet S, Ba N, Buffet C, Bedossa P** 2001 High glucose and hyperinsulinemia stimulate connective tissue growth factor expression: a potential mechanism involved in progression to fibrosis in nonalcoholic steatohepatitis. Hepatology 34:738–744
242. **Lin J, Chen A** 2011 Curcumin diminishes the impacts of hyperglycemia on the activation of hepatic stellate cells by suppressing membrane translocation and gene expression of glucose transporter-2. Mol Cell Endocrinol 333:160–171
243. **Könner AC, Brüning JC** 2011 Toll-like receptors: linking inflammation to metabolism. Trends Endocrinol Metab 22:16–23
244. **Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, Neyrinck AM, Fava F, Tuohy KM, Chabo C, Waget A, Delmée E, Cousin B, Sulpice T, Chamontin B, Ferrières J, Tanti JF, Gibson GR, Casteilla L, Delzenne NM, Alessi MC, Burcelin R** 2007 Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes 56:1761–1772
245. **Amyot J, Semache M, Ferdaoussi M, Fontés G, Poitout V** 2012 Lipopolysaccharides impair insulin gene expression in isolated islets of Langerhans via Toll-like receptor-4 and NF-κB signalling. PLoS ONE 7:e36200
246. **Lee JY, Zhao L, Youn HS, Weatherill AR, Tapping R, Feng L, Lee WH, Fitzgerald KA, Hwang DH** 2004 Saturated fatty acid activates but polyunsaturated fatty acid inhibits Toll-like receptor 2 dimerized with Toll-like receptor 6 or 1. J Biol Chem 279:16971–16979
247. **Schaeffler A, Gross P, Buettner R, Bollheimer C, Buechler C, Neumeier M, Kopp A, Schoelmerich J, Falk W** 2009 Fatty acid-induced induction of Toll-like receptor-4/nuclear factor-κB pathway in adipocytes links nutritional signalling with innate immunity. Immunology 126:233–245
248. **Manco M, Putignani L, Bottazzo GF** 2010 Gut microbiota, lipopolysaccharides, and innate immunity in the pathogenesis of obesity and cardiovascular risk. Endocr Rev 31:817–844
249. **Serino M, Luche E, Gres S, Baylac A, Bergé M, Cenac C, Waget A, Klopp P, Iacovoni J, Klopp C, Mariette J, Bouchez O, Lluch J, Ouarné F, Monsan P, Valet P, Roques C, Amar J, Bouloumié A, Théodorou V, Burcelin R** 2012 Metabolic adaptation to a high-fat diet is associated with a change in the gut microbiota. Gut 61:543–553
250. **Creely SJ, McTernan PG, Kusminski CM, Fisher M, Da Silva NF, Khanolkar M, Evans M, Harte AL, Kumar S** 2007 Lipopolysaccharide activates an innate immune system response in human adipose tissue in obesity and type 2 diabetes. Am J Physiol Endocrinol Metab 292:E740–E747
251. **Cornell RP** 1980 Mechanisms of acute hyperinsulinemia after Kupffer cell phagocytosis. Am J Physiol 238:E276–E283
252. **Byrne MM, Pluntke K, Wank U, Schirra J, Arnold R, Göke B, Katschinski M** 1998 Inhibitory effects of hyperglycaemia on fed jejunal motility: potential role of hyperinsulinaemia. Eur J Clin Invest 28:72–78
253. **Cuoco L, Montalto M, Jorizzo RA, Santarelli L, Arancio F, Cammarota G, Gasbarrini G** 2002 Eradication of small intestinal bacterial overgrowth and oro-cecal transit in diabetics. Hepatogastroenterology 49:1582–1586
254. **Erridge C, Attina T, Spickett CM, Webb DJ** 2007 A high-fat meal induces low-grade endotoxemia: evidence of a novel mechanism of postprandial inflammation. Am J Clin Nutr 86:1286–1292
255. **van der Poorten D, Milner KL, Hui J, Hodge A, Trenell MI, Kench JG, London R, Peduto T, Chisholm DJ, George J** 2008 Visceral fat: a key mediator of steatohepatitis in metabolic liver disease. Hepatology 48:449–457
256. **Ibrahim MM** 2010 Subcutaneous and visceral adipose tissue: structural and functional differences. Obes Rev 11:11–18
257. **Speliotes EK, Massaro JM, Hoffmann U, Vasan RS, Meigs JB, Sahani DV, Hirschhorn JN, O'Donnell CJ, Fox CS** 2010 Fatty liver is associated with dyslipidemia and dysglycemia independent of visceral fat: the Framingham Heart Study. Hepatology 51:1979–1987
258. **Fracanzani AL, Valenti L, Bugianesi E, Vanni E, Grieco A, Miele L, Consonni D, Fatta E, Lombardi R, Marchesini G, Fargion S** 2011 Risk of nonalcoholic steatohepatitis and

Downloaded from https://academic.oup.com/edrv/article/34/1/84/2354662 by U.S. Department of Justice user on 16 August 2022

fibrosis in patients with nonalcoholic fatty liver disease and low visceral adiposity. J Hepatol 54:1244–1249
259. **Machado MV, Cortez-Pinto H** 2011 No need for a large belly to have NASH. J Hepatol 54:1090–1093
260. **Malik VS, Popkin BM, Bray GA, Després JP, Willett WC, Hu FB** 2010 Sugar-sweetened beverages and risk of metabolic syndrome and type 2 diabetes: a meta-analysis. Diabetes Care 33:2477–2483
261. **Tappy L, Lê KA** 2010 Metabolic effects of fructose and the worldwide increase in obesity. Physiol Rev 90:23–46
262. **Steinbrecher A, Morimoto Y, Heak S, Ollberding NJ, Geller KS, Grandinetti A, Kolonel LN, Maskarinec G** 2011 The preventable proportion of type 2 diabetes by ethnicity: the multiethnic cohort. Ann Epidemiol 21:526–535
263. **Voulgari C, Katsilambros N, Tentolouris N** 2011 Smoking cessation predicts amelioration of microalbuminuria in newly diagnosed type 2 diabetes mellitus: a 1-year prospective study. Metabolism 60:1456–1464
264. **Molloy JW, Calcagno CJ, Williams CD, Jones FJ, Torres DM, Harrison SA** 2012 Association of coffee and caffeine consumption with fatty liver disease, nonalcoholic steatohepatitis, and degree of hepatic fibrosis. Hepatology 55: 429–436
265. **Kistler KD, Brunt EM, Clark JM, Diehl AM, Sallis JF, Schwimmer JB** 2011 Physical activity recommendations, exercise intensity, and histological severity of nonalcoholic fatty liver disease. Am J Gastroenterol 106:460–468; quiz 469
266. **Charlton M, Krishnan A, Viker K, Sanderson S, Cazanave S, McConico A, Masuoko H, Gores G** 2011 Fast food diet mouse: novel small animal model of NASH with ballooning, progressive fibrosis, and high physiological fidelity to the human condition. Am J Physiol Gastrointest Liver Physiol 301:G825–G834
267. **Kayaniyil S, Vieth R, Harris SB, Retnakaran R, Knight JA, Gerstein HC, Perkins BA, Zinman B, Hanley AJ** 2011 Association of 25(OH)D and PTH with metabolic syndrome and its traditional and nontraditional components. J Clin Endocrinol Metab 96:168–175
268. **Targher G, Bertolini L, Scala L, Cigolini M, Zenari L, Falezza G, Arcaro G** 2007 Associations between serum 25-hydroxyvitamin D3 concentrations and liver histology in patients with non-alcoholic fatty liver disease. Nutr Metab Cardiovasc Dis 17:517–524
269. **Barchetta I, Angelico F, Del Ben M, Baroni MG, Pozzilli P, Morini S, Cavallo MG** 2011 Strong association between non alcoholic fatty liver disease (NAFLD) and low 25(OH) vitamin D levels in an adult population with normal serum liver enzymes. BMC Med 9:85
270. **Manousou P, Kalambokis G, Grillo F, Watkins J, Xirouchakis E, Pleguezuelo M, Leandro G, Arvaniti V, Germani G, Patch D, Calvaruso V, Mikhailidis DP, Dhillon AP, Burroughs AK** 2011 Serum ferritin is a discriminant marker for both fibrosis and inflammation in histologically proven non-alcoholic fatty liver disease patients. Liver Int 31:730–739
271. **Ford ES, Cogswell ME** 1999 Diabetes and serum ferritin concentration among U.S. adults. Diabetes Care 22:1978–1983
272. **Sos BC, Harris C, Nordstrom SM, Tran JL, Balázs M, Caplazi P, Febbraio M, Applegate MA, Wagner KU, Weiss EJ** 2011 Abrogation of growth hormone secretion rescues fatty liver in mice with hepatocyte-specific deletion of JAK2. J Clin Invest 121:1412–1423
273. **García-Galiano D, Sánchez-Garrido MA, Espejo I, Montero JL, Costán G, Marchal T, Membrives A, Gallardo-Valverde JM, Muñoz-Castañeda JR, Arévalo E, De la Mata M, Muntané J** 2007 IL-6 and IGF-1 are independent prognostic factors of liver steatosis and non-alcoholic steatohepatitis in morbidly obese patients. Obes Surg 17:493–503
274. **Vardatsikos G, Sahu A, Srivastava AK** 2009 The insulin-like growth factor family: molecular mechanisms, redox regulation, and clinical implications. Antioxid Redox Signal 11:1165–1190
275. **Sandhu MS, Heald AH, Gibson JM, Cruickshank JK, Dunger DB, Wareham NJ** 2002 Circulating concentrations of insulin-like growth factor-1 and development of glucose intolerance: a prospective observational study. Lancet 359:1740–1745
276. **Colak Y, Senates E, Ozturk O, Yilmaz Y, Zemheri E, Yilmaz Enc F, Ulasoglu C, Aksaray S, Bozbeyoglu SG, Kiziltas S, Kurdas OO, Tuncer I** 2012 Serum concentrations of human insulin-like growth factor-1 and levels of insulin-like growth factor-binding protein-5 in patients with nonalcoholic fatty liver disease: association with liver histology. Eur J Gastroenterol Hepatol 24:255–261
277. **Hsueh WA, Wyne K** 2011 Renin-angiotensin-aldosterone system in diabetes and hypertension. J Clin Hypertens (Greenwich) 13:224–237
278. **Vinik AI, Maser RE, Ziegler D** 2011 Autonomic imbalance: prophet of doom or scope for hope? Diabet Med 28:643–651
279. **Polyzos SA, Kountouras J, Zafeiriadou E, Patsiaoura K, Katsiki E, Deretzi G, Zavos C, Tsarouchas G, Rakitzi P, Slavakis A** 2011 Effect of spironolactone and vitamin E on serum metabolic parameters and insulin resistance in patients with nonalcoholic fatty liver disease. J Renin Angiotensin Aldosterone Syst 12:498–503
280. **Byrne TJ, Parish JM, Somers V, Aqel BA, Rakela J** 2012 Evidence for liver injury in the setting of obstructive sleep apnea. Ann Hepatol 11:228–231
281. **Aron-Wisnewsky J, Minville C, Tordjman J, Lévy P, Bouillot JL, Basdevant A, Bedossa P, Clément K, Pépin JL** 2012 Chronic intermittent hypoxia is a major trigger for non-alcoholic fatty liver disease in morbid obese. J Hepatol 56:225–233
282. **Ronksley PE, Hemmelgarn BR, Heitman SJ, Hanly PJ, Faris PD, Quan H, Tsai WH** 2009 Obstructive sleep apnoea is associated with diabetes in sleepy subjects. Thorax 64:834–839
283. **Day CP, Saksena S** 2002 Non-alcoholic steatohepatitis: definitions and pathogenesis. J Gastroenterol Hepatol 17(Suppl 3):S377–S384
284. **Jamieson HA, Cogger VC, Twigg SM, McLennan SV, Warren A, Cheluvappa R, Hilmer SN, Fraser R, de Cabo R, Le Couteur DG** 2007 Alterations in liver sinusoidal endothelium in baboon model of type 1 diabetes. Diabetologia 50:1969–1976
285. **Bernuau D, Guillot R, Durand-Schneider AM, Poussier P, Moreau A, Feldmann G** 1985 Liver perisinusoidal fibrosis

Downloaded from https://academic.oup.com/edrv/article/34/1/84/2354662 by U.S. Department of Justice user on 16 August 2022

in BB rats with or without overt diabetes. Am J Pathol 120:38–45
286. **Latry P, Bioulac-Sage P, Echinard E, Gin H, Boussarie L, Grimaud JA, Balabaud C** 1987 Perisinusoidal fibrosis and basement membrane-like material in the livers of diabetic patients. Hum Pathol 18:775–780
287. **Harrison SA, Brunt EM, Goodman ZD, Di Bisceglie AM** 2006 Diabetic hepatosclerosis: diabetic microangiopathy of the liver. Arch Pathol Lab Med 130:27–32
288. **McGill M, Molyneaux L, Twigg SM, Yue DK** 2008 The metabolic syndrome in type 1 diabetes: does it exist and does it matter? J Diabetes Complications 22:18–23
289. **Obika M, Noguchi H** 2012 Diagnosis and evaluation of nonalcoholic fatty liver disease. Exp Diabetes Res 2012: 145754
290. **Smith BW, Adams LA** 2011 Nonalcoholic fatty liver disease and diabetes mellitus: pathogenesis and treatment. Nat Rev Endocrinol 7:456–465
291. **Szczepaniak LS, Nurenberg P, Leonard D, Browning JD, Reingold JS, Grundy S, Hobbs HH, Dobbins RL** 2005 Magnetic resonance spectroscopy to measure hepatic triglyceride content: prevalence of hepatic steatosis in the general population. Am J Physiol Endocrinol Metab 288: E462–E468
292. **Hamaguchi M, Kojima T, Itoh Y, Harano Y, Fujii K, Nakajima T, Kato T, Takeda N, Okuda J, Ida K, Kawahito Y, Yoshikawa T, Okanoue T** 2007 The severity of ultrasonographic findings in nonalcoholic fatty liver disease reflects the metabolic syndrome and visceral fat accumulation. Am J Gastroenterol 102:2708–2715
293. **Kawasaki S, Hasegawa O, Satoh S, Numata K, Terauchi Y** 2010 Sonographic assessment of fatty liver infiltration using the measurement of para- and perirenal fat thickness. J Clin Ultrasound 38:470–474
294. **Liu KH, Chan YL, Chan JCN, Chan WB, Kong WL** 2006 Mesenteric fat thickness as an independent determinant of fatty liver. Int J Obes 30:787–793
295. **Soares JB, Pimentel-Nunes P, Roncon-Albuquerque R, Leite-Moreira A** 2010 The role of lipopolysaccharide/toll-like receptor 4 signaling in chronic liver diseases. Hepatol Int 4:659–672
296. **Fracanzani AL, Valenti L, Bugianesi E, Andreoletti M, Colli A, Vanni E, Bertelli C, Fatta E, Bignamini D, Marchesini G, Fargion S** 2008 Risk of severe liver disease in nonalcoholic fatty liver disease with normal aminotransferase levels: a role for insulin resistance and diabetes. Hepatology 48:792–798
297. **Mofrad P, Contos MJ, Haque M, Sargeant C, Fisher RA, Luketic VA, Sterling RK, Shiffman ML, Stravitz RT, Sanyal AJ** 2003 Clinical and histologic spectrum of nonalcoholic fatty liver disease associated with normal ALT values. Hepatology 37:1286–1292
298. **Prati D, Taioli E, Zanella A, Della Torre E, Butelli S, Del Vecchio E, Vianello L, Zanuso F, Mozzi F, Milani S, Conte D, Colombo M, Sirchia G** 2002 Updated definitions of healthy ranges for serum alanine aminotransferase levels. Ann Intern Med 137:1–10
299. **Shlomai A, Kariv R, Leshno M, Beth-or A, Sheinberg B, Halpern Z** 2010 Large-scale population analysis reveals an extremely low threshold for 'non-healthy' alanine aminotransferase that predicts diabetes mellitus. J Gastroenterol Hepatol 25:1687–1691
300. **Kang HS, Um SH, Seo YS, An H, Lee KG, Hyun JJ, Kim ES, Park SC, Keum B, Kim JH, Yim HJ, Jeen YT, Lee HS, Chun HJ, Kim CD, Ryu HS** 2011 Healthy range for serum ALT and the clinical significance of "unhealthy" normal ALT levels in the Korean population. J Gastroenterol Hepatol 26:292–299
301. **Ratziu V, Charlotte F, Heurtier A, Gombert S, Giral P, Bruckert E, Grimaldi A, Capron F, Poynard T** 2005 Sampling variability of liver biopsy in nonalcoholic fatty liver disease. Gastroenterology 128:1898–1906
302. **Janiec DJ, Jacobson ER, Freeth A, Spaulding L, Blaszyk H** 2005 Histologic variation of grade and stage of non-alcoholic fatty liver disease in liver biopsies. Obes Surg 15: 497–501
303. **Vuppalanchi R, Unalp A, Van Natta ML, Cummings OW, Sandrasegaran KE, Hameed T, Tonascia J, Chalasani N** 2009 Effects of liver biopsy sample length and number of readings on sampling variability in nonalcoholic fatty liver disease. Clin Gastroenterol Hepatol 7:481–486
304. **Wong VW, Vergniol J, Wong GL, Foucher J, Chan HL, Le Bail B, Choi PC, Kowo M, Chan AW, Merrouche W, Sung JJ, de Lédinghen V** 2010 Diagnosis of fibrosis and cirrhosis using liver stiffness measurement in nonalcoholic fatty liver disease. Hepatology 51:454–462
305. **Castéra L, Foucher J, Bernard PH, Carvalho F, Allaix D, Merrouche W, Couzigou P, de Lédinghen V** 2010 Pitfalls of liver stiffness measurement: a 5-year prospective study of 13,369 examinations. Hepatology 51:828–835
306. **Petta S, Di Marco V, Cammà C, Butera G, Cabibi D, Craxì A** 2011 Reliability of liver stiffness measurement in non-alcoholic fatty liver disease: the effects of body mass index. Aliment Pharmacol Ther 33:1350–1360
307. **Myers R, Pomier-Layrargues G, Duarte-Rojo A, Wong D, Beaton M, Levstik M, Kirsch R, Pollett A, Crotty P, Sasso M, Landau M, Elkashab M** 2011 Performance of the Fibroscan XL probe for liver stiffness measurement in obese patients: a multicentre validation study. Canadian Digestive Diseases Week. Vancouver, British Colombia (Abstract A039)
308. **Myers RP, Pomier-Layrargues G, Kirsch R, Pollett A, Beaton M, Levstik M, Duarte-Rojo A, Wong D, Crotty P, Elkashab M** 2012 Discordance in fibrosis staging between liver biopsy and transient elastography using the FibroScan XL probe. J Hepatol 56:564–570
309. **de Lédinghen V, Wong VW, Vergniol J, Wong GL, Foucher J, Chu SH, Le Bail B, Choi PC, Chermak F, Yiu KK, Merrouche W, Chan HL** 2012 Diagnosis of liver fibrosis and cirrhosis using liver stiffness measurement: comparison between M and XL probe of FibroScan. J Hepatol 56:833–839
310. **Myers RP, Pomier-Layrargues G, Kirsch R, Pollett A, Duarte-Rojo A, Wong D, Beaton M, Levstik M, Crotty P, Elkashab M** 2012 Feasibility and diagnostic performance of the Fibroscan XL probe for liver stiffness measurement in overweight and obese patients. Hepatology 55:199–208
311. **Roulot D, Costes JL, Buyck JF, Warzocha U, Gambier N, Czernichow S, Le Clesiau H, Beaugrand M** 2011 Transient elastography as a screening tool for liver fibrosis and cir-

Downloaded from https://academic.oup.com/edrv/article/34/1/84/2354662 by U.S. Department of Justice user on 16 August 2022

rhosis in a community-based population aged over 45 years. Gut 60:977–984
312. **Palmeri ML, Wang MH, Rouze NC, Abdelmalek MF, Guy CD, Moser B, Diehl AM, Nightingale KR** 2011 Noninvasive evaluation of hepatic fibrosis using acoustic radiation force-based shear stiffness in patients with nonalcoholic fatty liver disease. J Hepatol 55:666–672
313. **Friedrich-Rust M, Romen D, Vermehren J, Kriener S, Sadet D, Herrmann E, Zeuzem S, Bojunga J** 2012 Acoustic radiation force impulse-imaging and transient elastography for non-invasive assessment of liver fibrosis and steatosis in NAFLD. Eur J Radiol 81:e325–e331
314. **Chen J, Talwalkar JA, Yin M, Glaser KJ, Sanderson SO, Ehman RL** 2011 Early detection of nonalcoholic steatohepatitis in patients with nonalcoholic fatty liver disease by using MR elastography. Radiology 259:749–756
315. **Guha IN, Parkes J, Roderick P, Chattopadhyay D, Cross R, Harris S, Kaye P, Burt AD, Ryder SD, Aithal GP, Day CP, Rosenberg WM** 2008 Noninvasive markers of fibrosis in nonalcoholic fatty liver disease: validating the European Liver Fibrosis Panel and exploring simple markers. Hepatology 47:455–460
316. **Wieckowska A, Zein NN, Yerian LM, Lopez AR, McCullough AJ, Feldstein AE** 2006 In vivo assessment of liver cell apoptosis as a novel biomarker of disease severity in nonalcoholic fatty liver disease. Hepatology 44:27–33
317. **Tsutsui M, Tanaka N, Kawakubo M, Sheena Y, Horiuchi A, Komatsu M, Nagaya T, Joshita S, Umemura T, Ichijo T, Matsumoto A, Yoshizawa K, Aoyama T, Tanaka E, Sano K** 2010 Serum fragmented cytokeratin 18 levels reflect the histologic activity score of nonalcoholic fatty liver disease more accurately than serum alanine aminotransferase levels. J Clin Gastroenterol 44:440–447
318. **Feldstein AE, Wieckowska A, Lopez AR, Liu YC, Zein NN, McCullough AJ** 2009 Cytokeratin-18 fragment levels as noninvasive biomarkers for nonalcoholic steatohepatitis: a multicenter validation study. Hepatology 50:1072–1078
319. **Pirola L, Balcerczyk A, Okabe J, El-Osta A** 2010 Epigenetic phenomena linked to diabetic complications. Nat Rev Endocrinol 6:665–675
320. **Wong VW, Chan HL** 2011 Should all patients with nonalcoholic fatty liver disease undergo oral glucose tolerance test? J Gastroenterol Hepatol 26:419–420
321. **Diabetes Prevention Program Research Group, Knowler WC, Fowler SE, Hamman RF, Christophi CA, Hoffman HJ, Brenneman AT, Brown-Friday JO, Goldberg R, Venditti E, Nathan DM** 2009 10-year follow-up of diabetes incidence and weight loss in the Diabetes Prevention Program Outcomes Study. Lancet 374:1677–1686
322. **The International Expert Committee** 2009 International Expert Committee report on the role of the A1C assay in the diagnosis of diabetes. Diabetes Care 32:1327–1334
323. **American Diabetes Association** 2012 Standards of medical care in diabetes–2012. Diabetes Care 35(Suppl 1):S11–S63
324. **Shiga T, Moriyoshi Y, Nagahara H, Shiratori K** 2009 Nonalcoholic fatty liver is a risk factor for postprandial hyperglycaemia, but not for impaired fasting glucose. J Gastroenterol 44:757–764
325. **Koga M, Kasayama S** 2010 Clinical impact of glycated albumin as another glycaemic control marker. Endocr J 57:751–762
326. **Cacciatore L, Cozzolino G, Giardina MG, De Marco F, Sacca L, Esposito P, Francica G, Lonardo A, Matarazzo M, Varriale A** 1988 Abnormalities of glucose metabolism induced by liver cirrhosis and glycosylated hemoglobin levels in chronic liver disease. Diabetes Res 7:185–188
327. **Hojo M, Watanabe S** 2011 Pharmacological therapy of nonalcoholic steatohepatitis. Hepatol Res 41:209–216
328. **Promrat K, Kleiner DE, Niemeier HM, Jackvony E, Kearns M, Wands JR, Fava JL, Wing RR** 2010 Randomized controlled trial testing the effects of weight loss on nonalcoholic steatohepatitis. Hepatology 51:121–129
329. **Harrison SA, Fecht W, Brunt EM, Neuschwander-Tetri BA** 2009 Orlistat for overweight subjects with nonalcoholic steatohepatitis: a randomized, prospective trial. Hepatology 49:80–86
330. **Marchesini G, Moscatiello S, Agostini F, Villanova N, Festi D** 2011 Treatment of non-alcoholic fatty liver disease with focus on emerging drugs. Expert Opin Emerg Drugs 16:121–136
331. **Musso G, Cassader M, Rosina F, Gambino R** 2012 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 55:885–904
332. **Lazo M, Solga SF, Horska A, Bonekamp S, Diehl AM, Brancati FL, Wagenknecht LE, Pi-Sunyer FX, Kahn SE, Clark JM** 2010 Effect of a 12-month intensive lifestyle intervention on hepatic steatosis in adults with type 2 diabetes. Diabetes Care 33:2156–2163
333. **Lim EL, Hollingsworth KG, Aribisala BS, Chen MJ, Mathers JC, Taylor R** 2011 Reversal of type 2 diabetes: normalisation of β cell function in association with decreased pancreas and liver triacylglycerol. Diabetologia 54:2506–2514
334. **Mingrone G, Panunzi S, De Gaetano A, Guidone C, Iaconelli A, Leccesi L, Nanni G, Pomp A, Castagneto M, Ghirlanda G, Rubino F** 2012 Bariatric surgery versus conventional medical therapy for type 2 diabetes. N Engl J Med 366:1577–1585
335. **Dixon JB, Alberti G, Rubino F, Zimmet P** 2011 Bariatric surgical and procedural interventions in the treatment of obese patients with type 2 diabetes: a position statement from the International Diabetes Federation Taskforce on Epidemiology and Prevention. Brussels: International Diabetes Federation
336. **Alamo M, Sepúlveda M, Gellona J, Herrera M, Astorga C, Manterola C** 2012 Sleeve gastrectomy with jejunal bypass for the treatment of type 2 diabetes mellitus in patients with body mass index <35 kg/m^2. A cohort study. Obes Surg 22:1097–1103
337. **Chavez-Tapia NC, Tellez-Avila FI, Barrientos-Gutierrez T, Mendez-Sanchez N, Lizardi-Cervera J, Uribe M** 2010 Bariatric surgery for non-alcoholic steatohepatitis in obese patients. Cochrane Database Syst Rev 1:CD007340
338. **Weiner RA** 2010 Surgical treatment of non-alcoholic steatohepatitis and non-alcoholic fatty liver disease. Dig Dis 28:274–279
339. **Dixon JB, Bhathal PS, O'Brien PE** 2006 Weight loss and non-alcoholic fatty liver disease: falls in γ-glutamyl trans-

Downloaded from https://academic.oup.com/edrv/article/34/1/84/2354662 by U.S. Department of Justice user on 16 August 2022

ferase concentrations are associated with histologic improvement. Obes Surg 16:1278–1286
340. **Mathurin P, Hollebecque A, Arnalsteen L, Buob D, Leteurtre E, Caiazzo R, Pigeyre M, Verkindt H, Dharancy S, Louvet A, Romon M, Pattou F** 2009 Prospective study of the long-term effects of bariatric surgery on liver injury in patients without advanced disease. Gastroenterology 137: 532–540
341. **Miller GD, Nicklas BJ, Fernandez A** 2011 Serial changes in inflammatory biomarkers after Roux-en-Y gastric bypass surgery. Surg Obes Relat Dis 7:618–624
342. **Huang H, Kasumov T, Gatmaitan P, Heneghan HM, Kashyap SR, Schauer PR, Brethauer SA, Kirwan JP** 2011 Gastric bypass surgery reduces plasma ceramide subspecies and improves insulin sensitivity in severely obese patients. Obesity 19:2235–2240
343. **Appachi S, Kelly KR, Schauer PR, Kirwan JP, Hazen S, Gupta M, Kashyap SR** 2011 Reduced cardiovascular risk following bariatric surgeries is related to a partial recovery from "adiposopathy." Obes Surg 21:1928–1936
344. **Kral JG, Thung SN, Biron S, Hould FS, Lebel S, Marceau S, Simard S, Marceau P** 2004 Effects of surgical treatment of the metabolic syndrome on liver fibrosis and cirrhosis. Surgery 135:48–58
345. **Satapathy SK, Sanyal AJ** 2010 Novel treatment modalities for nonalcoholic steatohepatitis. Trends Endocrinol Metab 21:668–675
346. **Ribeireiro T, Swain J, Sarr M, Kendrick M, Que F, Sanderson S, Krishnan A, Viker K, Watt K, Charlton M** 2011 NAFLD and insulin resistance do not increase the risk of postoperative complications among patients undergoing bariatric surgery—a prospective analysis. Obes Surg 21: 310–315
347. **Bonekamp S, Barone BB, Clark J, Stewart KJ** 2008 The effect of an exercise training intervention on hepatic steatosis (Abstract 1119). Hepatology 48(Suppl 1):806A
348. **St George A, Bauman A, Johnston A, Farrell G, Chey T, George J** 2009 Independent effects of physical activity in patients with nonalcoholic fatty liver disease. Hepatology 50:68–76
349. **Hallsworth K, Fattakhova G, Hollingsworth KG, Thoma C, Moore S, Taylor R, Day CP, Trenell MI** 2011 Resistance exercise reduces liver fat and its mediators in non-alcoholic fatty liver disease independent of weight loss. Gut 60: 1278–1283
350. **Kawaguchi T, Shiba N, Maeda T, Matsugaki T, Takano Y, Itou M, Sakata M, Taniguchi E, Nagata K, Sata M** 2011 Hybrid training of voluntary and electrical muscle contractions reduces steatosis, insulin resistance, and IL-6 levels in patients with NAFLD: a pilot study. J Gastroenterol 46:746–757
351. **Rabøl R, Petersen KF, Dufour S, Flannery C, Shulman GI** 2011 Reversal of muscle insulin resistance with exercise reduces postprandial hepatic de novo lipogenesis in insulin resistant individuals. Proc Natl Acad Sci USA 108:13705–13709
352. **Hamaguchi E, Takamura T, Sakurai M, Mizukoshi E, Zen Y, Takeshita Y, Kurita S, Arai K, Yamashita T, Sasaki M, Nakanuma Y, Kaneko S** 2010 Histological course of nonalcoholic fatty liver disease in Japanese patients: tight glycemic control, rather than weight reduction, ameliorates liver fibrosis. Diabetes Care 33:284–286
353. **Wang P, Kang D, Cao W, Wang Y, Liu Z** 2012 Diabetes mellitus and risk of hepatocellular carcinoma: a systematic review and meta-analysis. Diabetes Metab Res Rev 28: 109–122
354. **Currie CJ, Poole CD, Gale EAM** 2009 The influence of glucose-lowering therapies on cancer risk in type 2 diabetes. Diabetologia 52:1766–1777
355. **Renehan AG, Yeh HC, Johnson JA, Wild SH, Gale EA, Møller H** 2012 Diabetes and cancer (2): evaluating the impact of diabetes on mortality in patients with cancer. Diabetologia 55:1619–1632
356. **Johnson JA, Carstensen B, Witte D, Bowker SL, Lipscombe L, Renehan AG, Diabetes and Cancer Research Consortium** 2012 Diabetes and cancer (1): evaluating the temporal relationship between type 2 diabetes and cancer incidence. Diabetologia 55:1607–1618
357. **Nar A, Gedik O** 2009 The effect of metformin on leptin in obese patients with type 2 diabetes mellitus and nonalcoholic fatty liver disease. Acta Diabetol 46:113–118
358. **Torres DM, Jones FJ, Shaw JC, Williams CD, Ward JA, Harrison SA** 2011 Rosiglitazone versus rosiglitazone and metformin versus rosiglitazone and losartan in the treatment of nonalcoholic steatohepatitis in humans: a 12-month randomized, prospective, open-label trial. Hepatology 54:1631–1639
359. **Gupta AK, Bray GA, Greenway FL, Martin CK, Johnson WD, Smith SR** 2010 Pioglitazone, but not metformin, reduces liver fat in type-2 diabetes mellitus independent of weight changes. J Diabetes Complications 24:289–296
360. **Rakoski MO, Singal AG, Rogers MA, Conjeevaram H** 2010 Meta-analysis: insulin sensitizers for the treatment of non-alcoholic steatohepatitis. Aliment Pharmacol Ther 32:1211–1221
361. **Lavine JE, Schwimmer JB, Van Natta ML, Molleston JP, Murray KF, Rosenthal P, Abrams SH, Scheimann AO, Sanyal AJ, Chalasani N, Tonascia J, Ünalp A, Clark JM, Brunt EM, Kleiner DE, Hoofnagle JH, Robuck PR** 2011 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
362. **Ben Sahra I, Laurent K, Loubat A, Giorgetti-Peraldi S, Colosetti P, Auberger P, Tanti JF, Le Marchand-Brustel Y, Bost F** 2008 The antidiabetic drug metformin exerts an antitumoral effect in vitro and in vivo through a decrease in cyclin D1 level. Oncogene 27:3576–3586
363. **Donadon V, Balbi M, Ghersetti M, Grazioli S, Perciaccante A, Della Valentina G, Gardenal R, Dal Mas M, Casarin P, Zanette G, Miranda C** 2009 Antidiabetic therapy and increased risk of hepatocellular carcinoma in chronic liver disease. World J Gastroenterol 15:2506–2511
364. **Hassan MM, Curley SA, Li D, Kaseb A, Davila M, Abdalla EK, Javle M, Moghazy DM, Lozano RD, Abbruzzese JL, Vauthey JN** 2010 Association of diabetes duration and diabetes treatment with the risk of hepatocellular carcinoma. Cancer 116:1938–1946
365. **Musso G, Gambino R, Cassader M, Pagano G** 2010 A meta-analysis of randomized trials for the treatment of nonalcoholic fatty liver disease. Hepatology 52:79–104
366. **Aithal GP, Thomas JA, Kaye PV, Lawson A, Ryder SD,**

Downloaded from https://academic.oup.com/edrv/article/34/1/84/2354662 by U.S. Department of Justice user on 16 August 2022

Spendlove I, Austin AS, Freeman JG, Morgan L, Webber J 2008 Randomized, placebo-controlled trial of pioglitazone in nondiabetic subjects with nonalcoholic steatohepatitis. Gastroenterology 135:1176–1184

367. **Sanyal AJ, Chalasani N, Kowdley KV, McCullough A, Diehl AM, Bass NM, Neuschwander-Tetri BA, Lavine JE, Tonascia J, Unalp A, Van Natta M, Clark J, Brunt EM, Kleiner DE, Hoofnagle JH, Robuck PR** 2010 Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. N Engl J Med 362:1675–1685
368. **Belfort R, Harrison SA, Brown K, Darland C, Finch J, Hardies J, Balas B, Gastaldelli A, Tio F, Pulcini J, Berria R, Ma JZ, Dwivedi S, Havranek R, Fincke C, DeFronzo R, Bannayan GA, Schenker S, Cusi K** 2006 A placebo-controlled trial of pioglitazone in subjects with nonalcoholic steatohepatitis. N Engl J Med 355:2297–2307
369. **Armstrong MJ, Houlihan DD, Rowe IA** 2010 Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. N Engl J Med 363:1185; author reply 186
370. **Bloomgarden ZT** 2011 Type 2 diabetes: uses of thiazolidinediones and insulin. Diabetes Care 34:e11–e16
371. **Lincoff AM, Wolski K, Nicholls SJ, Nissen SE** 2007 Pioglitazone and risk of cardiovascular events in patients with type 2 diabetes mellitus. JAMA 298:1180–1188
372. **Kelly IE, Han TS, Walsh K, Lean MEJ** 1999 Effects of a thiazolidinedione compound on body fat and fat distribution of patients with type 2 diabetes. Diabetes Care 22: 288–293
373. **Hernandez AV, Usmani A, Rajamanickam A, Moheet A** 2011 Thiazolidinediones and risk of heart failure in patients with or at high risk of type 2 diabetes mellitus: a meta-analysis and meta-regression analysis of placebo-controlled randomized clinical trials. Am J Cardiovasc Drugs 11:115–128
374. **Nissen SE, Wolski K** 2010 Rosiglitazone revisited: an updated meta-analysis of risk for myocardial infarction and cardiovascular mortality. Arch Intern Med 170:1191–1201
375. **Graham DJ, Ouellet-Hellstrom R, MaCurdy TE, Ali F, Sholley C, Worrall C, Kelman JA** 2010 Risk of acute myocardial infarction, stroke, heart failure, and death in elderly Medicare patients treated with rosiglitazone or pioglitazone. JAMA 304:411–418
376. **Lewis JD, Ferrara A, Peng T, Hedderson M, Bilker WB, Quesenberry Jr CP, Vaughn DJ, Nessel L, Selby J, Strom BL** 2011 Risk of bladder cancer among diabetic patients treated with pioglitazone: interim report of a longitudinal cohort study. Diabetes Care 34:916–922
377. **Loke YK, Singh S, Furberg CD** 2009 Long-term use of thiazolidinediones and fractures in type 2 diabetes: a meta-analysis. CMAJ 180:32–39
378. **Deacon CF** 2011 Dipeptidyl peptidase-4 inhibitors in the treatment of type 2 diabetes: a comparative review. Diabetes Obes Metab 13:7–18
379. **Vilsbøll T, Christensen M, Junker AE, Knop FK, Gluud LL** 2012 Effects of glucagon-like peptide-1 receptor agonists on weight loss: systemic review and meta-analyses of randomised controlled trials. BMJ 344:d7771
380. **Kenny PR, Brady DE, Torres DM, Ragozzino L, Chalasani N, Harrison SA** 2010 Exenatide in the treatment of diabetic patients with non-alcoholic steatohepatitis: a case series. Am J Gastroenterol 105:2707–2709
381. **Klonoff DC, Buse JB, Nielsen LL, Guan X, Bowlus CL, Holcombe JH, Wintle ME, Maggs DG** 2008 Exenatide effects on diabetes, obesity, cardiovascular risk factors and hepatic biomarkers in patients with type 2 diabetes treated for at least 3 years. Curr Med Res Opin 24:275–286
382. **Sharma S, Mells JE, Fu PP, Saxena NK, Anania FA** 2011 GLP-1 analogs reduce hepatocyte steatosis and improve survival by enhancing the unfolded protein response and promoting macroautophagy. PLoS ONE 6:e25269
383. **Ben-Shlomo S, Zvibel I, Shnell M, Shlomai A, Chepurko E, Halpern Z, Barzilai N, Oren R, Fishman S** 2011 Glucagon-like peptide-1 reduces hepatic lipogenesis via activation of AMP-activated protein kinase. J Hepatol 54:1214–1223
384. **Svegliati-Baroni G, Saccomanno S, Rychlicki C, Agostinelli L, De Minicis S, Candelaresi C, Faraci G, Pacetti D, Vivarelli M, Nicolini D, Garelli P, Casini A, Manco M, Mingrone G, Risaliti A, Frega GN, Benedetti A, Gastaldelli A** 2011 Glucagon-like peptide-1 receptor activation stimulates hepatic lipid oxidation and restores hepatic signalling alteration induced by a high-fat diet in nonalcoholic steatohepatitis. Liver Int 31:1285–1297
385. **Kim MK, Chae YN, Kim HD, Yang EK, Cho EJ, Choi SH, Cheong YH, Kim HS, Kim HJ, Jo YW, Son MH, Kim SH, Shin CY** 2012 DA-1229, a novel and potent DPP4 inhibitor, improves insulin resistance and delays the onset of diabetes. Life Sci 90:21–29
386. **Miyazaki M, Kato M, Tanaka K, Tanaka M, Kohjima M, Nakamura K, Enjoji M, Nakamuta M, Kotoh K, Takayanagi R** 2012 Increased hepatic expression of dipeptidyl peptidase-4 in non-alcoholic fatty liver disease and its association with insulin resistance and glucose metabolism. Mol Med Report 5:729–733
387. **Gupta NA, Mells J, Dunham RM, Grakoui A, Handy J, Saxena NK, Anania FA** 2010 Glucagon-like peptide-1 receptor is present on human hepatocytes and has a direct role in decreasing hepatic steatosis in vitro by modulating elements of the insulin signaling pathway. Hepatology 51: 1584–1592
388. **Trevaskis JL, Griffin PS, Wittmer C, Neuschwander-Tetri BA, Brunt EM, Dolman CS, Erickson MR, Napora J, Parkes DG, Roth JD** 2012 Glucagon-like peptide-1 receptor agonism improves metabolic, biochemical, and histopathological indices of nonalcoholic steatohepatitis in mice. Am J Physiol Gastrointest Liver Physiol 302:G762–G772
389. **Schuppan D, Gorrell MD, Klein T, Mark M, Afdhal NH** 2010 The challenge of developing novel pharmacological therapies for non-alcoholic steatohepatitis. Liver Int 30: 795–808
390. **Elashoff M, Matveyenko AV, Gier B, Elashoff R, Butler PC** 2011 Pancreatitis, pancreatic, and thyroid cancer with glucagon-like peptide-1-based therapies. Gastroenterology 141:150–156
391. **Foster T, Budoff MJ, Saab S, Ahmadi N, Gordon C, Guerci AD** 2011 Atorvastatin and antioxidants for the treatment of nonalcoholic fatty liver disease: the St Francis Heart Study randomized clinical trial. Am J Gastroenterol 106: 71–77
392. **Arendt BM, Allard JP** 2011 Effect of atorvastatin, vitamin

Downloaded from https://academic.oup.com/edrv/article/34/1/84/2354662 by U.S. Department of Justice user on 16 August 2022

E and C on nonalcoholic fatty liver disease: is the combination required? Am J Gastroenterol 106:78–80
393. **Fogari R, Maffioli P, Mugellini A, Zoppi A, Lazzari P, Derosa G** 2012 Effects of losartan and amlodipine alone or combined with simvastatin in hypertensive patients with nonalcoholic hepatic steatosis. Eur J Gastroenterol Hepatol 24:164–171
394. **Nelson A, Torres DM, Morgan AE, Fincke C, Harrison SA** 2009 A pilot study using simvastatin in the treatment of nonalcoholic steatohepatitis: a randomized placebo-controlled trial. J Clin Gastroenterol 43:990–994
395. **Athyros VG, Tziomalos K, Daskalopoulos GN, Karagiannis A, Mikhailidis DP** 2011 Statin-based treatment for cardiovascular risk and non-alcoholic fatty liver disease. Killing two birds with one stone? Ann Med 43:167–171
396. **Cohen DE, Anania FA, Chalasani N** 2006 An assessment of statin safety by hepatologists. Am J Cardiol 97:77C–81C
397. **Zheng S, Hoos L, Cook J, Tetzloff G, Davis Jr H, van Heek M, Hwa JJ** 2008 Ezetimibe improves high fat and cholesterol diet-induced non-alcoholic fatty liver disease in mice. Eur J Pharmacol 584:118–124
398. **Deushi M, Nomura M, Kawakami A, Haraguchi M, Ito M, Okazaki M, Ishii H, Yoshida M** 2007 Ezetimibe improves liver steatosis and insulin resistance in obese rat model of metabolic syndrome. FEBS Lett 581:5664–5670
399. **Yoshida M** 2011 Novel role of NPC1L1 in the regulation of hepatic metabolism: potential contribution of ezetimibe in NAFLD/NASH treatment. Curr Vasc Pharmacol 9:121–123
400. **Yoneda M, Fujita K, Nozaki Y, Endo H, Takahashi H, Hosono K, Suzuki K, Mawatari H, Kirikoshi H, Inamori M, Saito S, Iwasaki T, Terauchi Y, Kubota K, Maeyama S, Nakajima A** 2010 Efficacy of ezetemibe for the treatment of non-alcoholic steatohepatitis: an open-label, pilot study. Hepatol Res 40:613–621
401. **Park H, Shima T, Yamaguchi K, Mitsuyoshi H, Minami M, Yasui K, Itoh Y, Yoshikawa T, Fukui M, Hasegawa G, Nakamura N, Ohta M, Obayashi H, Okanoue T** 2011 Efficacy of long-term ezetimibe therapy in patients with nonalcoholic fatty liver disease. J Gastroenterol 46:101–107
402. **Svegliati-Baroni G, Candelaresi C, Saccomanno S, Ferretti G, Bachetti T, Marzioni M, De Minicis S, Nobili L, Salzano R, Omenetti A, Pacetti D, Sigmund S, Benedetti A, Casini A** 2006 A model of insulin resistance and nonalcoholic steatohepatitis in rats: role of peroxisome proliferator-activated receptor-α and n-3 polyunsaturated fatty acid treatment on liver injury. Am J Pathol 169:846–860
403. **Basaranoglu M, Acbay O, Sonsuz A** 1999 A controlled trial of gemfibrozil in the treatment of patients with nonalcoholic steatohepatitis. J Hepatol 31:384
404. **Kallwitz ER, McLachlan A, Cotler SJ** 2008 Role of peroxisome proliferator-activated receptors in the pathogenesis and treatment of nonalcoholic fatty liver disease. World J Gastroenterol 14:22–28
405. **Laurin J, Lindor KD, Crippin JS, Gossard A, Gores GJ, Ludwig J, Rakela J, McGill DB** 1996 Ursodeoxycholic acid or clofibrate in the treatment of non-alcohol-induced steatohepatitis: a pilot study. Hepatology 23:1464–1467
406. **Hirose A, Ono M, Saibara T, Nozaki Y, Masuda K, Yoshioka A, Takahashi M, Akisawa N, Iwasaki S, Oben JA, Onishi S** 2007 Angiotensin II type 1 receptor blocker inhibits fibrosis in rat nonalcoholic steatohepatitis. Hepatology 45:1375–1381
407. **Georgescu EF** 2008 Angiotensin receptor blockers in the treatment of NASH/NAFLD: could they be a first-class option? Adv Ther 25:1141–1174
408. **Fujita K, Yoneda M, Wada K, Mawatari H, Takahashi H, Kirikoshi H, Inamori M, Nozaki Y, Maeyama S, Saito S, Iwasaki T, Terauchi Y, Nakajima A** 2007 Telmisartan, an angiotensin II type 1 receptor blocker, controls progress of nonalcoholic steatohepatitis in rats. Dig Dis Sci 52:3455–3464
409. **Georgescu EF, Ionescu R, Niculescu M, Mogoanta L, Vancica L** 2009 Angiotensin-receptor blockers as therapy for mild-to-moderate hypertension-associated non-alcoholic steatohepatitis. World J Gastroenterol 15:942–954
410. **Wada T, Kenmochi H, Miyashita Y, Sasaki M, Ojima M, Sasahara M, Koya D, Tsuneki H, Sasaoka T** 2010 Spironolactone improves glucose and lipid metabolism by ameliorating hepatic steatosis and inflammation and suppressing enhanced gluconeogenesis induced by high-fat and high-fructose diet. Endocrinology 151:2040–2049
411. **Bjelakovic G, Nikolova D, Gluud LL, Simonetti RG, Gluud C** 2008 Antioxidant supplements for prevention of mortality in healthy participants and patients with various diseases. Cochrane Database Syst Rev 2:CD007176
412. **Bjelakovic G, Nikolova D, Gluud LL, Simonetti RG, Gluud C** 2007 Mortality in randomized trials of antioxidant supplements for primary and secondary prevention: systemic review and meta-analysis. JAMA 297:842–857
413. **Miller 3rd ER, Pastor-Barriuso R, Dalal D, Riemersma RA, Appel LJ, Guallar E** 2005 Meta-analysis: high-dosage vitamin E supplementation may increase all-cause mortality. Ann Intern Med 142:37–46
414. **Lindor KD, Kowdley KV, Heathcote EJ, Harrison ME, Jorgensen R, Angulo P, Lymp JF, Burgart L, Colin P** 2004 Ursodeoxycholic acid for treatment of nonalcoholic steatohepatitis: results of a randomized trial. Hepatology 39:770–778
415. **Ratziu V, de Ledinghen V, Oberti F, Mathurin P, Wartelle-Bladou C, Renou C, Sogni P, Maynard M, Larrey D, Serfaty L, Bonnefont-Rousselot D, Bastard JP, Rivière M, Spénard J** 2011 A randomized controlled trial of high-dose ursodeoxycholic acid for nonalcoholic steatohepatitis. J Hepatol 54:1011–1019
416. **Leuschner UF, Lindenthal B, Herrmann G, Arnold JC, Rössle M, Cordes HJ, Zeuzem S, Hein J, Berg T** 2010 High-dose ursodeoxycholic acid therapy for nonalcoholic steatohepatitis: a double-blind, randomized, placebo-controlled trial. Hepatology 52:472–479
417. **Haedrich M, Dufour JF** 2011 UDCA for NASH: end of story? J Hepatol 54:856–858
418. **Pietu F, Guillaud O, Walter T, Vallin M, Hervieu V, Scoazec JY, Dumortier J** 2012 Ursodeoxycholic acid with vitamin E in patients with nonalcoholic steatohepatitis: long-term results. Clin Res Hepatol Gastroenterol 36:146–155
419. **Zein CO, Yerian LM, Gogate P, Lopez R, Kirwan JP, Feldstein AE, McCullough AJ** 2011 Pentoxifylline improves

Downloaded from https://academic.oup.com/edrv/article/34/1/84/2354662 by U.S. Department of Justice user on 16 August 2022

nonalcoholic steatohepatitis: a randomized placebo-controlled trial. Hepatology 54:1610–1619

420. **Schnabl B** 2010 Peroxisome proliferator-activated receptor-δ as emerging target in liver disease. Drug Dev Res 71:106–111
421. **Musso G, Gambino R, Cassader M, Pagano G** 2012 A novel approach to control hyperglycaemia in type 2 diabetes: sodium glucose co-transport (SGLT) inhibitors. Systemic review and meta-analysis of randomized trials. Ann Med 44:375–393
422. **Schalkwijk CG, Miyata T** 2012 Early- and advanced non-enzymatic glycation in diabetic vascular complications: the search for therapeutics. Amino Acids 42:1193–1204
423. **Fayad ZA, Mani V, Woodward M, Kallend D, Abt M, Burgess T, Fuster V, Ballantyne CM, Stein EA, Tardif JC, Rudd JH, Farkouh ME, Tawakol A** 2011 Safety and efficacy of dalcetrapib on atherosclerotic disease using novel non-invasive multimodality imaging (de-PLAQUE): a randomised clinical trial. Lancet 378:1547–1559
424. **Candia R, Riquelme A, Baudrand R, Carvajal CA, Morales M, Solís N, Pizarro M, Escalona A, Carrasco G, Boza C, Pérez G, Padilla O, Cerda J, Fardella CE, Arrese M** 2012 Overexpression of 11 β-hydroxysteroid dehydrogenase type 1 in visceral adipose tissue and portal hypercortisolism in non-alcoholic fatty liver disease. Liver Int 32:392–399
425. **El-Serag HB** 2011 Hepatocellular carcinoma. N Engl J Med 365:1118–1127
426. **Ertle J, Dechêne A, Sowa JP, Penndorf V, Herzer K, Kaiser G, Schlaak JF, Gerken G, Syn WK, Canbay A** 2011 Non-alcoholic fatty liver disease progresses to hepatocellular carcinoma in the absence of apparent cirrhosis. Int J Cancer 128:2436–2443

Downloaded from https://academic.oup.com/edrv/article/34/1/84/2354662 by U.S. Department of Justice user on 16 August 2022

Save the Date for ENDO 2013

June 15 - 18, 2013, San Francisco, California

www.endo-society.org/endo2013