Internal Medicine Grand Rounds
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Non-Alcoholic Fatty Liver Disease

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Jeffrey D. Browning, M.D., has disclosed no financial interests or other relationships with commercial concerns directly or indirectly related to this program. Dr. Browning will be discussing off-label uses in his presentation.

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Obesity, insulin resistance, and nonalcoholic fatty liver disease (NAFLD)

One major metabolic consequence of obesity is insulin resistance. Obesity and insulin resistance are both strongly associated with the deposition of triglycerides in the liver. Hepatic steatosis can either be a benign, non-inflammatory condition that has no adverse sequelae or it can be associated with steatohepatitis (NASH); a condition that can result in end-stage liver disease that accounts for up to 14% of liver transplants in the U.S. [8]. Non-alcoholic fatty liver disease (NAFLD) is a clinicopathological term that encompasses a spectrum of disease ranging from simple triglyceride accumulation in hepatocytes (hepatic steatosis) to hepatic steatosis with inflammation (steatohepatitis), fibrosis, and cirrhosis [9]. NAFLD is the most frequent cause of abnormal liver function tests (LFTs) in the U.S. [10-11] and it has been associated with a wide variety of metabolic abnormalities, including obesity, insulin-resistant diabetes, hyperlipidemia, and certain drugs/toxins (**Table 1**) [12-14]. However, the most common metabolic disorders associated with hepatic steatosis are insulin resistance and obesity [11]. As such, it has been proposed that NAFLD be considered a component of the metabolic syndrome [15].

Table 1. Diseases or conditions associated with hepatic steatosis [16]

abolic Abnormal	Inborn Errors	ırgical Procedur	Drugs/ Toxins	Miscellaneous
Obesity	Wilson's disease	Jejunoileal bypass	Amiodarone	ute fatty liver of pregnar
Diabetes	Abetalipoproteinemia	liopancreatic diversi	Glucocorticoids	anal diverticulosis; bacte overgrowth
Hyperlipidemia	Hypobetalipoproteinemia	small bowel resectio	Synthetic estrogens	Weber-Christian disease
Lipodystrophy	Tyrosinemia	Gastroplasty	Tamoxifen	Tuberculosis
Acute starvation	Glycogen storage disease		Isoniazid	Hepatitis C
TPN	Homocystinuria		Coumadin	ETOH ingestion
Rapid weight loss	Hereditary fructose intolerance		Tetracycline	Reye's syndrome
	Carnitine deficiency		Bleomycin	
	Galactosemia		Methotrexate	
			L-Asparaginase	
			Hydralazine	
			Several metals	

The estimated prevalence of NAFLD varies depending on the population studied. Older estimates based primarily on autopsy studies reported a prevalence of 14-24% in the U.S. [17-18]. However, the prevalence of NAFLD in certain populations is dramatically higher. In obese individuals, the prevalence of NAFLD is as high as 95% in some series, but ranges from 60-95% in different study populations [12, 18-22]. In a literature survey of 41 original articles that contained liver morphology from 1515 obese patients (in adults, a BMI of >25 kg/m^2 is considered overweight and a BMI >30 kg/m^2 is considered obese), liver biopsies were normal in only 12% of the cases [23].

Most prevalence studies have used qualitative or semi-quantitative measures to determine liver fat content. The available techniques to measure liver fat include liver biopsy, ultrasound, computed tomography, and magnetic resonance. Liver biopsies, although previously considered the gold standard, typically use a qualitative grading system to estimate hepatic fat [24]. Liver histology is prone to processing artifacts that can lead to overestimation of liver fat, or to underestimation owing to microvesicular steatosis [25-26]. Liver biopsies also suffer from sampling error [27]. Radiologic studies (summarized below) can be very suggestive of NAFLD, but none can provide an estimate of inflammation or fibrosis.

1) **Sonography**-Liver with fatty change is often described as a "bright liver" because of the increased echogenicity and sound attenuation. These findings are very difficult to distinguish from other disease processes that present with diffuse increased echogenicity since fibrosis from any cause can have similar sonographic appearance. Older reports show that ultrasonography is 89-95% sensitive and 84-93% specific for steatosis, but only 57-77% sensitive and 85-89% specific for fibrosis [28].

2) **Computed Tomography**-The most accurate CT method to characterize hepatic steatosis is unenhanced CT. The difference in attenuation values between the spleen and liver are measured and, if greater than –10 Hounsfield units, the criteria for hepatic steatosis are fulfilled [29]. Normal liver has greater attenuation than spleen.

3) **Magnetic Resonance Imaging**-MR characteristics of fat can be used to assess hepatic steatosis, however, the diagnosis is often more easily made by using other imaging modalities. There is a reasonably close correlation between MRI assessment and histological evaluation of hepatic steatosis [30].

4) **Magnetic Resonance Spectroscopy**-Chemical shift-sensitive MR is the most sensitive and specific noninvasive test to detect fat in liver and it is the only method that is quantitative [31]. The measurement of fat is robust because it merely requires the evaluation of two dominant peaks (water and lipid) within the MR spectrum (see **Fig. 1**) [5]. This method is the gold standard for noninvasive measurements of fat content; however, it is not readily available at most institutions.

We recently performed the largest study to date that determined the prevalence of hepatic steatosis in a U.S. population. Our group [3] studied participants in the Dallas Heart Study (DHS) [32], a multiethnic, population-based probability sample of Dallas County that was weighted to include 50% blacks and 50% non-blacks. Multiple measurements of 2,287 individuals were obtained including: blood pressure, BMI, insulin resistance, plasma glucose, plasma lipids, plasma LFTs, and ^{1}H-MRS of liver to quantify liver fat content. This was the first large study to use NMR to quantify liver fat content in a general population.

A normal range for liver fat was established in the DHS population by excluding those individuals who had known risk factors for hepatic steatosis. The 95th percentile of hepatic triglyceride content in normal subjects was 5.5%. Using 5.5% fat as the upper limit of normal, they found that 708 (31%) of the 2,287 DHS subjects had hepatic steatosis. Correction for population sampling indicated that the overall prevalence of hepatic steatosis in Dallas County is 34%. If this prevalence is

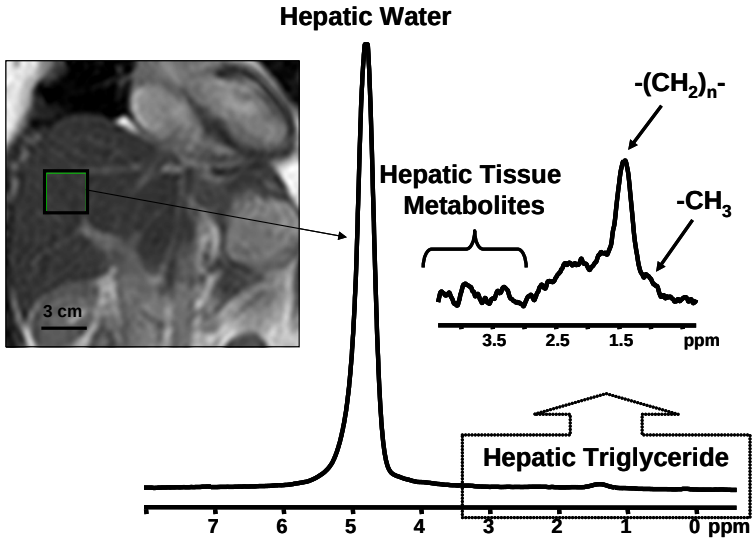


Fig. 1. ^{1}H-MRS spectra. Hepatic triglyceride content is calculated as the ratio of signal from the methylene in fatty acid chains of hepatic triglyceride, represented by the area under the [-(CH_2)n-] resonance and the total signal generated by the combination of the methylene signal and water signal. From Szczepaniak *et al.* [5].

representative of the ~200,000,000 people over the age of 18 then ~68 million individuals have hepatic steatosis in the U.S.

An unexpected finding that emerged from this study was that significant differences exist in the prevalence of hepatic steatosis among the three major ethnic groups represented in the DHS (**Fig. 2**). Hispanics had a significantly higher prevalence of hepatic steatosis compared to whites (45% vs. 33%), whereas blacks had a significantly lower prevalence of hepatic steatosis compared to whites (24% vs. 33%). Consistent with previous smaller studies, the liver fat content was positively correlated with BMI and insulin resistance. The higher prevalence of hepatic steatosis in Hispanics was due to the higher prevalence of obesity and insulin resistance. However, these risk factors could not explain the lower prevalence of hepatic steatosis in blacks, since Hispanics and blacks had a similar prevalence of obesity and insulin resistance. Of note, the prevalence of hypertriglyceridemia was also lower in blacks. The reason for the difference in the prevalence of hepatic steatosis in blacks could not be ascertained in this study. No difference in the prevalence of hepatic steatosis between men and women was present in blacks or Hispanics. However, in whites the prevalence of hepatic steatosis was 2-fold higher in men than in women.

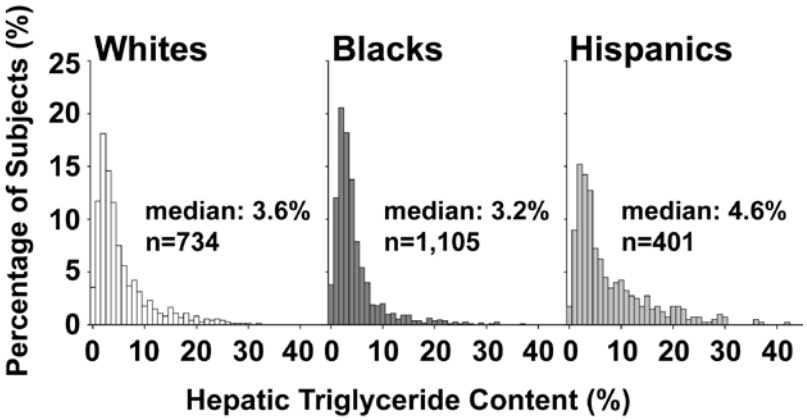


Fig. 2. Distribution of hepatic triglyceride content in the DHS population by ethnicity [3].

This study also confirmed results from previous smaller studies regarding the utility of alanine aminotransferase (ALT) measurements in identifying those who have hepatic steatosis. Although the prevalence of elevated ALTs was clearly higher in those with hepatic steatosis compared to those with normal liver fat content (21% vs. 9%), the vast majority (79%) with hepatic steatosis had *normal* ALTs. Thus, NAFLD is very common and ALTs cannot be used as a screening test to identify those with the condition.

Finally, the presence of hepatic steatosis in the DHS population was strongly associated with components of the metabolic syndrome. As has been reported in previous studies [15], individuals with hepatic steatosis were significantly more likely to be obese, diabetic, hypertriglyceridemic, and hypertensive. Overall, the correlation coefficients were highest for BMI and insulin resistance, 0.585 and 0.503, respectively.

Pathogenesis of hepatic steatosis in obesity and Type 2 diabetes

The liver is the principal organ responsible for the intermediary metabolism of carbohydrates, lipids, and proteins. In the fed state, all mammals preferentially burn carbohydrates to generate ATP and convert excess carbohydrates into fatty acids, which are stored as triglycerides in adipocytes. Under normal conditions, there is constant cycling of fatty acids between adipose tissue and liver. Fatty acids in the liver are derived from one of four sources: 1) hydrolysis of adipose tissue triglycerides; 2) hydrolysis of dietary triglycerides; 3) direct uptake of chylomicron remnants in the postprandial state; or 4) synthesis from acetyl-CoA. The relative rates of uptake and synthesis from these pathways largely depend on insulin levels and nutritional status. Normally, the liver handles these large fluxes of fatty acids without difficulty. Key metabolic changes must develop to alter the normal balance of synthesis, uptake, export, and oxidation to result in triglyceride accumulation in liver. It has become evident that two important

metabolic alterations associated with NAFLD are the development of **insulin resistance** and resulting **hyperinsulinemia**.

Determinants of insulin sensitivity, such as insulin-mediated glucose disposal and insulin-mediated suppression of hepatic glucose output correlate inversely with BMI [33]. Using the homeostasis model assessment method to measure insulin resistance, Marchesini *et al.* [4] reported that the strongest predictor of NAFLD was insulin resistance, irrespective of BMI, fat distribution, or glucose tolerance. Limitations of this study were that they selected only patients with abnormal LFTs and used ultrasound as the criterion for the presence of steatosis.

These studies were extended to NAFLD patients with chronically elevated ALTs but with BMIs <30 kg/m^2 and normal fasting glucose levels [15]. Of the 30 patients studied 21 (70%) had histologic evidence of NASH and 9 had pure steatosis. Fasting plasma insulin levels were increased 3-fold on average (124 *vs*. 44 pmol/L) in the 30 patients despite normal fasting and post-load glucose levels. Euglycemic clamp studies demonstrated that NAFLD patients had a 50% reduction in glucose disposal (**Fig. 3**). In addition, NAFLD patients had moderately elevated fasting basal levels of plasma free fatty acids and *reduced* insulin-induced suppression of lipolysis. Finally, the normal ability of insulin to suppress glucose output from the liver was also attenuated in individuals with NAFLD (**Fig. 6**). Thus, even in non-obese individuals with NAFLD and normal glucose tolerance, physiologic hallmarks of insulin resistance are present.

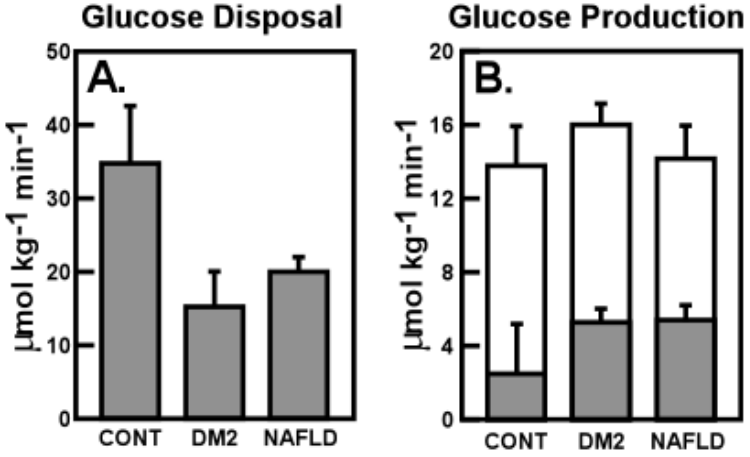


Fig. 3. Glucose disposal (A) and hepatic glucose production (B) during the course of the clamp study in controls (CONT), patients with type 2 diabetes (DM2) and patients with NAFLD. Shaded bars in B represent the hepatic glucose production at the end of the clamp study [4].

Molecular mediators of lipogenesis and their role in hepatic steatosis

The molecular and subsequent metabolic changes that occur as a result of insulin resistance have been most extensively studied in rodent models of hepatic steatosis. **Figure 4** summarizes a series of molecular and physiologic alterations that occur in the setting of insulin resistance resulting in the accumulation of triglycerides in liver. The conventional explanation for hepatic triglyceride accumulation is that obesity and insulin resistance result in increased release of free fatty acids (FFA) from adipocytes. Increased adipocyte mass and increased hydrolysis of triglycerides through increased hormone-sensitive lipase activity contribute to elevated plasma levels of FFAs [34]. The rate of FFA uptake in liver is unregulated and, therefore, directly proportional to plasma FFA concentrations [35].

FFAs taken up by the liver are metabolized by one of two pathways: 1) oxidation to generate adenosine triphosphate (ATP); or 2) esterification to produce triglycerides, which are either incorporated into very-low-density lipoprotein (VLDL) particles for export, or stored within the hepatocyte. Defects in one or both of these pathways can lead to hepatic steatosis.

A central metabolic function of liver is to maintain plasma glucose levels regardless of the nutritional state. In the setting of energy excess, glucose is converted to fatty acids via the conversion of

glucose to pyruvate, which enters the Krebs cycle in the mitochondria. Citrate formed in the Krebs cycle is shuttled to the cytosol where it is converted to acetyl-CoA, which is the 2-carbon precursor required for fatty acid synthesis. The fatty acids are then used to synthesize triglycerides--the primary source of energy storage and transport. Humans [36] and mice [37] with hepatic steatosis accumulate excess oleic acid, the end-product of *de novo* fatty acid synthesis. This suggests that fatty acid synthetic rates are increased in insulin-resistant livers.

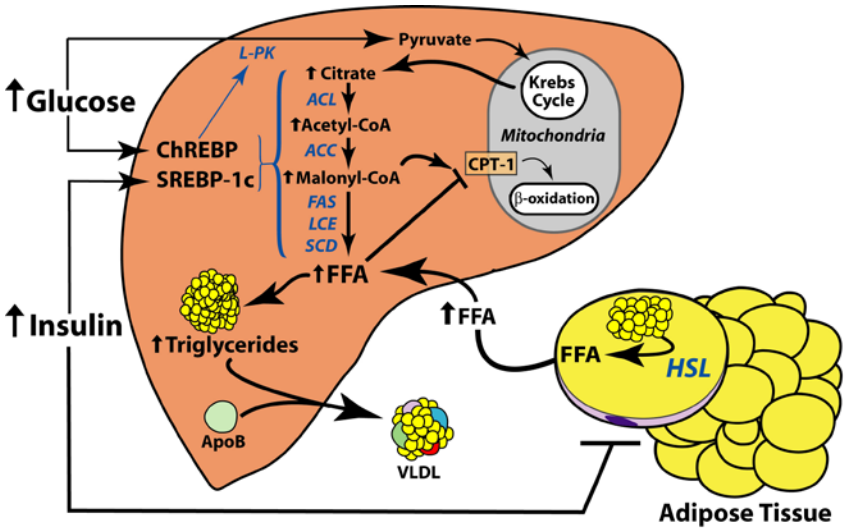


Fig. 4. Metabolic alterations resulting in hepatic triglyceride accumulation in insulin-resistant states [2].

De novo synthesis of fatty acids in liver is regulated independently by insulin and glucose [38]. Insulin's ability to activate lipogenesis is transcriptionally mediated by the membrane-bound transcription factor, sterol regulatory element-binding protein-1c (SREBP-1c) [39-40]. Insulin signaling results in increased SREBP-1c expression in liver. SREBP-1c then transcriptionally activates all genes required for fatty acid synthesis [41]. The overexpression of SREBP-1c in transgenic mouse livers leads to the development of a classic "fatty liver" due to increased lipogenesis [41].

Hyperinsulinemia and increased hepatic glucose production are hallmarks of insulin resistance [42]. It might be anticipated that SREBP-1c would not be activated in states of insulin resistance. Surprisingly, even in the presence of profound insulin resistance, insulin-mediated activation of hepatic SREBP-1c transcription remains intact, resulting in increased rates of *de novo* fatty acid biosynthesis [43]. The importance of SREBP-1c activation in the development of hepatic steatosis in insulin-resistant livers has been determined in *ob/ob* mice. *Ob/ob* mice are severely obese and insulin resistant due to a mutation in the leptin gene and as a consequence, these mice have hepatic steatosis [44]. Inactivation of the SREBP-1 gene in the livers of *ob/ob* mice results in a ~50% reduction in hepatic triglycerides [45].

SREBP-1c also activates ACC2, an isoform of ACC that produces malonyl-CoA at the mitochondrial membrane [46]. High concentrations of malonyl-CoA reduce the oxidation of fatty acids by inhibiting carnitine palmitoyl transferase-1 (CPT-1), the protein that shuttles fatty acids into mitochondria [47]. Thus, the activation of SREBP-1c increases fat accumulation in liver by directly stimulating fatty acid synthesis and indirectly inhibiting fat oxidation via the increased production of malonyl-CoA.

Carbohydrate (glucose)-mediated stimulation of fatty acid synthesis is transcriptionally mediated by a second transcription factor, designated carbohydrate response element binding protein (ChREBP) [48]. Glucose activates ChREBP by regulating the entry of ChREBP from the cytosol into the nucleus and by activating the binding of the transcription factor to DNA [49]. ChREBP binds to the promoter of liver-type pyruvate kinase (L-PK), a key regulatory enzyme in glycolysis. L-PK catalyzes the conversion of phospho*enol*pyruvate to pyruvate, which enters the Krebs cycle to generate citrate, the

principal source of acetyl-CoA used for fatty acid synthesis. Recently, ChREBP knockout mice have been developed and characterized [50]. As predicted, the expression of L-PK was reduced by ~90% in livers of ChREBP knockout mice. The unexpected finding was that the expression of all fatty acid synthesis enzymes also was reduced by ~50%. Thus, ChREBP stimulates both glycolysis and lipogenesis, thereby facilitating the conversion of glucose to fatty acids under conditions of energy excess. Whether inactivation of ChREBP will attenuate the development of fatty livers in insulin-resistant states is currently under investigation, however, it would be predicted that excessive stimulation of lipogenesis by ChREBP stimulation would be important only after the development of hyperglycemia.

A third transcription factor that may participate in the development of hepatic steatosis is peroxisome proliferator-activated receptor γ (PPARγ). PPARγ is a member of the nuclear hormone receptor superfamily and is required for normal adipocyte differentiation [51]. Normally, PPARγ is expressed at very low levels in liver; however, in animal models with insulin resistance and fatty livers, the expression of PPARγ is markedly induced [52]. Liver-specific gene deletions of PPARγ in insulin-resistant mouse models markedly attenuated the development of hepatic steatosis, independent of the hyperinsulinemia or hyperglycemia [53]. The precise molecular events mediated by PPARγ that promote triglyceride deposition in the liver have not been fully defined. It is also not known whether PPARγ expression is increased in human livers with steatosis.

The most recent transcription factor identified as potentially important in the development of hepatic steatosis in the insulin-resistant liver is Foxa2. Foxa2, also designated hepatocyte nuclear factor-3β, belongs to the forkhead family of transcription factors [54]. The function of Foxa2 is to activate the transcription of genes involved in mitochondrial and peroxisomal fatty acid oxidation, ketogenesis, and glycolysis [54]. Thus, Foxa2 is active during fasting or starvation when fat must be oxidized as a source of energy. In response to insulin signaling (fed state), Foxa2 is phosphorylated, which leads to its nuclear export into the cytosol where it is no longer active [55].

In livers of insulin-resistant mice, Foxa2 is exclusively located in the cytosol under starved and fed conditions [54]. This suggests that insulin signaling pathway that regulates Foxa2 phosphorylation is also intact in livers of insulin-resistant mice and that the hyperinsulinemia present in these mice leads to the permanent inactivation of Foxa2. Foxa2 inactivation results in reduced expression of CPT-1 and several genes involved in fat oxidation. If a constitutively active version of Foxa2 is expressed in livers of insulin-resistant mice, rates of fat oxidation increase, plasma insulin and glucose levels fall, and liver triglyceride content is reduced ~3-fold [54].

The available data suggest that the insulin-resistant liver exhibits a mixed pattern for insulin signaling. The hyperinsulinemia present in insulin-resistant states continues to signal normally in some pathways, but is defective in others. Defective insulin signaling is manifested by the inability of insulin to suppress gluconeogenesis and glucose output from the liver. Insulin-mediated activation of SREBP-1c, however, remains intact. SREBP-1c activation results in the induction of lipogenesis and inhibition of β-oxidation through the increased production of malonyl-CoA. The insulin-mediated inactivation of Foxa2 also is intact in insulin-resistant livers. Inactivation of Foxa2 reduces the expression of CPT-1 and several proteins involved in fat oxidation, thus further suppressing rates of β-oxidation in the hepatocyte. Combined, increased lipogenesis and reduced β-oxidation cause a dual metabolic defect that leads to increased hepatic triglyceride content and hepatic steatosis.

Is fat in liver bad?

There is a growing body of literature suggesting that the accumulation of fat in liver is associated with adverse outcomes. Direct cause and effect has not been clearly established in most instances; however,

there is mounting evidence that suggests guilt by association. A summary of these associations is provided below.

NAFLD and Cryptogenic Cirrhosis

It is likely that NAFLD is the most common cause of cryptogenic cirrhosis. Cryptogenic cirrhosis constitutes 3-30% of all cases of cirrhosis and ~7% of all orthotopic liver transplants in the U.S. [56-57]. Powell and colleagues [20] first suggested that NAFLD may cause cryptogenic cirrhosis as shown in their study describing the histologic course of 42 patients with NASH. All patients were obese except 2 who had lipodystrophy. Twenty had steatosis and 22 had steatosis with some degree of fibrosis. Unfortunately, only 13 underwent serial biopsies, but of those 13, 6 remained unchanged over 1-9 years. Six showed disease progression, 3 of which initially only had steatosis on the initial biopsy. One patient died of hepatocellular carcinoma. A larger study by Ratziu *et al*. [58] reported that 73% of patients with cryptogenic cirrhosis were overweight, 88% had diabetes, and 56% had hypertriglyceridemia; implying that NAFLD was the etiology of their liver disease.

Hepatic steatosis tends to regress as the liver fibrosis progresses to cirrhosis; therefore, it is difficult to ascertain the actual number of patients with NAFLD as the primary etiology of cryptogenic cirrhosis. However, it is estimated that 30-70% of cryptogenic cirrhosis is due to NAFLD [59-60].

NAFLD and Liver Transplantation

The percentage of transplants performed for NAFLD has not been carefully studied. The Mayo Clinic has reported that 2.9% of their liver transplants are for patients with end-stage liver disease from NAFLD [56]. They reported their experience from 15 of these patients after transplant and found that at 1 year, 60% of the patients had evidence of recurrent steatosis and 33% had fibrosis on a repeat biopsy. Two patients subsequently developed cirrhosis, one of which required re-transplant at 27 months. Combining the results of several studies, it appears that 25-50% of patients receiving a liver transplant for cryptogenic cirrhosis develop NAFLD in the newly grafted liver [61-63]. Also, the estimates of recurrence are likely underestimated, since only those patients with abnormal LFTs underwent repeat liver biopsy and the number of years followed after transplant has been relatively few.

The prevalence of NAFLD in liver donors also has significantly limited the availability of suitable organs for transplant. Steatosis of the donor liver is associated with increased rates of primary nonfunction in the allograft and poor outcomes [64]. In general, livers with >30% steatosis as graded by histologic analysis are not used for transplantation. In patients receiving grafts with ~30% steatosis, 5.1% developed primary nonfunction in the allograft compared to only 1.8% of those transplanted with livers devoid of fat [64]. At 2 years, 70% of the grafts with steatosis had survived compared to 82% of those without steatosis.

The presence of NAFLD is an important consideration in the evaluation of living donors for transplantation. Many centers exclude potential living donors with a BMI >28 and most try to select living donors a liver fat content of <10%. The routine use of biopsy for living donors is controversial and its use varies from center to center. Proposed mechanisms for poor graft survival of steatotic livers are summarized in **Table 2**.

Table 2. Proposed mechanisms of poor graft function in steatotic livers [65]

Mechanism	Pathophysiology
Diminished portal flow	Ballooned hepatocytes distort sinusoidal lumen causing increased resistance, reduced blood flow, and ischemia
Inefficient anaerobic metabolism	Steatotic hepatocytes express increased uncoupling proteins and have decreased mitochondrial ATP production
Physical properties of lipids	Altered plasma membrane fluidity of steatotic hepatocytes leads to increased Kupffer cell adhesion and activation on reperfusion. Lipid solidifies during cold preservation and may cause physical disruption of hepatocytes
Oxidative stress	Steatotic liver is predisposed to OS at baseline. Tocopherol, an oxygen radical scavenger, improves survival of rats model of NAFLD exposed to ischemia/reperfusion injury

NAFLD and Hepatitis C

Hepatitis C (HCV) is characterized by inflammation, slowly progressive fibrosis, and the development of hepatocellular carcinoma. The prevalence of hepatitis C in the U.S. population is ~1.8%. The prevalence of steatosis in liver biopsies from patients with chronic HCV ranges from 30-70% [66], which is stated to be 2- to 3-fold higher than the general population; however, it is difficult to be confident in the true increase above the NAFLD background because of the lack of NAFLD prevalence data in the same studies.

Alterations in lipid metabolism have been reported in patients with HCV, but not other viral forms of hepatitis. Hepatic steatosis in patients infected with genotype 3a resolves in two-thirds of the patients if the virus is successfully eradicated [67]. It also appears the degree of steatosis may correlate with the level of viral replication [67]. These results, plus the fact that transgenic mouse models of hepatitis C have steatosis [68], suggest that the virus itself is responsible for the fat accumulation. Some studies have suggested that the mechanism responsible is the inhibition of VLDL secretion from liver via the inhibition of microsomal triglyceride transfer protein by the HCV core protein [69]. Other studies have shown that insulin resistance is independently associated with HCV [70-73]. Currently, it is not clear whether the virus first induces insulin resistance and then steatosis develops, or whether the virus induces steatosis, leading to insulin resistance.

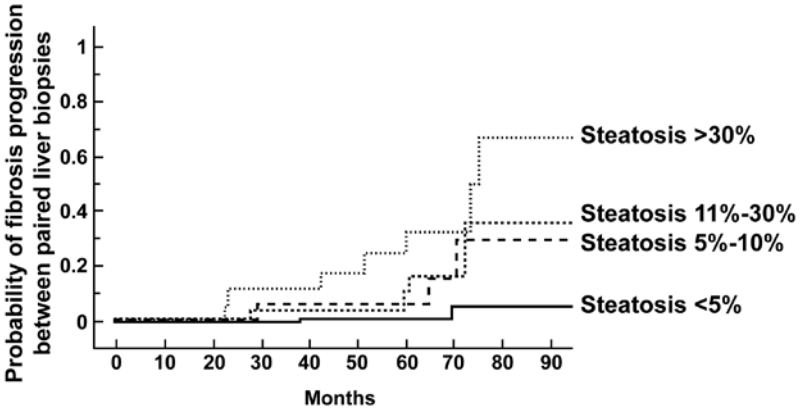


Fig. 5. Cumulative probability of fibrosis progression according to the percentage of steatosis on the initial biopsy [1].

NAFLD also may alter the progression of HCV. There is a positive correlation between severity of steatosis on the index biopsy and increased progression of fibrosis in HCV-infected patients irrespective of the virus genotype [1, 74-77]. The most recent study by Fartoux *et al*. [1] evaluated 135 patients with mild HCV and found steatosis in 46% of the patients, yet only 5% had BMIs >30. The mean duration between liver biopsies was 62 months. In the multivariate analysis, steatosis was the only independent predictor for the progression of fibrosis (**Fig. 5**).

NAFLD and Hepatocellular Carcinoma

Hepatocellular carcinoma (HCC) accounts for 84% of all liver cancers in the U.S. The age-adjusted incidence rates of HCC increased 2-fold from 1985 to 1998 [78]. Most of the increase has been attributed to an increase in HCV-associated HCC. Overall, approximately 50% of HCC cases have evidence of HCV and 15% HBV; however, 33% have no known risk factors [79]. HCC may represent a late complication of NAFLD-related cirrhosis [7, 58, 62, 78, 80-81]. **Figure 6** shows the prevalence of conditions associated with NAFLD in 614 HCC patients with cirrhosis [7]. Those with HCC associated with cryptogenic cirrhosis have a higher prevalence of conditions associated with NAFLD than those associated with viral infections or ethanol use. Others have estimated that 13% of HCC is a result of NAFLD-induced cirrhosis [81].

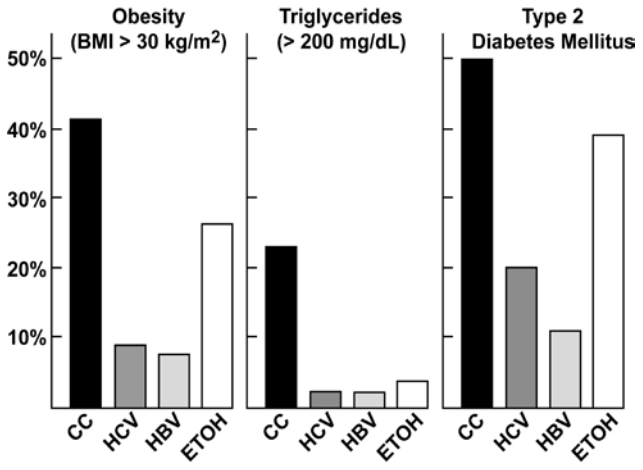


Fig. 6. Prevalence of obesity, hypertriglyceridemia, and diabetes in patients with HCC, grouped according to etiology [7]. Abbrev. CC, cryptogenic cirrhosis; ETOH, ethanol.

NAFLD and Diabetes

The association between type-2 diabetes and NAFLD is more variable (20-55%) than that for obesity. Some of this variation is due to differences in patient selection in the various studies and to differences in the criteria used to define type-2 diabetes. However, given the strong association of NAFLD with obesity, it is not surprising that type-2 diabetes is the second most common metabolic abnormality associated with NAFLD. The prevalence of diagnosed type-2 diabetes has also increased from 4.9% in 1990 to 7.3% in 2000 [82]. If undiagnosed diabetes is considered, ~10% of the U.S. population currently has type-2 diabetes, which represents ~16 million Americans.

The current consensus is that type-2 diabetes confers an increased risk for the subsequent development of fibrosis and cirrhosis in NAFLD. NAFLD occurs in up to 75% of patients with type-2 diabetes. Hepatic fibrosis was more common and prominent in obese patients that have hyperglycemia; it was also associated with a higher rate of fibrosis in a large longitudinal study of 103 patients (**Table 3**) [83-84].

Table 3. Type-2 diabetes and the incidence of cirrhosis and liver-related deaths [83]

Characteristic	Diabetes (n=42)	Normoglycemic (n=84)
Age at diagnosis	57 ± 11	54 ± 14
Triglycerides (mg/dl)	489 ± 312	226 ±115
Development of cirrhosis (%)	24	1
Liver-related deaths (%)	19	2

In general, hepatobiliary disorders occur more frequently in patients with diabetes, and cirrhosis is one of the leading causes of death. The Verona Diabetes Study is a population-based study of 7148

patients with known type-2 diabetes [85]. At the end of 5 years, 1550 subjects with diabetes had died. Cardiovascular disease accounted for 40% of the deaths, whereas cirrhosis was responsible for 4.4%. The standardized mortality ratio (SMR) was calculated using the >300,000 inhabitants of Verona as the reference. As expected, diabetics had a higher overall mortality risk than the general population (SMR =1.42). The SMR for cardiovascular disease in this population was 1.34. Interestingly, the highest SMR for patients with diabetes was for cirrhosis. Individuals with diabetes had more than a 2-fold greater risk of dying from cirrhosis than the general population (SMR=2.52). The SMR for cirrhosis in patients being treated with insulin was even higher (6.84). This could be a direct consequence of insulin administration or could reflect the duration of the diabetes.

NAFLD and Ethanol

By definition, primary NAFLD requires the exclusion of excessive ethanol intake. Excessive ethanol ingestion produces histologic liver lesions very similar to those observed in NAFLD. Studies looking at the effect of modest ethanol ingestion on the development of NAFLD have been mixed. Bellentani *et al*. [18] reported that the prevalence of steatosis was 2.8-fold higher in heavy drinkers and 5.8-fold higher in obese heavy drinkers. Other studies have found that moderate alcohol consumption actually reduces the risk of NAFLD. Dixon *et al*. [86] reported that ethanol ingestion was associated with reduced hepatic fat content in his obese patients. Browning *et al.* [3] also reported similar findings in white women of the DHS population. The reduced prevalence of hepatic fat in those who drink alcohol may result from an ethanol-associated increase in insulin sensitivity [3, 87-88].

NAFLD and Ethnicity

Several studies have strongly suggested that the susceptibility for developing NAFLD differs significantly between ethnic groups. In the NHANES III report of the 167.7 million adults, 8% of the population had liver enzyme elevations, but only 1/3 had an identifiable cause. The elevations were more common in Mexican-Americans (14.9%) than in non-Hispanic blacks (8.1%) and whites (7.1%) [10]. Three studies have reported that Hispanics with NAFLD appear to progress to NASH and end-stage liver disease more frequently than either blacks or whites [6, 89-90]. One study from Dallas found that Hispanics had a disproportionately high prevalence of NAFLD-related cirrhosis, while that of blacks was low [6]. As shown in **Figure 7**, the combined studies from Dallas suggest that Hispanics are more susceptible to NAFLD and NAFLD-induced liver damage than whites or blacks.

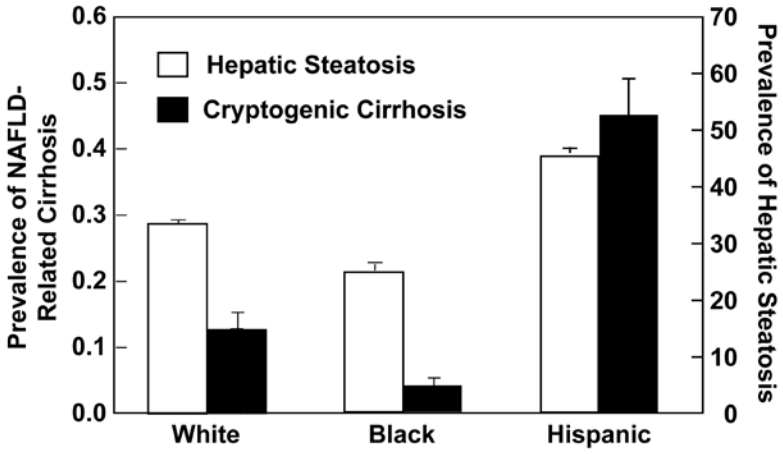


Fig. 7. Prevalence of NAFLD-related cirrhosis and hepatic steatosis in the three major ethnic groups of Dallas County, Texas [3, 6].

Weston *et al*. [90] also reported that Hispanics with NAFLD were significantly overrepresented in a cohort of 742 newly-diagnosed patients with chronic liver disease compared to the general membership in a Kaiser Permanente Medical Care Program. African-Americans with NAFLD-induced liver disease were underrepresented. These data also are consistent with those found in the DHS [3].

NAFLD and Mortality

To date, few long-term studies of the impact of NAFLD on mortality have been performed. Adams *et al.* followed 420 patients with a diagnosis of NAFLD in Olmstead County, Minnesota between 1980 and 2000 [84]. Of the 420 patients followed, 53 died over the period of observation. In this group, mortality was greater than that of the general population, with malignancy and cardiovascular disease accounting for the majority of deaths. However, liver disease was the 3rd leading cause of death, compared to 13th in the general population. It is apparent from this study that age-adjusted mortality of patients with fibrotic NAFLD/NASH increase as a result of liver disease; however, the absolute risk of liver-related death is relatively low (7-year liver related mortality: 1.7%).

A seperate long-term study from Ekstedt *et al.* followed 129 subjects with biopsy-proven NAFLD for a median of 13.7 years [91]. Importantly, mortality was not increased in subjects with steatosis alone but was significantly increased in those subjects with NASH (**Figure 8**). The primary cause of death among NASH subjects was cardiovascular disease followed by malignancy and then liver-related death. In this study, 5.4% of subjects developed cirrhosis.

NAFLD and Cardiovascular Disease

As can be seen by the mortality data above, cardiovascular disease is a primary cause of death in individuals with NAFLD. In a study from Targher *et al.* 85 subjects were extensively evaluated with regard to the presence of hepatic steatosis and carotid intimal thickness [92]. They found that carotid intimal wall thickness was 20% greater in subjects with NAFLD. A follow-up study by this same group found that carotid intimal wall thickness was predicted by the histologic features of NASH present on liver biopsy: as steatosis, necroinflammation, and fibrosis increased so did intimal wall thickness [93].

Villanova *et al.* assessed cardiovascular risk in subjects with NAFLD by evaluating flow-mediated vasodialtion (FMV), a measure of endothelial function [94]. In the group of 52 subjects with NAFLD, FMV was markedly reduced as compared to age- and sex-matched controls. In addition, those NAFLD subjects with NASH had a greater impairment in FMV as compared to those with simple steatosis. These studies indicate that there is a strong association between atherosclerotic disease and NAFLD, particularly in the setting of NASH.

Disease progression: Steatosis to NASH

Despite the high prevalence of NAFLD and its potential for serious sequelae, the underlying factors that determine disease progression to cirrhosis remain poorly understood. Studies to clearly define the molecular and physiologic changes that mediate the presumed transition from hepatic steatosis to NASH have been limited by several factors. First, no animal models incorporate all features of human hepatic steatohepatitis. Second, the available noninvasive techniques to study hepatic metabolism in humans are limited. Third, liver biopsies are required to identify individuals with NASH, precluding large population-based studies. Therefore, our current understanding of the mechanisms by which hepatic steatosis progresses to NASH is based almost exclusively on correlative data from animal models. How

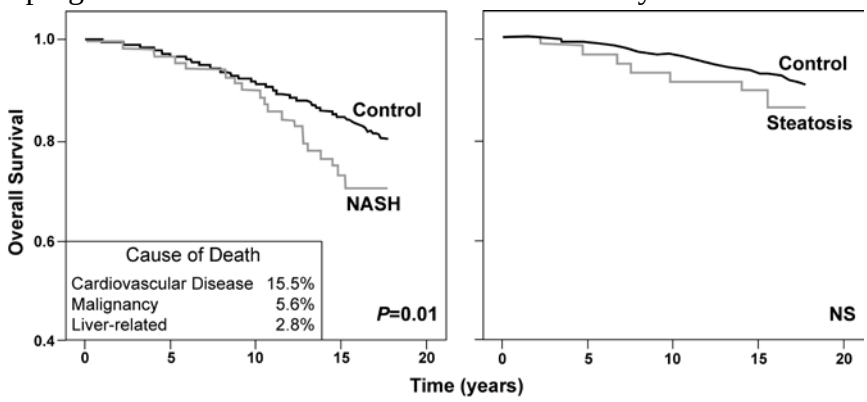
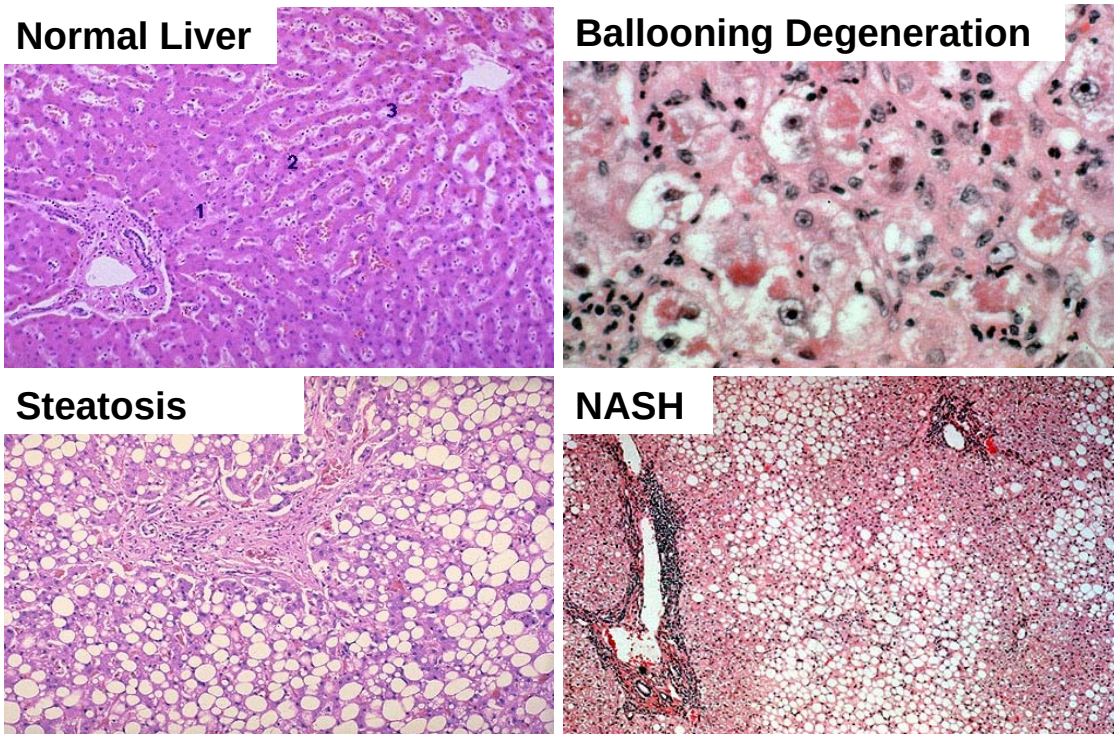


Fig. 8. Longterm survival of 129 subjects with biopsy-proven NAFLD followed for a median of 13.7 years. Increased mortality was only seen in subjects with NASH (91).

well these animal models reflect the human pathophysiology of NASH is not known.

Although ~34% of the population has NAFLD, only an estimated 10-20% of those will ultimately progress to clinically significant disease [95]. In this respect, NAFLD is similar to ethanol-induced liver disease in which less than 10% of heavy drinkers ultimately develop cirrhosis [96]. This led Day and James [97] to propose a "2-hit hypothesis" for the development of NASH. The "first hit" is the underlying metabolic process that results in fat accumulation and the "second hit" is a cellular event that leads to inflammation, fibrosis, and ultimately, cirrhosis. Many investigators feel that multiople "hits" may, in fact, be required for disease progression. The best prevalence estimate for NASH comes from a large autopsy series that reported histologic evidence of NASH in 6.3% of all patients [14]. Unfortunately, this study is now 20 years old. In patients with unexplained elevations in liver function tests, NASH is found in at least 26% of all biopsies [98]. The liver histology of NASH is characterized by the following:

a) **Macrovesicular fat deposits.** Cytoplasmic lipid droplets composed of triglycerides and some cholesteryl esters that stain positively with Oil red-O.



b) **Ballooning degeneration.** Hepatocellular injury results in two different morphologic manifestations, either ballooning degeneration or acidophilic degeneration. The ballooning results from intracellular fluid accumulation and the cells are typically located in zone 3 (pericentral).

c) **Focal necrosis with mixed polymorphonuclear inflammatory cells**. The inflammation of NASH is typically mild and is predominantly lobular rather than portal. Neutrophilic cells in the lobular inflammatory infiltrates are a distinguishing feature from other forms of acute and chronic liver injury.

d) **Sinusoidal fibrosis.** The patterns of fibrosis are one of the characteristic findings in NASH. Deposition of collagen initially occurs in the perivenular and perisinusoidal spaces of zone 3. The collagen envelops single cells in a pattern that is commonly referred to as "chicken-wire fibrosis." This pattern of fibrosis distinguishes NASH and alcohol-induced fibrosis from other forms of chronic liver disease in which the fibrosis is initially periportal.**Mallory bodies**. Mallory's hyaline is an intracytoplasmic inclusion that consists of many aggregated cytoskeletal peptides, some of which include cytokeratins 7, 18, 19 and

ubiquitin. It is generally located in ballooned hepatocytes in zone 3. In adult studies, the incidence of Mallory's hyaline ranges from 9.5-90% [20-21].

Potential mediators of the "second hit" leading to NASH

Hypoxia

One of the striking histologic features of NASH, as compared to other types of hepatitis, is the predominance of Zone 3 (perivenular) involvement. The smallest functional unit within liver is termed the acinus [99], with its architecture based solely on blood supply. Zone 1 of the acinus is composed of the portal vein, hepatic artery, and bile ducts, constituting the portal triad. Zone 3 is composed of the area surrounding the hepatic (a.k.a., central) vein. Oxygen and nutrient rich blood is fed to the acinus via the hepatic artery and portal vein, respectively. This blood flows from Zone 1 via the hepatic sinusoids to the central vein. As a result of this architectural arrangement, an oxygen gradient is established such that the partial pressure of oxygen in the periportal region is 60-65 mmHg and falls to 30-35 mmHg in the perivenular region [100]. This creates a condition of relative hypoxia within Zone 3 of the liver, a situation that may place this region of liver at high risk for NAFLD.

Li *et al.* have demonstrated in lean mice that chronic intermittent hypoxia leads to hyperlipidemia and fatty liver [101]. Importantly, intermittent hypoxia leads to the upregulation of SREBP-1 in liver, an important regulator of *de novo* lipogenesis. A follow-up study by this group confirmed that the genes for fatty acid biosynthesis were upregulated in liver of these mice, the majority of which were under the direct control of SREBP-1 [102]. Though these changes were not observed in obese mice (*ob/ob*) subjected to intermittent hypoxia, data from humans with obstructive sleep apnea (OSA: a condition of chronic intermittent hypoxia) provides evidence that such a scenario may be occurring in human NAFLD. Tanné *et al.* studied 163 consecutive patients undergoing nocturnal polysomnography for clinical suspicion of OSA [103]. They found that the severity of OSA, defined by the number of hypoxic episodes per hour, was significantly correlated with both the presence of elevated liver enzymes as well as the histologic severity of NAFLD.

Vascular Abnormalities

As noted above, the perivenular region of liver is dependent upon a sustained supply of oxygen for normal functioning. Conditions that limit the availability of oxygen (OSA, congestive heart failure, hypotension) place this region of liver at risk for damage. There is evidence to suggest that excess liver fat can directly impair the microvascular circulation in liver, thereby impeding the delivery of oxygenated blood to this region. McCuskey *et al.* have shown in a mouse model of steatohepatitis that sinusoidal diameter and sinusoidal blood flow decrease as liver fat accumulates and steatohepatitis develops [104]. Likewise, perfusion studies of human livers harvested for transplantation using *in vivo* laser Doppler flowmetry have demonstrated that hepatic steatosis is associated with impaired microcirculation [105], a condition that appears to be reversible solely by reducing liver fat content [106].

The basis for these circulatory changes is unclear; however, there is evidence that they are a direct consequence of fatty infiltration of the liver. As hepatocytes become engorged with fat they impinge upon the hepatic sinusoids leading to an increased resistance to blood flow [104]. An exhaustive review of human liver biopsy specimens by Wanless *et al.* has provided additional insight into the pathogenesis of these vascular abnormalities [107]. Their work demonstrates that lipid released from hepatocytes as a result of injury tend to coalesce in the sinusoids leading to obstruction of blood flow. In addition, these extracellular lipids lead to a vigorous inflammatory response that is characterized by neutrophilic and macrophage infiltration, ultimately causing the obliteration of small

hepatic veins. Such obliteration is proportional to the degree of Zone 3 fibrosis and can lead to parenchymal collapse. As a result of these findings, the authors have proposed a 4-step process for the development of NASH: 1) the development of hepatic steatosis as a result of insulin resistance; 2) hepatocyte injury as a result of hypoxia or other events (as discussed below); 3) the release of lipid into the extracellular space leading to vascular impairment and inflammation; and 4) obstruction of hepatic veins leading to fibrous collapse and, ultimately, cirrhosis.

Oxidative Stress

Oxidative stress results from an imbalance between prooxidant and antioxidant chemical species that leads to oxidative damage of cellular macromolecules [108]. The predominant prooxidant chemicals in fatty livers are singlet oxygen molecules, superoxide anions**,** hydrogen peroxide, and hydroxyl radicals; molecules collectively referred to as reactive oxygen species (ROS). The oxidation of fatty acids is an important source of ROS in fatty livers [109-112]. Some of the consequences of increased ROS include DNA damage, alterations in protein stability, depletion of ATP and nicotinamide dinucleotide, the destruction of membranes via lipoperoxidation, and the release of proinflammatory cytokines [108, 113]. Increased production of ROS in the presence of excess free fatty acids has been validated in animal models of NASH [112, 114]. Human livers with NASH have increased levels of byproducts of lipid peroxidation, providing evidence of an increase in oxidative stress in this condition [115].

Mitochondrial Dysfunction

Ac*c*umulation of triglyceride (TG) is characteristic of most insulin resistant tissues [116-117]. The connection between lipid metabolism and insulin resistance hinges not on TG accumulation, per se, but on the pathologic catabolism of TG and generation of lipid-derived signaling molecules which impair insulin action. This process originates with the impaired [116], or incomplete mitochondrial catabolism [117] of intracellular lipid in insulin resistant skeletal muscle. A similar mitochondrial limitation in fatty acid oxidation in liver [118] would provide a plausible basis for the development of NAFLD during insulin resistance [116]. However, NAFLD appears to result predominantly from increased adipose lipolysis, de novo lipogenesis and perhaps saturated lipoprotein TG export [119-121], with sparse *in vivo* data to address the role of mitochondrial metabolism in the pathogenesis or progression of NAFLD.

Mitochondrial metabolism is indispensible for liver function [122]. Mitochondrial β-oxidation is upregulated 10-fold to accommodate large differentials in lipid influx and insulin action [123] during fasting. Induction of lipid oxidation is required for the endergonic steps of gluconeogenesis and ureagenesis, pathways that are partially localized in liver mitochondria and constitutively upregulated during insulin resistance. Thus, unlike skeletal muscle, the insulin resistant and fatty liver may activate oxidative metabolism [124-125]. Chronic activation of mitochondria in the setting of lipid overload may predispose the liver to oxidative stress and cellular damage, events thought to drive the development of NASH [126]. In fact, defects in hepatic mitochondria or the function of oxidative phosphorylation similar to skeletal muscle are typically observed only during NASH or diabetes [127-130]. Thus mitochondrial metabolism almost certainly plays a role in hepatic insulin resistance and NAFLD [131], but the nature of this role is poorly understood.

Lipid oxidation is the principal source of energy generation in liver and its dysregulation represents a potential metabolic link between NAFLD, mediators of insulin resistance and hepatocellular damage. In a recent study, we demonstrated that subjects with NAFLD had a ~2-fold induction in oxidative flux through TCA cycle ($P = 0.002$). Liver TG content was positively correlated with hepatic TCA cycle flux ($r = 0.71$; $P = 0.002$) indicating that oxidative metabolism in the mitochondrial TCA cycle is not impaired even at very high IHTG levels in humans. However, ketone production assessed by

tracer dilution of β-hydroxybutyrate was not different between subjects with low and high IHTG. These data indicated that excess acetyl-CoA was selectively partitioned to oxidation in the TCA cycle rather than ketogenesis, perhaps due to increased energy requirements of the fatty liver. There was a strong correlation between TCA cycle flux and PC / PEPCK (anaplerosis and cataplerosis) ($r = 0.87$; $P < 0.001$; **Figure 1E**), consistent with the induction of gluconeogenesis by fat oxidation [132-134], and also suggesting that part of the increased energy demand/production of the fatty liver is associated with increased gluconeogenesis.

Two principal hypotheses prevail regarding the function of mitochondrial metabolism during insulin resistance and NAFLD. First is the concept that impaired mitochondrial capacity results in the pathologic formation of lipid metabolites which deactivate the insulin signaling cascade through a PKC dependent mechanism [116, 135]. This hypothesis is compelling because it addresses both the underlying etiology of insulin resistance and the concurrent intracellular lipid accumulation. Mitochondrial dysfunction has been reported in insulin resistant skeletal muscle as morphologic defects, decreased mitochondrial content, respiration, ATP synthesis, and TCA cycle turnover [136-138]. In liver, several reports indicate defects in *in vivo* ATP synthesis in individuals with NASH [129] or diabetes [127], but the majority of evidence is indirect. Hepatic lipid overload by short-term high-fat feeding [135], lipid infusion [139] or inhibition of fat oxidation [140] results in hepatic insulin resistance, while increasing mitochondrial fat oxidation is sufficient to suppress liver PKC [135] and improve insulin sensitivity. Certainly the data in the present study supports increased hepatic lipid burden, as indicated by elevated lipolysis, but surprisingly, the data revealed no indication of a mitochondrial insufficiency.

A second hypothesis holds that chronic lipid overload in the liver of individuals with NAFLD induces mitochondrial oxidation resulting in oxidative stress and eventual damage to cellular components including mitochondria, ushering in inflammation, cell death and the progression from benign steatosis to NASH [126, 141-143]. Indeed, individuals with NASH have elevated circulating ketones [124] and obese humans have a 2-fold increase in hepatic ^{11}C-palmitate oxidation by Positron Emission Tomography [125]. We found that increased lipolytic rates were associated with elevated β-hydroxybutyrate turnover, although no differences were detected between the low and high IHTG groups. This might be related to the fact that we were unable to determine acetoacetate contribution to ketone turnover because of unexpectedly low enrichments and concentrations. Nonetheless, elevated hepatic TCA cycle flux demonstrated that mitochondrial oxidative activity is increased in people with high liver fat. Although histology was not characterized in these subjects, elevated oxidative metabolism may be an important contributor to the oxidative stress that attends the progression to NASH.

Inasmuch as mitochondrial TCA cycle activity is closely governed by energy demand, we interpret this finding to indicate increased energy demand during NAFLD. This interpretation is distinct from lipid overload, *per se*, because it implies that the downstream pathways requiring oxidative metabolism are elevated in addition to the oversupply of oxidative substrate (lipid). Congruent with this interpretation is an increase in mitochondrial anaplerosis with IHTG content ($r = 0.53$; $P = 0.035$) leading to a 30% higher rate of gluconeogenesis in individuals with elevated IHTG content. In this respect, lipid overload induces constitutive activation of mitochondrial activity and gluconeogenesis, a pathway with substantial endergonic requirements and a phenomenon that is well known in humans [132-134]. Nonetheless, elevated gluconeogenesis is unlikely sufficient to account for the 2-fold higher TCA cycle activity in individuals with NAFLD, suggesting that other endergonic processes may also be involved. In addition to activation of specific intermediary pathways during insulin resistance, ongoing

liver damage during NAFLD and concomitant hepatocellular regeneration may be sufficient to increase the energy requirements of the liver, as occurs in other forms of liver damage [144].

Alternatively, mitochondria may simply be less efficient during NAFLD, a possibility supported by mitochondrial damage [130, 143] and uncoupling [145] in the liver of NASH patients. The cause of mitochondrial damage has been proposed to be the result of constitutive over-activation of oxidative metabolism during NAFLD; a view substantiated by the current findings. The oxidative stress associated with elevated hydride production in the TCA cycle may be sufficient to damage the electron transport chain during chronic steatosis; a condition functionally manifest by impaired ATP synthesis in people with NASH [129] or diabetes [127]. This would result in a degenerative spiral whereby damaged electron transport chain in turn requires elevated TCA cycle activity to produce sufficient reducing equivalents for normal ATP and cellular homeostasis.

Lipid Peroxidation

ROS are relatively short-lived molecules that exert local effects. However, they can attack polyunsaturated fatty acids (PUFAs) and initiate lipid peroxidation within the cell, resulting in the formation of aldehyde by-products such as *trans*-4-hydroxy-2-nonenal (HNE) and malondialdehyde (MDA) [146]. These molecules have longer half-lives than ROS and have the potential to diffuse from their site of origin to reach distant intracellular and extracellular targets, thereby amplifying the effects of oxidative stress. The formation of HNE and MDA occurs only from the peroxidation of PUFAs, which are preferentially oxidized, owing to decreased carbon-hydrogen bond strength in methylene groups between unsaturated carbon pairs [146-147]. As the number of double bonds in PUFAs increase, their rate of peroxidation increases exponentially. The formation of aldehyde by-products from lipid peroxidation may decrease the content of intracellular and membrane PUFAs. Mitochondria have a substantial concentration of phospholipids containing docosahexaenoic (22:6n-3), which may be essential for functional assembly of the MRC. Peroxidation of these mitochondrial membrane components could lead to further diminution of MRC activity and increased cellular oxidative stress.

In addition to the deleterious effects of lipid peroxidation on organelle function, aldehyde by-products also are detrimental to cellular homeostasis. Aldehyde by-products impair nucleotide and protein synthesis, increase production of the pro-inflammatory cytokine TNF-α, promote influx of inflammatory cells, and activate stellate cells, leading to collagen deposition and fibrosis [reviewed in references [146, 148]]. These effects have the potential to directly initiate an inflammatory process within the liver, inducing hepatocyte death, and triggering the deposition of collagen and liver fibrosis.

Role of Cytokines

In alcohol-induced liver disease, endotoxin and endotoxin-inducible cytokines, including tumor necrosis factor alpha (TNFα) and certain TNF-inducible cytokines such as interleukins-6 and -8, have been incriminated in the pathogenesis of steatohepatitis and cirrhosis. Several lines of evidence suggest that, at least in rodents, these cytokines could be involved in the progression of liver disease to NASH. The leptin-deficient *ob/ob* mouse develops severe obesity, insulin resistance, and fatty livers. Basal TNFα expression levels are increased in livers and adipose tissue of *ob/ob* mice as well as in adipose tissue from obese humans [149]. The administration of anti-TNFα antibodies to *ob/ob* mice significantly reduces the liver triglyceride content [150]. TNFα may contribute to NAFLD by interfering with insulin receptor-mediated signal transduction, which is important for the development of insulin resistance in mice inasmuch as *ob/ob* mice that lack TNFα are protected from insulin resistance [151]. Recent studies have also shown that several animal models of hepatic steatosis have increased NF-κB activity [152-153]. NF-κB is a master regulator that controls the expression of several proinflammatory mediators,

including TNFα and interleukin-6. Inhibiting NF-κB signaling in rodent models of hepatic steatosis improves markers of insulin resistance and significantly reduces the accumulation triglycerides in liver [152-153].

Activation of Stellate Cells

In all forms of liver disease, the final path leading to cirrhosis passes through the stellate cell (also referred to as Ito cells, fat-storing cells, or lipocytes) [154]. As in other parenchymal tissues, normal liver contains an epithelial component (hepatocytes), an endothelial lining, tissue macrophages (Kupffer cells), and a perivascular mesenchymal cell, the stellate cell. Stellate cells comprise ~15% of the total number of cells in liver. They have long cytoplasmic processes that facilitate their interactions with neighboring cell types. Following hepatic injury, stellate cells undergo a process referred to as "activation." This process transforms the quiescent vitamin A-storing cells into proliferative, fibrogenic, and contractile myofibroblasts [155].

The stimuli that initiate stellate cell activation in NASH are very poorly characterized. Injury to all cell types can ultimately result in the production of substances that may initiate the activation of stellate cells. Hepatocytes and Kupffer cells are capable of producing ROS, which makes them leading candidate cells responsible for stellate cell activation in NASH. The major lipid peroxidative products, malondialdehyde and 4-hydroxy-nonenal can activate cultured stellate cells [155].

During the initiation of stellate cell activation, rapid changes in gene expression occur that change the phenotype of the cell so that it can respond to extracellular signals. A cascade of events within the cell results in an increase in extracellular matrix synthesis (ECM), expression of growth factors, cytokine receptors, contractile structures, and metalloproteinases. This results in a cell that has proliferative, synthetic, and contractile properties. The proteins produced by activated stellate cells remodel the ECM in the subendothelial space, changing it from the normal low-density basement membrane matrix to an interstitial-type matrix containing fibril-forming collagens [154]. These events do not seem to be specific to NAFLD, but are a general wound-healing response.

Conclusions

Obesity and its associated co-morbidities are the most prevalent and challenging conditions confronting the medical profession. A major metabolic consequence of obesity is insulin resistance, which is strongly associated with deposition of triglycerides in the liver. Hepatic steatosis can either be a benign, noninflammatory condition that appears to have no adverse sequelae, or it can be associated with steatohepatitis; a condition that can result in end-stage liver disease and accounting for up to 14% of liver transplants in the U.S. [8]. Fat accumulation in primary NAFLD is likely the result of insulin resistance although other as yet unidentified factors, either environmental or genetic, clearly contribute to the pathogenesis. We still have a paucity of outcome studies detailing the natural history of this condition. Studies designed to identify those individuals at risk for histological progression are required so that those most likely to benefit from potential therapies can be targeted for further investigation and possible treatment. Although several therapeutic studies are encouraging, available pharmacologic therapies are limited. At present, weight reduction and lifestyle modification should be the mainstay of therapy. Based on the known physiologic alterations responsible for fat accumulation in liver, therapies targeted to increase insulin sensitivity seem to be the best candidates for future study. Patients who develop end-stage liver disease from NASH should be evaluated for liver transplantation. The overall outcome of liver transplantation in these patients seems to be good, although NASH can recur in the transplanted liver.

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Potential Conflicts of Interest

Naga Chalasani, MD, FACG has received compensation for providing consulting related to NAFLD and NASH from Amylin, Gilead, Genentech, and Mochida and he has received research support from Amylin, Eli Lilly, Intercept, and Cumberland Pharmaceuticals in the last 3 years. Over the last 3 years, he has received compensation for providing consultation related to drug hepatotoxicity from J & J, Merck, GlaxoSmithKline, Karo Bio, Salix, Advanced Life Sciences, BMS, Teva Pharmaceuticals, Abbott, Biolex, Sanofi-Aventis, and Vertex.

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