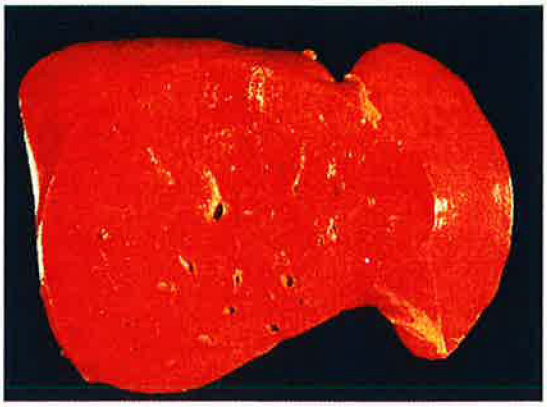
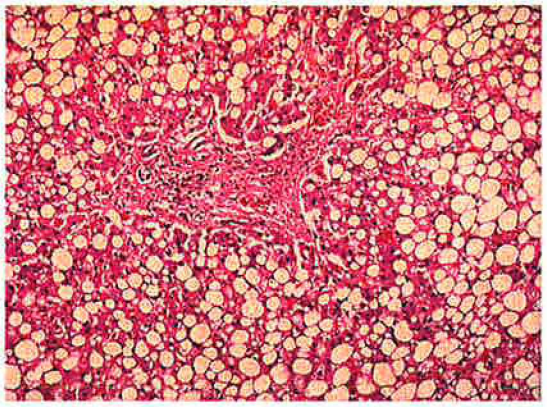
Internal Medicine Grand Rounds

February 24, 2005

Fast Food

To Fatty Liver





Jay D. Horton, M.D.

This is to acknowledge that Jay D. Horton, M.D., has disclosed financial interests or other relationships with commercial concerns directly or indirectly related to this program. Dr. Horton will be discussing off-label uses in his presentation.

Jay D. Horton, M.D. is an Associate Professor in the Department of Internal Medicine, Division of Gastroenterology and the Department of Molecular Genetics. His research is focused on the study of lipid metabolism.

Introduction

Since the 1950s, fast food has increasingly become a staple of most American diets. It is served at stadiums, airports, zoos, schools, universities, Kmarts, Wal-Marts, gas stations, and even hospitals. In 1970, Americans spent ~$6 billion on fast food; in 2001 alone, we spent more than $110 billion. In the book entitled Fast Food Nation by Eric Schlosser he states, "Americans now spend more money on fast food than on higher education, personal computers, computer software, or new cars. We also spend more money on fast food than on movies, books, magazines, newspapers, videos, and recorded music-combined."

The most successful fast-food chain is McDonald's. McDonald's was founded in the mid 1950s and by 1960 operated ~1000 restaurants. Today there are more than 31,000 McDonald's in 119 countries. McDonald's opens almost two thousand new restaurants each year. Currently, they employ ~1.5 million people and an estimated one out of every eight workers in the U.S. has at some point been employed by McDonald's. Not surprisingly, McDonald's is the nation's largest purchaser of beef, pork, and potatoes and the second largest purchaser of chicken. What is less well appreciated is that it also is one of the nation's largest distributors of toys. A survey of U.S. schoolchildren found that 96% could identify Ronald McDonald, a percentage only surpassed by Santa Claus. Therefore, it is difficult to underestimate the extraordinary influence of this institution. All told, there are now more than 280,000 fast food outlets in the U.S. alone.

The epidemic of obesity in the U.S. is well-chronicled (see **Fig. 1**). Approximately 65% of adults are overweight and 31% are obese (11). Consumption of fast food by children and adults has paralleled the increase in the prevalence of obesity. In children, fast food consumption accounted for 2% of the total energy intake in the 1970s but by the 1990s this had risen to 10% (14).

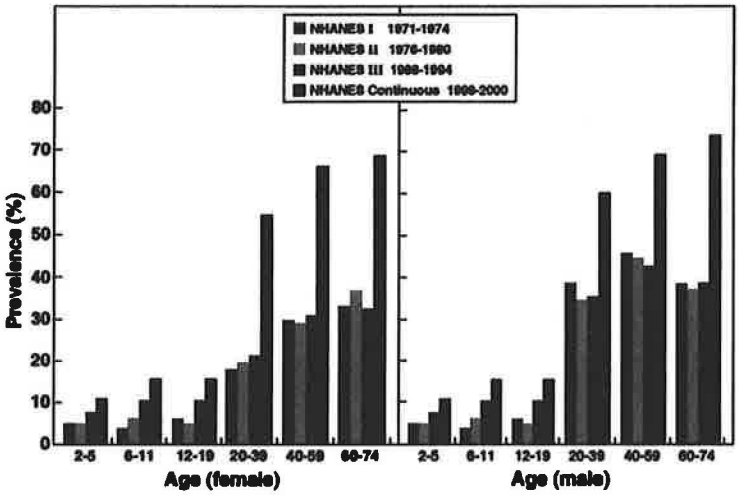


Fig. 1. Trends in the age-specific prevalence of overweight status from 1960-2000. For children (ages 2-19), the shaded bars represent the percentage of children with a weight-for-length ≥ the 95th percentile. For adults (ages 20-74), the bars represent the prevalence with a body mass index (BMI) >25 (11-13).

In the January 1, 2005 issue of *The Lancet*, Pereira *et al.* (5) provided the first documentation that young adults who frequently eat at fast-food restaurants gain more weight and have a greater prevalence of insulin resistance in early middle age. This multi-center population-based prospective trial followed 3031 young adults (ages 18-30) as part of The Coronary Artery Risk Development in Young Adults (CARDIA) study. After 15 years, those who ate at fast-food restaurants at least twice each week had gained

an additional ten pounds and had a two-fold greater increase in insulin resistance compared to those who consumed fast food less than once a week (**Fig. 2**).

One reason for the weight gain is that a single meal from fast-food restaurants often contains enough calories to satisfy a person's total caloric requirement for an entire day. A second reason is that adolescents will overconsume fast food regardless of body weight. Unfortunately, this phenomenon is even more pronounced in adolescents who are overweight (15). Compared to lean adolescents, overweight adolescents also are less likely to adjust their energy intake throughout the day to compensate for the excess energy obtained from fast food.

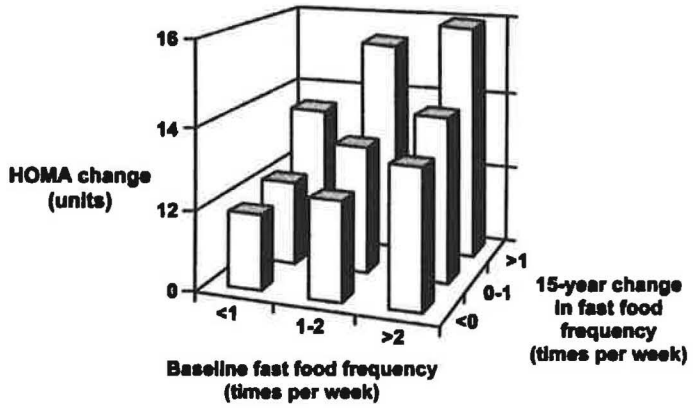


Fig. 2 Changes in frequency of fast food consumption and HOMA insulin resistance over a 15-year period (5).

Increased caloric intake is not limited to children. Per capita, all Americans are consuming significantly more calories, whether at fast-food restaurants or at home. Between 1973 and 1999, U.S. food intake increased by 716 calories per day (~25%), the equivalent of 64 pounds of fat per year. We also appear to be equal opportunity consumers. As shown in **Fig. 3**, since 1950 our per capita consumption of meat, fruits, vegetables, grains, added fats, and sugars has increased significantly (1). The only decreases have been in dairy and egg consumption. The combination of increased caloric intake and more sedentary jobs has led to the "epidemic of obesity."

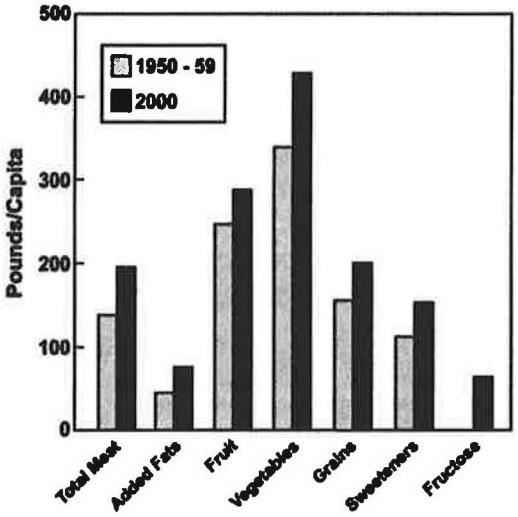


Fig. 3. Per capita annual averages of dietary intake from 1950-1959 compared to 2000 listed in pounds (1).

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. (16). 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 (17). NAFLD is the most frequent cause of abnormal liver function tests (LFTs) in the U.S. (18, 19) and it has been associated with a wide variety of metabolic abnormalities, including obesity, insulin-resistant diabetes, hyperlipidemia, and certain drugs/toxins (**Table 1**) (20-22). However, the most common metabolic disorders associated with hepatic steatosis are insulin resistance and obesity (19). As such, it has been proposed that NAFLD be considered a component of the metabolic syndrome (23).

Table 1. Diseases or conditions associated with hepatic steatosis (24)

Metabolic Abnormalities	Inborn Errors	Surgical Procedures	Drugs/ Toxins	Miscellaneous
Obesity	Wilson's disease	Jejunoileal bypass	Amiodarone	Acute fatty liver of pregnancy
Diabetes	Abetalipoproteinemia	Biliopancreatic diversion	Glucocorticoids	Jejunal diverticulosis; bacterial overgrowth
Hyperlipidemia	Hypobetalipoproteinemia	Small bowel resection	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. (25, 26). 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 (20, 26-30). 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 (31).

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 (32). Liver histology is prone to processing artifacts that can lead to overestimation of liver fat, or to underestimation owing to microvesicular steatosis (33, 34). Liver biopsies also suffer from sampling error (35). 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 (36).

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 (37). 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 (38).

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 (39). 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. 4**) (7). This method is the gold standard for noninvasive measurements of fat content; however, it is not readily available at most institutions.

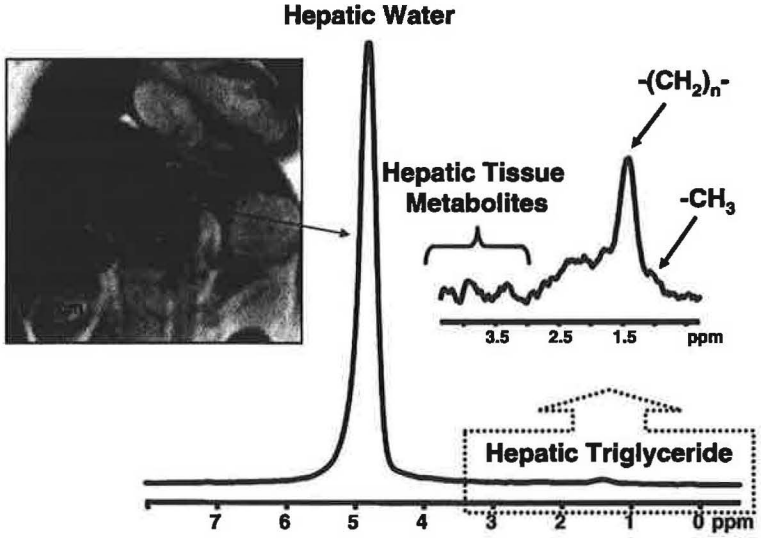


Fig. 4. ^{1}H-MRS spectra. Hepatic triglyceride content is calculated as the ratio of signal from the methylene in fatty acid chains of hepatic triglyceride, represented by the area under the [-(CH_2)n-] resonance and the total signal generated by the combination of the methylene signal and water signal. From Szczepaniak *et al.* (7).

The largest study to date that determined the prevalence of hepatic steatosis in a U.S. population was recently published by investigators at UT Southwestern. Browning *et al.* (4) studied participants in the Dallas Heart Study (DHS) (40), 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 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. 5**). 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.

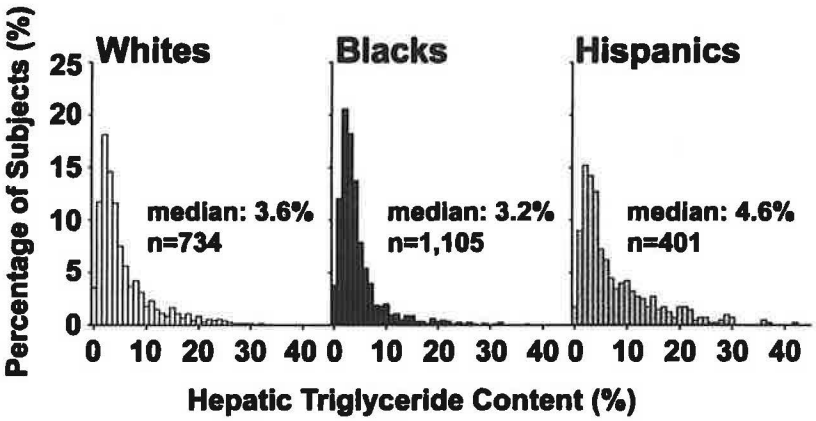


Fig. 5. Distribution of hepatic triglyceride content in the DHS population by ethnicity (4).

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 (23), 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 (41). Using the homeostasis model assessment method to measure insulin resistance, Marchesini *et al.* (6) 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 (23). 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. 6**). 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.

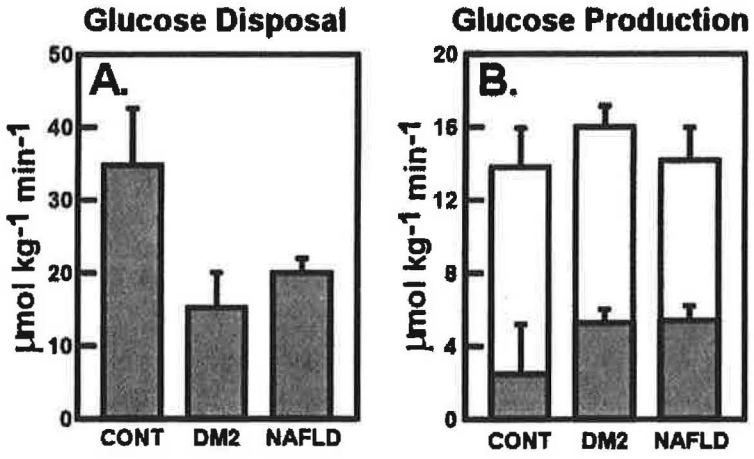


Fig. 6. Glucose disposal (A) and hepatic glucose production (B) during the course of the clamp study in controls (CONT), patients with type 2 diabetes (DM2) and patients with NAFLD. Shaded bars in B represent the hepatic glucose production at the end of the clamp study (6).

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. **Fig. 7** 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 (42). The rate of FFA uptake in liver is unregulated and, therefore, directly proportional to plasma FFA concentrations (43).

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.

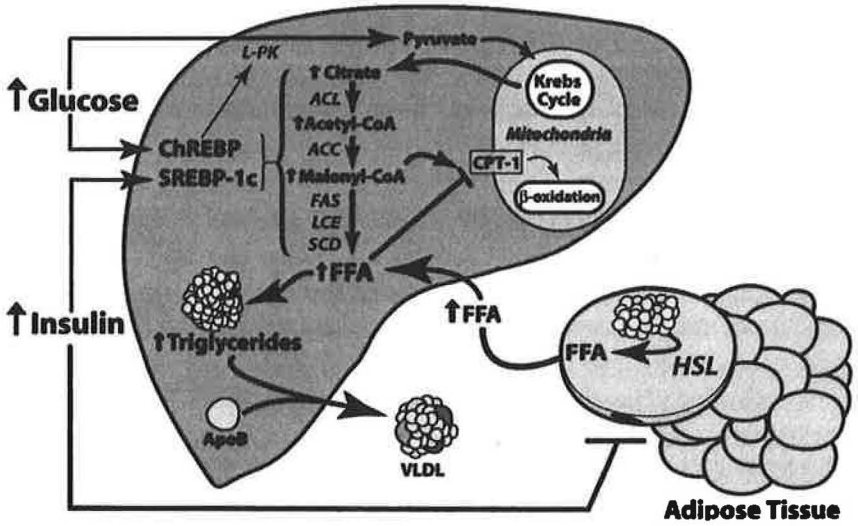


Fig. 7. Metabolic alterations resulting in hepatic triglyceride accumulation in insulin-resistant states (3).

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 (44) and mice (45) 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.

De novo synthesis of fatty acids in liver is regulated independently by insulin and glucose (46). Insulin's ability to activate lipogenesis is transcriptionally mediated by the membrane-bound transcription factor, sterol regulatory element-binding protein-1c (SREBP-1c) (47, 48). Insulin signaling results in increased SREBP-1c expression in liver. SREBP-1c then transcriptionally activates all genes required for fatty acid synthesis (49). The overexpression of SREBP-1c in transgenic mouse livers leads to the development of a classic "fatty liver" due to increased lipogenesis (49).

Hyperinsulinemia and increased hepatic glucose production are hallmarks of

insulin resistance (50). 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 (51). 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 (52). Inactivation of the SREBP-1 gene in the livers of *ob/ob* mice results in a ~50% reduction in hepatic triglycerides (53).

SREBP-1c also activates ACC2, an isoform of ACC that produces malonyl-CoA at the mitochondrial membrane (54). 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 (55). 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) (56). 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 (57). 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 (58). 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 (59). 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 (60). Liver-specific gene deletions of PPARγ in insulin-resistant mouse models markedly attenuated the development of hepatic steatosis, independent of the hyperinsulinemia or hyperglycemia (61). 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 (62). The function of Foxa2 is to activate the transcription of genes involved in

mitochondrial and peroxisomal fatty acid oxidation, ketogenesis, and glycolysis (62). 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 (63).

In livers of insulin-resistant mice, Foxa2 is exclusively located in the cytosol under starved and fed conditions (62). 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 (62).

The available data suggest that the insulin-resistant liver exhibits a mixed pattern for insulin siganling. 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 quilt 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. (64, 65). Powell and colleagues (28) 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.* (66) 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 (67, 68).

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 (64). 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 (69-71). 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 (72). 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 (72). 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 (73)

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% (74), 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 (75). It also appears the degree of steatosis may correlate with the level of viral replication (75). These results, plus the fact that transgenic mouse models of hepatitis C have steatosis (76), 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 (77). Other studies have shown that insulin resistance is independently associated with HCV (78-81). 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.

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 (2, 82-85). The most recent study by Fartoux *et al.* (2) 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. 8**).

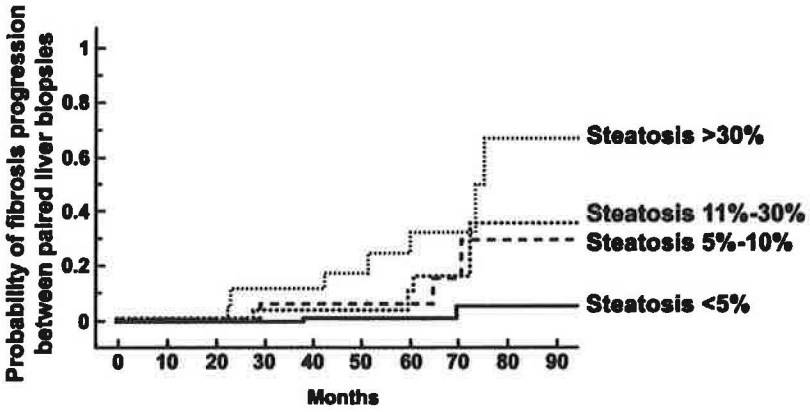


Fig. 8. Cumulative probability of fibrosis progression according to the percentage of steatosis on the initial biopsy (2).

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 (86). 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 (87). HCC may represent a late complication of NAFLD-related cirrhosis (10, 66, 70, 88-90). **Fig. 9** shows the prevalence of conditions associated with NAFLD in 614 HCC patients with cirrhosis (10). 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 (89).

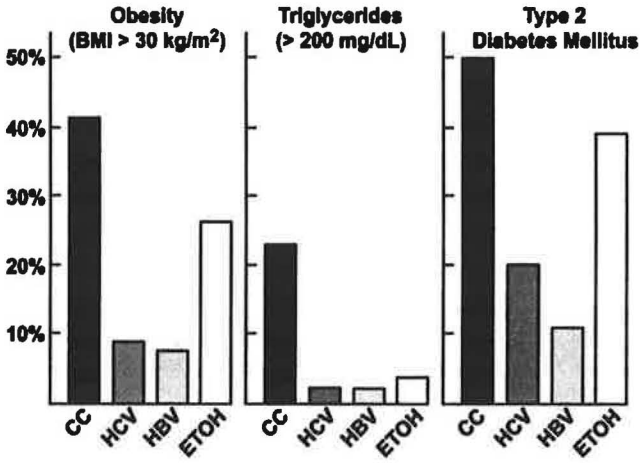


Fig. 9. Prevalence of obesity, hypertriglyceridemia, and diabetes in patients with HCC, grouped according to etiology (10). Abbrev. CC, cryptogenic cirrhosis; ETOH, ethanol.

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 (91). 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**) (92, 93).

Table 3. Type 2 diabetes and the incidence of cirrhosis and liver-related deaths (92)

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 (94). 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.* (26) 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.* (95) reported that ethanol ingestion was associated with reduced hepatic fat content in his obese patients. Browning *et al.* (4) 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 (4, 96, 97).

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%) (18). Three studies has reported that Hispanics with NAFLD appear to progress to NASH and end-stage liver disease more frequently than either blacks or whites (9, 98, 99). One study from Dallas found that Hispanics had a disproportionately high prevalence of NAFLD-related cirrhosis, while that of blacks was low (9). As shown in **Fig. 10**, the combined studies from Dallas suggest that Hispanics are more susceptible to NAFLD and NAFLD-induced liver damage than whites or blacks.

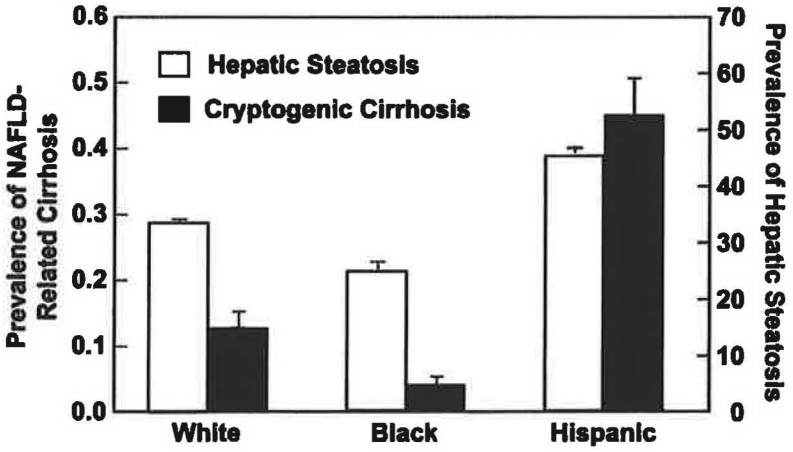


Fig. 10. Prevalence of NAFLD-related cirrhosis and hepatic steatosis in the three major ethnic groups of Dallas County, Texas (4, 9).

Weston *et al.* (99) 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 (4).

NAFLD and mortality

The majority of obese patients with NAFLD-related cirrhosis die of their liver disease despite the high prevalence of concomitant cardiovascular disease (100). This is somewhat surprising since the largest studies of patients with cirrhosis indicate that patients die of other causes at a rate that approximates that of cirrhosis-related deaths (8). However, the mortality of cirrhotic patients for non-liver-related causes of death is 5-fold higher than the general population (8). **Fig. 11** shows the 10-year survival curves of over 10,000 Danish patients diagnosed with cirrhosis grouped by cause (8). NAFLD patients were not specifically identified in this study, but were included in the non-specified cirrhosis group. Those with non-specified cirrhosis had the worst overall prognosis with an ~50% survival at 5 years.

Very few controlled trials have specifically addressed the long-term outcome of patients with NAFLD. Hui *et al.* (100) followed 23 patients with NASH and cirrhosis and compared their outcomes to matched patients with HCV-related cirrhosis. They found the prognosis was similar between the two groups both for morbidity and mortality. The probability of complication-free survival of NASH patients was 83%, 77%, and 48% at 1, 3, and 10 years, respectively.

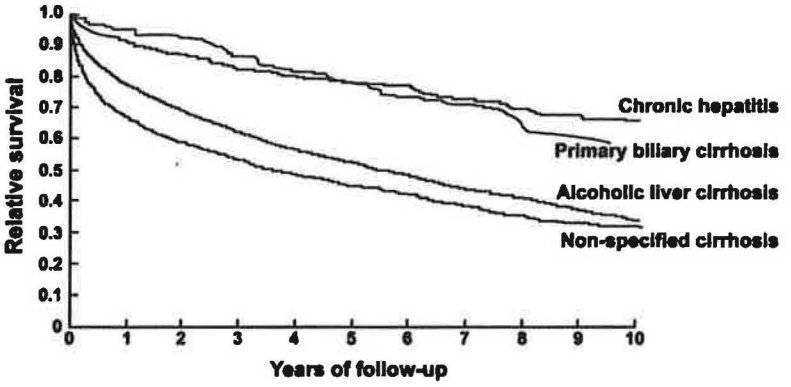


Fig. 11. Relative survival curves for 10,154 patients with liver cirrhosis during a 10-year follow-up period (8).

Disease progression: Steatosis to NASH

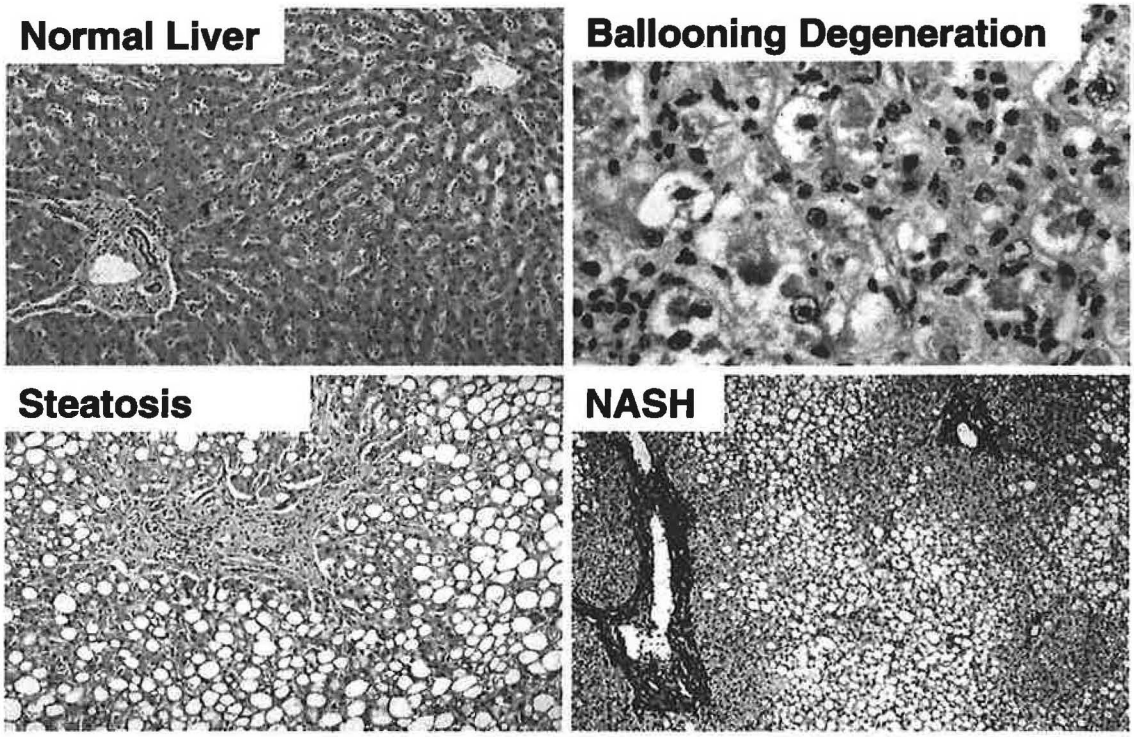
Despite the high prevalence of NAFLD and its potential for serious sequelae, the underlying factors that determine disease progression to cirrhosis remain poorly understood. Studies to clearly define the molecular and physiologic changes that mediate the presumed transition from hepatic steatosis to 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 well these animal models reflect the human pathophysiology of NASH is not known.

Non-alcoholic steatohepatitis (NASH)

Although ~34% of the population has NAFLD, only an estimated 10-20% of those will ultimately progress to clinically significant disease (101). In this respect, NAFLD is similar to ethanol-induced liver disease in which less than 10% of heavy drinkers ultimately develop cirrhosis (102). This led Day and James (103) 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. The best prevalence estimate for NASH comes from a large autopsy series that reported histologic evidence of NASH in 6.3% of all patients (22). Unfortunately, this study is now 15 years old. In patients with unexplained elevations in liver function tests, NASH is found in at least 26% of all biopsies (104). 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.
e) **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% (28, 29).



Potential mediators of the "second hit" leading to NASH

Inasmuch as NASH is histologically similar to alcohol-induced steatohepatitis, many of the factors implicated in the development of alcoholic steatohepatitis are also associated with NASH. These factors can be grouped into two broad categories: 1) Factors causing an increase in oxidative stress; and 2) Factors promoting expression of pro-inflammatory cytokines. These pathways are summarized in **Fig. 12.**

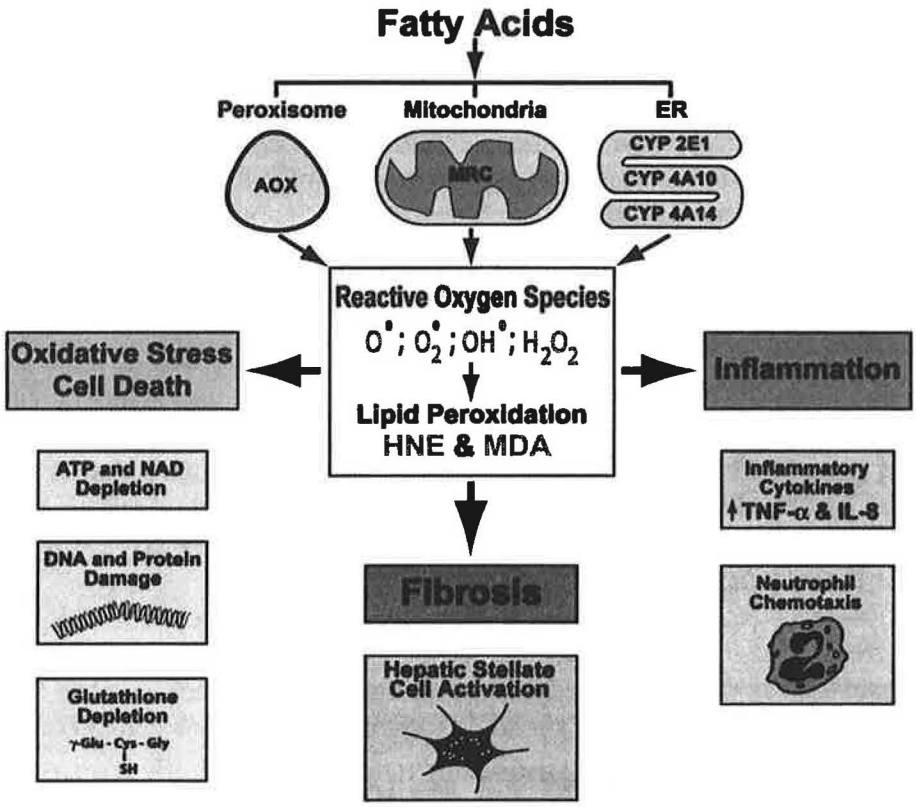


Fig. 12. Mechanisms of lipid-induced cellular injury in NAFLD. Reactive oxygen species (ROS) are formed through oxidative processes within the cell. In the mitochondria, impaired respiratory chain (MRC) activity leads to the formation of superoxide anions and hydrogen peroxide. The accumulation of fatty acids in the cytosol increases fatty acid oxidation in peroxisomes and the endoplasmic reticulum (ER). The initial reaction in peroxisomal β-oxidation is catalyzed by acyl-CoA oxidase (AOX) that forms hydrogen peroxide through the donation of electrons to molecular oxygen. Microsomal ω-oxidation is catalyzed by cytochrome P450 (CYP) enzymes 2E1, 4A10, and 4A14, which form ROS through flavoprotein-mediated donation of electrons to molecular oxygen. Polyunsaturated fatty acids (PUFAs) are extremely susceptible to lipid peroxidation by ROS. By-products of PUFA peroxidation are aldehydes, such as *trans*-4-hydroxy-2-nonenal (HNE) and malondialdehyde (MDA). These aldehydes are themselves cytotoxic and can freely diffuse into the extracellular space to affect distant cells. ROS and aldehydes induce oxidative stress and cell death via ATP and NAD depletion, DNA and protein damage, and glutathione depletion. Additionally, they induce inflammation through the production of proinflammatory cytokines, which leads to neutrophil chemotaxis. Within the extracellular space, HNE and MDA also are themselves potent chemoattractants for neutrophils. Finally, ROS and products of lipid peroxidation can lead to fibrosis by activating hepatic stellate cells, which synthesize collagen and perpetuate the inflammatory response (3).

Oxidative stress

Oxidative stress results from an imbalance between prooxidant and antioxidant chemical species that leads to oxidative damage of cellular macromolecules (105). 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 (106-109). 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 (105, 110). Increased production of ROS in the presence of excess free fatty acids has been validated in animal models of NASH (109, 111). Human livers with NASH have increased levels of byproducts of lipid peroxidation, providing evidence of an increase in oxidative stress in this condition (112).

Mitochondrial dysfunction

Mitochondrial β-oxidation is the dominant oxidative pathway for the disposition of fatty acids under normal physiologic conditions, but it also can be a major source of ROS (113). Several lines of evidence suggest that mitochondrial function is impaired in patients with NASH. Ultrastructural mitochondrial abnormalities have been documented in patients with NASH (114). Similar mitochondrial lesions are found in liver biopsy specimens from patients treated with 4,4'-diethylaminoethoxyhexestrol, a drug that inhibits mitochondrial respiratory chain activity and mitochondrial β-oxidation (115). Prolonged treatment with this agent is associated with hepatic steatosis and steatohepatitis that is histologically indistinguishable from NAFLD in humans (115). The ultrastructural mitochondrial defects in patients with NAFLD may be indicative of defective oxidative-phosphorylation, since these patients also have reduced mitochondrial respiratory chain activity (116) and impaired ATP synthesis after a fructose challenge (117).

Mitochondrial respiratory chain dysfunction can directly lead to the production of ROS. If electron flow is interrupted at any point in the respiratory chain, the preceding respiratory intermediates can transfer electrons to molecular oxygen to produce superoxide anions and hydrogen peroxide (108, 109).

As the oxidative capacity of the mitochondria becomes impaired, cytosolic fatty acids accumulate. Alternative pathways in the peroxisomes (β-oxidation) and in microsomes (ω-oxidation) are activated, resulting in the formation of additional ROS (115, 118, 119). In the initial step of peroxisomal β-oxidation, hydrogen peroxide is formed by the action of acyl-CoA oxidase, which donates electrons directly to molecular oxygen (107). Microsomal ω-oxidation of fatty acids, catalyzed primarily by cytochrome P450 enzymes 2E1, 4A10, and 4A14, forms ROS through flavoprotein-mediated donation of electrons to molecular oxygen (106). Dicarboxylic acids, another product of microsomal fatty acid ω-oxidation, can impair mitochondrial function by uncoupling oxidative-phosphorylation (120). Protonated dicarboxylic acids cycle from the inner to the outer mitochondrial membrane, resulting in dissipation of the mitochondrial proton gradient without concomitant ATP production (121). The cumulative effect of extramitochondrial fatty acid oxidation is a further increase in oxidative stress and mitochondrial impairment.

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) (122). 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 (122, 123). 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 (122, 124)]. 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 (125). The administration of anti-TNFα antibodies to *ob/ob* mice significantly reduces the liver triglyceride content (126). 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 (127). Recent studies have also shown that several animal models of hepatic steatosis have increased NF-κB activity (128, 129). 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 (128, 129).

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) (130). 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 (131).

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 (131).

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 (130). These events do not seem to be specific to NAFLD, but are a general wound-healing response.

Evaluation of patients with suspected NAFLD

NAFLD patients typically present for evaluation of other conditions and are found to have abnormal LFTs or incidental hepatomegaly (20). The majority of patients with NAFLD are asymptomatic (45-100%) but, occasionally, right upper quadrant pain, fatigue, and malaise are reported (**Table 4**) (132). The most common liver function test abnormalities are 2- to 5-fold elevations of ALT and aspartate aminotransferase (AST) (133). However, elevated LFTs are insensitive and nonspecific and the degree of elevation also does not correlate with the level of histologic damage. In 60-90% of NAFLD patients, the ratio of AST/ALT is <1, which is typically the reverse of that measured in serum from patients with alcohol-induced liver injury. Unfortunately, this ratio commonly reverses in NAFLD if the liver disease progresses to cirrhosis. Serum gamma-glutamyltranferase (GGT) and alkaline phosphatase levels are elevated in less than 50% of the cases. Therefore, the laboratory abnormalities are rather non-specific and cannot be used exclusively to provide the diagnosis of NAFLD.

Table 4. Symptoms and signs associated with NAFLD

Symptoms	Signs	Laboratory Values
Fatigue Malaise RUQ pain	Hepatomegaly (~75%) Splenomegaly (~25%) Rarely portal hypertension	2-5 fold increase in ALT and AST +/- Increased alk. phos. and GGT Increased cholesterol and triglycerides Hyperglycemia

The diagnosis of NAFLD is ultimately made by excluding other known causes of liver disease such as chronic viral hepatitis, autoimmune chronic active hepatitis, hemochromatosis, inherited metabolic abnormalities (Wilson's disease and α1-antitrypsin deficiency), as well as alcohol use and reactions to medications. Laboratory testing, including serologic tests for viral hepatitis, iron studies, ceruloplasmin levels, phenotype, and levels of α-1 antitrypsin, antimitochondrial and antinuclear antibodies should be measured to detect treatable causes of chronic liver diseases.

Who should be biopsied?

No noninvasive tests are available to diagnose and stage NAFLD. Liver biopsy remains the most sensitive modality, but it cannot distinguish between causes. Determining whether a liver biopsy is necessary would be easier if the true natural history of the disease was known. The natural history of NAFLD appears to vary according to the histologic type. The presence of bland hepatic fat seems to be a benign condition, and progression to fibrosis and cirrhosis is less common (92, 134). It should be noted, however, that only five relatively small studies have specifically assessed the natural history of NAFLD using serial liver biopsies (28, 29, 101, 134, 135). The follow-up period for the vast majority of patients was <10 years. Increased BMI and diabetes were independent predictors of progression in most studies. The conclusions from the larger studies suggest that ~1/3 of patients with NAFLD will histologically progress within 3-4 years (93, 135).

The largest studies also predicted the rate of progression to cirrhosis is approximately ½ the rate proposed for HCV, assuming the development of fibrosis is a linear process (93, 135). Unfortunately, there are significant individual differences in the rates of progression that limit the usefulness of the estimated rate. In particular, those with higher BMIs, diabetes, and lower fibrosis scores on the first biopsy progressed up to five times faster than the overall estimated rate. Inasmuch as a majority of patients with NAFLD do not have NASH, there is a clear need for indicators that could predict which patients are more likely to progress to fibrosis and cirrhosis.

Whether patients with suspected NAFLD require a liver biopsy is still debated. As will be discussed in the following section, no treatment is currently available for NAFLD, so one could argue the results will not significantly impact the patient's course. On the other hand, the result of a liver biopsy in NAFLD may have some predictive value and could identify those patients most likely to benefit from future clinical trials and for treatment once available. Currently, no formal guidelines currently exist for the use of liver biopsies in NAFLD.

Treatment

At this time there are no recommended treatments available for NAFLD other than gradual weight loss. As presented above, most patients with NAFLD have a relatively benign course and there is currently no way to identify those who will progress to fibrosis and/or cirrhosis. Since the true natural history of NAFLD remains largely unknown, it is difficult to recommend routine treatment strategies. Several small trials in highly selective populations have been performed and are discussed below.

Weight loss

NAFLD may resolve with weight loss but the actual benefits seem to be inconsistent and most studies have reported mixed results. A systematic review of all publications prior to 2003 concluded that there was little data to support or refute the benefits of weight loss (136). Since this review, two additional studies reported improvement in liver histology in patients undergoing gastric procedures for obesity (137, 138). Dixon *et al.* (137) studied 36 patients undergoing a laparoscopic gastric band placement. The patients lost 34 kg on average, 82% of the patients had improvement in all histologic scores for NASH, 9% showed selective improvement, and 9% had no change. Although weight loss should be recommended for all obese patients, the optimal rate of weight loss and the amount required for normalization of the liver histology have not been determined. It is also not known whether nonobese NAFLD patients will benefit from weight loss.

Pharmacologic therapies

Relevant published therapeutic trials for NAFLD are listed in **Table 5**. To date, published studies have included a very small number of patients and most have been uncontrolled open-label trials. A summary of the most interesting therapeutic agents is provided below.

Table 5. Therapeutic trials for the treatment of NAFLD

Study	Drug	# of Pts	Type of Study	Months	LFTs	Histology	Benefit
Laurin (139)	UDCA	24	Open label	12	Yes	Yes	Yes
Lindor (140)	UDCA	126	Placebo-controlled	24	Yes	Yes	No
Laurin (139)	Clofibrate	16	Open label	12	Yes	Yes	No
Basaranoglu (141)	Gemfibrozil	46	Randomized	1	Yes	No	No
Abdelmalek (142)	Betaine	8	Open label	12	Yes	Yes	Yes
Lavine (143)	Vitamin E	11*	Open label	4-10	Yes	No	Yes
Kugelmas (144)	Vitamin E	16	Open label	3	Yes	No	No
Schenker (145)	Vitamin E and C	45	Placebo-controlled	6	Yes	Yes	No
Caldwell (146)	Troglitazone	10	Open label	6	Yes	Yes	?
Assy (147)	Orlistat	8	Open-label	6	Yes	Yes	Yes
Marchesini (148)	Metformin	20	Open label	4	Yes	No	Yes
Perrillo (149)	Metformin	15	Open label	12	Yes	No	?
Bacon (150)	Rosiglitazone	30	Open label	12	Yes	Yes	Yes
Hoofnagle (151)	Pioglitazone	18	Open label	12	Yes	Yes	Yes
Rallidis (152)	Pravastatin	5	Open label	6	Yes	Yes	Yes
Nakamura (153)	Losartan	8	Open label	12	Yes	Yes	Yes
Merat (154)	Probucol	30	Open label	6	Yes	No	Yes
Adams (155)	Pentoxifylline	20	Open label	12	Yes	No	Yes

* The 11 patients were children.

1) **Ursodeoxycholic acid** is a hydrophilic bile acid that may protect cells from apoptosis (156). A large-scale randomized trial reported last year showed that UDCA treatment for 2 years was well-tolerated and safe, but no better than placebo for patients with NASH (140).

2) **Clofibrate and gemfibrozil** are fibric acid derivatives used as lipid-lowering drugs. The mechanisms by which they reduce plasma lipids are multiple but at the molecular level, they activate the nuclear receptor PPARα. PPARα transcriptionally activates several genes involved in oxidation of fatty acids. The longest trial with clofibrate was in 16 patients that were treated with 2 g/d for 1 year (139). No significant improvements in LFTs or liver histology were found at the end of the 12 months.

3) **Betaine** is a normal component of the methionine metabolic cycle. Ethanol feeding to rats alters methionine metabolism by decreasing the activity of methionine synthetase, the enzyme that converts homocysteine to methionine. This causes a reduction in S-adenosylmethionine, which is the activated form of methionine. Betaine administration to rats significantly increased S-adenosylmethionine and protected them from ethanol-induced fat accumulation in liver (157). Abdelmalek *et al.* (142) reported a pilot study in which 8 patients with NASH were treated with betaine (20 g/d) for 12 months. Seven patients completed the study and 3 of these had a 50% reduction in their LFTs. Overall, 50% of the patients had histologic improvement. The authors concluded that the results were encouraging enough to warrant a large controlled trial.

4) **Thiazolidinediones** are PPARγ activators used to treat type 2 diabetes. Two recent open label trials using thiazolidinediones in patients with NASH have generated significant enthusiasm. Neuschwander-Tetri *et al.* (150) conducted a 48-week trial with rosiglitazone 4 mg BID in 26 nondiabetic patients with NASH. Mean ALT levels were significantly decreased and liver histology improved in 10 of the 26 patients. Insulin sensitivity was improved despite significant weight gain in ~70% of the participants. The second trial administered pioglitazone for 48 weeks to 18 nondiabetic patients with NASH. The majority (89%) of the initial biopsies of these patients had fibrosis, although none had cirrhosis. During the treatment period ALT levels fell in all patients and normalized in 72%. The repeat liver biopsies following treatment showed an overall decrease in the NASH activity scores of ~50%. In both studies, the majority of the patients gained weight but had improved measures of insulin sensitivity. This supports the hypothesis that the primary metabolic alteration for NAFLD is insulin resistance and not merely obesity.

It should be noted that 6 patients had little or no response and 3 of these normalized their ALT levels. This again points out that ALTs cannot be used as a surrogate marker for liver damage and that all clinical trials must include before and after liver biopsies. A clear shortcoming of these studies is the lack of a placebo control group. In the one large placebo-controlled trial of UDCA, ~60 of the placebo-treated patients had improvement in hepatic steatosis, although there was no change in the indices of inflammation and fibrosis.

Although these pilot studies are encouraging it is important to remember that the first generation thioglitazone (troglitazone) was withdrawn from the market after evidence of hepatotoxicity. It appears that the second generation thioglitazones are safer and have less hepatotoxicity. It is noteworthy, however, that 1 of the 10 patients receiving pioglitazone plus vitamin E had a significant increase in ALT level and was withdrawn from the study. Similarly, 1 patient taking rosiglitazone in the second pilot trial also discontinued therapy because of increased ALT levels. Pioglitazone is currently approved by the FDA for use in type 2 diabetes, but concomitant active liver disease or the presence of ALT more than 2.5 times normal are contraindications for its use. On the other hand, presence of type 2 diabetes is associated with the development of advanced liver fibrosis in NASH patients, and type 2 diabetes is an important risk factor for mortality in patients with this liver disease. Thus, this subgroup of NASH patients with diabetes who are expected to derive the most benefit from medical treatment are currently contraindicated from thioglitazone therapy outside of approved clinical trials.

5) Metformin is an attractive candidate for study in NAFLD because it improves hepatic and peripheral sensitivity to insulin, suppresses gluconeogenesis, often results in weight loss, and does not cause overt hypoglycemia. Lin *et al.* (158) demonstrated that metformin resulted in significantly reduced liver fat in a mouse model of fatty liver, the *ob/ob* mouse. *Ob/ob* mice and lean controls were treated with metformin for 4 weeks. The treatment resulted in a marked improvement of LFTs, a normalization of liver weight and a markedly improved liver histology. The mice were administered much higher doses of metformin than is typically used in humans. None of the mice developed lactic acidosis, a known side effect.

The encouraging results in mice have led to two pilot studies in humans. Marchesini *et al.* (148) treated 14 patients with histologic evidence of steatohepatitis with metformin (500 mg TID) for 4 months. All patients in the study had normal fasting glucose levels and oral glucose tolerance tests. When compared with the six individuals not complying with treatment, 50% of the actively treated patients had a complete normalization of their transaminase levels, but no liver biopsies were performed. Insulin sensitivity improved significantly and liver volume was decreased on average by 20%.

A second pilot study was recently reported in patients with NAFLD treated with metformin for 1 year (149). Only 10 patients had repeat liver biopsies after 1 year of treatment. In these 10 patients, 3 had improved steatosis, 2 had improvement in inflammation, and 1 had improvement in fibrosis. Insulin sensitivity was improved after 3 months on metformin, but this was not maintained after 1 year of treatment. In both pilot studies, no patients developed significant lactic acidosis.

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. (16). 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 pilot studies are encouraging, solid therapeutic recommendations must still await the results of controlled randomized clinical trials with clinically relevant end-points. 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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References

1. Agriculture*, U. S. D. o. (2003) in *Agriculture Fact Book 2001-2002* (U.S. Government Printing Office, pp. 13-21.
2. Fartoux, L., Chazouilleres, O., Wendum, D., Poupon, R. & Serfaty, L. (2005) *Hepatology* **41,** 82-87.
3. Browning, J. D. & Horton, J. D. (2004) *J. Clin. Invest.* **114,** 147-152.
4. Browning, J. D., Szczepaniak, L. S., Dobbins, R., Nuremberg, P., Horton, J. D., Cohen, J. C., Grundy, S. M. & Hobbs, H. H. (2004) *Hepatology* **40,** 1387-1395.
5. Pereira, M. A., Kartashov, A. I., Ebbeling, C. B., Van Horn, L., Slattery, M. L., Jacobs Jr, P. D. R. & Ludwig, D. S. (2005) *The Lancet* **365,** 36-42.
6. Marchesini, G., Brizi, M., Morselli-Labate, A. M., Bianchi, G., Bugianesi, E., McCullough, A. J., Forlani, G. & Melchionda, N. (1999) *Am. J. Med.* **107,** 450-455.
7. Szczepaniak, L. S., Nurenberg, P., Leonard, D., Browning, J. D., Reingold, J. S., Grundy, S., Hobbs, H. H. & Dobbins, R. L. (2005) *Am. J. Physiol. Endocrinol. Metab.* **288,** E462-8. Epub 2004 Aug 31.
8. Sorensen, H. T., Thulstrup, A. M., Mellemkjar, L., Jepsen, P., Christensen, E., Olsen, J. H. & Vilstrup, H. (2003) *J. Clin. Epidemiol.* **56,** 88-93.
9. Browning, J. D., Kumar, K. S., Saboorian, M. H. & Thiele, D. L. (2004) *Am. J. Gastroenterol.* **99,** 292-298.
10. Bugianesi, E., Leone, N., Vanni, E., Marchesini, G., Brunello, F., Carucci, P., Musso, A., De Paolis, P., Capussotti, L., Salizzoni, M. & Rizzetto, M. (2002) *Gastroenterology* **123,** 134-140.
11. Flegal, K. M., Carroll, M. D., Ogden, C. L. & Johnson, C. L. (2002) *JAMA* **288,** 1723-1727.
12. Ogden, C. L., Flegal, K. M., Carroll, M. D. & Johnson, C. L. (2002) *JAMA* **288,** 1728-1732.
13. Flegal, K. M., Carroll, M. D., Kuczmarski, R. J. & Johnson, C. L. (1998) *Int. J. Obes. Relat. Metab. Disord.* **22,** 39-47.
14. Guthrie, J. F., Lin, B. H. & Frazao, E. (2002) *J. Nutr. Educ. Behav.* **34,** 140-150.
15. Ebbeling, C. B., Sinclair, K. B., Pereira, M. A., Garcia-Lago, E., Feldman, H. A. & Ludwig, D. S. (2004) *JAMA* **291,** 2828-2833.
16. Bell, S. H., Beringer, K. C. & Detre, K. M. (1996) in *Clinical Transplantation 1995*, eds. Cecka, J. M. & Terasaki, P. I. (UCLA Tissue Typing Laboratory, Los Angeles).
17. Neuschwander-Tetri, B. & Caldwell, S. (2003) *Hepatology* **37,** 1202-1219.
18. Clark, J. M., Brancati, F. L. & Diehl, A. M. (2003) *Am. J. Gastroenterol.* **98,** 960-967.
19. Angulo, P. (2002) *N. Engl. J. Med.* **346,** 1221-1231.
20. Ludwig, J., Viggiano, T. R., McGill, D. B. & Oh, B. J. (1980) *Mayo Clin. Proc.* **55,** 434-438.
21. Adler, M. A. & Schaffner, F. (1979) *Am. J. Med. Sci.* **67,** 811-816.
22. Wanless, I. R. & Lentz, J. S. (1990) *Hepatology* **12,** 1106-1110.
23. Marchesini, G., Brizi, M., Bianchi, G., Tomassetti, S., Bugianesi, E., Lenzi, M., McCullough, A. J., Natale, S., Forlani, G. & Melchionda, N. (2001) *Diabetes* **50,** 1844-1850.
24. Diehl, A. M. (1999) *Semin. Liver Dis.* **19,** 221-228.
25. Hilden, M., Christoffersen, P., Juhl, E. & Dalgaard, J. B. (1977) *Scand. J. Gastroenterol.* **12,** 593-597.
26. Bellentani, S., Saccoccio, G., Masutti, F., Croce, L. S., Brandi, G., Sasso, F., Cristanini, G. & Tiribelli, C. (2000) *Ann. Intern. Med.* **132,** 112-117.
27. Angulo, P., Keach, J. C., Batts, K. P. & Lindor, K. D. (1999) *Hepatology* **30,** 1356-1362.
28. Powell, E. E., Cooksley, W. G., Hanson, R., Searle, J., Halliday, J. W. & Powell, L. W. (1990) *Hepatology* **11,** 74-80.
29. Lee, R. G. (1989) *Hum. Pathol.* **20,** 594-598.
30. Diehl, A. M., Goodman, Z. & Ishak, K. G. (1988) *Gastroenterology* **95,** 1056-1062.
31. Anderson, T. & Gluud, C. (1984) *Int. J. Obes.* **8,** 97-106.
32. Brunt, E. M., Janney, C. G., Di Bisceglie, A. M., Neuschwander-Tetri, B. A. & Bacon, B. R. (1999) *Am. J. Gastroenterol.* **94,** 2467-2474.
33. Bedoucha, M., Atzpodien, E. & Boelsterli, U. A. (2001) *J. Hepatol.* **35,** 17-23.
34. Peterson, K. F., West, A. B., Reuben, A., Rothman, D. L. & Shulman, G. I. (1996) *Hepatology* **24,** 114-117.

35. Thomsen, C., Becker, U., Winkler, K., Christoffersen, P., Jensen, M. & Henriksen, O. (1994) *Magn. Reson. Imaging* **12,** 487-495.
36. Joseph, A. E., Saverymuttu, S. H., al-Sam, S., Cook, M. G. & Maxwell, J. D. (1991) *Clin. Radiol.* **43,** 26-31.
37. Saadeh, S., Younossi, Z. M., Remer, E. M., Gramlich, T., Ong, J. P., Hurley, M., Mullen, K. D., Cooper, J. N. & Sheridan, M. J. (2002) *Gastroenterology* **123,** 745-750.
38. MacDonald, G. A. & Peduto, A. J. (2000) *J. Gastroenterol. Hepatol.* **15,** 980-991.
39. Longo, R., Pollesello, P., Ricci, C., Masutti, F., Kvam, B. J., Bercich, L., Croce, L. S., Grigolato, P., Paoletti, S., de Bernard, B. & et al. (1995) *J. Magn. Reson. Imaging* **5,** 281-285.
40. Victor, R. G., Haley, R. W., Willett, D., Peshock, R. M., Vaeth, P. C., Leonard, D., Basit, M., Cooper, R. S., Iannacchione, V. G., Visscher, W., Staab, J. & Hobbs, H. H. (2004) *Am. J. Cardiol.* **93,** 1473-1480.
41. Abate, N., Garg, A., Peshock, R. M., Stray-Gundersen, J. & Grundy, S. M. (1995) *J. Clin. Invest.* **96,** 88-98.
42. Lewis, G. F., Carpentier, A., Adeli, K. & Giacca, A. (2002) *Endocr. Rev.* **23,** 201-229.
43. Wahren, J., Sato, Y., Ostman, J., Hagenfeldt, L. & Felig, P. (1984) *J. Clin. Invest.* **73,** 1367-1376.
44. Araya, J., Rodrigo, R., Videla, L., Thielemann, L., Orellana, M., Pettinelli, P. & Poniachik, J. (2004) *Clin. Sci. (Lond).* In press.
45. Shimomura, I., Shimano, H., Korn, B. S., Bashmakov, Y. & Horton, J. D. (1998) *J. Biol. Chem.* **273,** 35299-35306.
46. Koo, S.-H., Dutcher, A. K. & Towle, H. C. (2001) *J. Biol. Chem.* **276,** 9437-9445.
47. Shimomura, I., Bashmakov, Y., Ikemoto, S., Horton, J. D., Brown, M. S. & Goldstein, J. L. (1999) *Proc. Natl. Acad. Sci. USA* **96,** 13656-13661.
48. Foretz, M., Guichard, C., Ferre, P. & Foufelle, F. (1999) *Proc. Natl. Acad. Sci. USA* **96,** 12737-12742.
49. Horton, J. D., Goldstein, J. L. & Brown, M. S. (2002) *J. Clin. Invest.* **109,** 1125-1131.
50. Lam, T. K. T., Carpentier, A., Lewis, G. F., van de Werve, G., Fantus, I. G. & Giacca, A. (2003) *Am. J. Physiol. Endocrinol. Metab.* **284,** E863-E873.
51. Shimomura, I., Bashmakov, Y. & Horton, J. D. (1999) *J. Biol. Chem.* **274,** 30028-30032.
52. Halaas, J. L., Gajiwala, K. S., Maffei, M., Cohen, S. L., Chait, B. T., Rabinowitz, D., Lallone, R. L., Burley, S. K. & Friedman, J. M. (1995) *Science* **269,** 543-546.
53. Yahagi, N., Shimano, H., Hasty, A. H., Matsuzaka, T., Ide, T., Yoshikawa, T., Amemiya-Kudo, M., Tomita, S., Okazaki, H., Tamura, Y., Iizuka, Y., Ohashi, K., Osuga, J.-i., Harada, K., Gotoda, T., Nagai, R., Ishibashi, S. & Yamada, N. (2002) *J. Biol. Chem.* **277,** 19353-19357.
54. Abu-Elheiga, L., Brinkley, W. R., Zhong, L., Chirala, S. S., Woldegiorgis, G. & Wakil, S. J. (2000) *Proc. Natl. Acad. Sci. USA* **97,** 1444-1449.
55. McGarry, J. D., Mannaerts, G. P. & Foster, D. W. (1977) *J. Clin. Invest.* **60,** 265-270.
56. Yamashita, H., Takenoshita, M., Sakurai, M., Bruick, R. K., Henzel, W. J., Shillinglaw, W., Arnot, D. & Uyeda, K. (2001) *Proc. Natl. Acad. Sci. USA* **98,** 9116-9121.
57. Kawaguchi, T., Takenoshita, M., Kabashima, T. & Uyeda, K. (2001) *Proc. Natl. Acad. Sci. USA* **98,** 13710-13715.
58. Iizuka, K., Bruick, R. K., Liang, G., Horton, J. D. & Uyeda, K. (2004) *Proc. Natl. Acad. Sci. USA* **101,** 7281-7286.
59. Tontonoz, P., Hu, E. & Spiegelman, B. M. (1994) *Cell* **79,** 1147-1156.
60. Edvardsson, U., Bergstrom, M., Alexandersson, M., Bamberg, K., Ljung, B. & Dahllof, B. (1999) *J. Lipid Res.* **40,** 1177-1184.
61. Matsusue, K., Haluzik, M., Lambert, G. Y., S. H., Gavrilova, O., Ward, J. M., Brewer, B., Jr., Reitman, M. L. & Gonzalez, F. J. (2003) *J. Clin. Invest.* **111,** 737-747.
62. Wolfrum, C., Asilmaz, E., Luca, E., Friedman, J. M. & Stoffel, M. (2004) *Nature* **432,** 1027-1032.
63. Wolfrum, C., Besser, D., Luca, E. & Stoffel, M. (2003) *Proc. Natl. Acad. Sci. USA* **100,** 11624-11629.
64. Charlton, M., Kasparova, P., Weston, S., Lindor, K., Maor-Kendler, Y., Wiesner, R. H., Rosen, C. B. & Batts, K. P. (2001) *Liver Transpl.* **7,** 608-614.
65. Caldwell, S. H. & Crespo, D. M. (2004) *J. Hepatol.* **40,** 578-584.
66. Ratziu, V., Bonyhay, L., Di Martino, V., Charlotte, F., Cavallaro, L., Sayegh-Tainturier, M. H., Giral, P., Grimaldi, A., Opolon, P. & Poynard, T. (2002) *Hepatology* **35,** 1485-1493.

67. Caldwell, S. H., Oelsner, D. H., Iezzoni, J. C., Hespenheide, E. E., Battle, E. H. & Driscoll, C. J. (1999) *Hepatology* **29,** 664-669.
68. Poonawala, A., Nair, S. P. & Thuluvath, P. J. (2000) *Hepatology* **32,** 689-692.
69. Contos, M. J., Cales, W., Sterling, R. K., Luketic, V. A., Shiffman, M. L., Mills, A. S., Fisher, R. A., Ham, J. & Sanyal, A. J. (2001) *Liver Transpl.* **7,** 363-373.
70. Ong, J., Younossi, Z. M., Reddy, V., Price, L. L., Gramlich, T., Mayes, J. & Boparai, N. (2001) *Liver Transpl.* **7,** 797-801.
71. Sutedja, D. S., Gow, P. J., Hubscher, S. G. & Elias, E. (2004) *Transplantation Proceedings* **36,** 2334-2337.
72. Marsman, W. A., Wiesner, R. H., Rodriguez, L., Batts, K. P., Porayko, M. K., Hay, J. E., Gores, G. J. & Krom, R. A. (1996) *Transplantation* **62,** 1246-1251.
73. Burke, A. & Lucey, M. R. (2004) *Am. J. Transplant.* **4,** 686-693.
74. Lonardo, A., Adinolfi, L. E., Loria, P., Carulli, N., Ruggiero, G. & Day, C. P. (2004) *Gastroenterology* **126,** 586-597.
75. Kumar, D., Farrell, G. C., Fung, C. & George, J. (2002) *Hepatology* **36,** 1266-1272.
76. Lerat, H., Honda, M., Beard, M. R., Loesch, K., Sun, J., Yang, Y., Okuda, M., Gosert, R., Xiao, S. Y., Weinman, S. A. & Lemon, S. M. (2002) *Gastroenterology* **122,** 352-365.
77. Perlemuter, G., Sabile, A., Letteron, P., Vona, G., Topilco, A., Chretien, Y., Koike, K., Pessayre, D., Chapman, J., Barba, G. & Brechot, C. (2002) *FASEB J.* **16,** 185-194.
78. Allison, M. E., Wreghitt, T., Palmer, C. R. & Alexander, G. J. (1994) *J. Hepatol.* **21,** 1135-1139.
79. Mason, A. L., Lau, J. Y., Hoang, N., Qian, K., Alexander, G. J., Xu, L., Guo, L., Jacob, S., Regenstein, F. G., Zimmerman, R., Everhart, J. E., Wasserfall, C., Maclaren, N. K. & Perrillo, R. P. (1999) *Hepatology* **29,** 328-33.
80. Caronia, S., Taylor, K., Pagliaro, L., Carr, C., Palazzo, U., Petrik, J., O'Rahilly, S., Shore, S., Tom, B. D. & Alexander, G. J. (1999) *Hepatology* **30,** 1059-63.
81. Mehta, S. H., Brancati, F. L., Sulkowski, M. S., Strathdee, S. A., Szklo, M. & Thomas, D. L. (2000) *Ann. Intern. Med.* **133,** 592-599.
82. Hourigan, L. F., MacDonald, G. A., Purdie, D., Whitehall, V. H., Shorthouse, C., Clouston, A. & Powel, E. E. (1999) *Hepatology* **29,** 1215-1219.
83. Adinolfi, L. E., Gambardella, M., Andreana, A., Tripodi, M. F., Utili, R. & Ruggiero, G. (2001) *Hepatology* **33,** 1358-1364.
84. Westin, J., Nordlinder, H., Lagging, M., Norkrans, G. & Wejstal, R. (2002) *J. Hepatol.* **37,** 837-842.
85. Castera, L., Hezode, C., Roudot-Thoraval, F., Bastie, A., Zafrani, E.-S., Pawlotsky, J.-M. & Dhumeaux, D. (2003) *Gut* **52,** 288-292.
86. El-Serag, H. B. (2004) *Gastroenterology* **127,** S27-34.
87. Di Bisceglie, A. M., Lyra, A. C., Schwartz, M., Reddy, R. K., Martin, P., Gores, G., Lok, A. S., Hussain, K. B., Gish, R., Van Thiel, D. H., Younossi, Z., Tong, M., Hassanein, T., Balart, L., Fleckenstein, J., Flamm, S., Blei, A. & Befeler, A. S. (2003) *Am. J. Gastroenterol.* **98,** 2060-2063.
88. Shimada, M., Hashimoto, E., Taniai, M., Hasegawa, K., Okuda, H., Hayashi, N., Takasaki, K. & Ludwig, J. (2002) *J. Hepatol.* **37,** 154-160.
89. Marrero, J. A., Fontana, R. J., Su, G. L., Conjeevaram, H. S., Emick, D. M. & Lok, A. S. (2002) *Hepatology* **36,** 1349-1354.
90. El-Serag, H. B., Tran, T. & Everhart, J. E. (2004) *Gastroenterology* **126,** 460-468.
91. Mokdad, A. H., Ford, E. S., Bowman, B. A., Dietz, W. H., Vinicor, F., Bales, V. S. & Marks, J. S. (2003) *JAMA* **289,** 76-79.
92. Matteoni, C. A., Younossi, Z. M., Gramlich, T., Boparai, N., Liu, Y. C. & McCullough, A. J. (1999) *Gastroenterology* **116,** 1413-1419.
93. Adams, L. A., Sanderson, S., Lindor, K. D. & Angulo, P. (2005) *J. Hepatol.* **42,** 132-138.
94. de Marco, R., Locatelli, F., Zoppini, G., Verlato, G., Bonora, E. & Muggeo, M. (1999) *Diabetes Care* **22,** 756-761.
95. Dixon, J. B., Bhathal, P. S. & O'Brien, P. E. (2001) *Gastroenterology* **121,** 91-100.
96. Facchini, F., Chen, Y. D. & Reaven, G. M. (1994) *Diabetes Care* **17,** 115-119.
97. Kiechl, S., Willeit, J., Poewe, W., Egger, G., Oberhollenzer, F., Muggeo, M. & Bonora, E. (1996) *B.M.J.* **313,** 1040-1044.

98. Caldwell, S. H., Harris, D. M., Patrie, J. T. & Hespenheide, E. E. (2002) *Am. J. Gastroenterol.* **97,** 1496-1500.
99. Weston, S. E., Leyden, W., Murphy, R., Bass, N. M., Bell, B. P., Manos, M. M. & Terrault, N. A. (2005) *Hepatology* **41,** 372-379.
100. Hui, J., Kench, J., Chitturi, S., Sud, A., Farrell, G., Byth, K., Hall, P., Khan, M. & George, J. (2003) *Hepatology* **38,** 420-427.
101. Ratziu, V., Giral, P., Charlotte, F., Bruckert, E., Thibault, V., Theodorou, I., Khalil, L., Turpin, G., Opolon, P. & Poynard, T. (2000) *Gastroenterology* **118,** 1117-1123.
102. Bellentani, S., Saccoccio, G., Costa, G., Tiribelli, C., Manenti, F., Sodde, M., Saveria Croce, L., Sasso, F., G., P., Cristanini, G. & Brandi, G. (1997) *Gut* **41,** 845-850.
103. Day, C. P. & James, O. F. W. (1998) *Gastroenterology* **114,** 842-845.
104. Daniel, S., Ben-Menachem, T., Vasudevan, G., Ma, C. K. & Blumenkehl, M. (1999) *Am. J. Gastroenterol.* **94,** 3010-3014.
105. Robertson, G., Leclercq, I. & Farrell, G. C. (2001) *Am. J. Physiol. Gastrointest. Liver Physiol.* **281,** G1135-G1139.
106. Lieber, C. S. (2004) *Hepatology Research* **28,** 1-11.
107. Mannaerts, G. P., Van Veldhoven, P. P. & Casteels, M. (2000) *Cell Biochem. Biophys.* **32,** 73-87.
108. Garcia-Ruiz, C., Colell, A., Morales, A., Kaplowitz, N. & Fernandez-Checa, J. C. (1995) *Mol. Pharmacol.* **48,** 825-834.
109. Hensley, K., Kotake, Y., Sang, H., Pye, Q. N., Wallis, G. L., Kolker, L. M., Tabatabaie, T., Stewart, C. A., Konishi, Y., Nakae, D. & Floyd, R. A. (2000) *Carcinogenesis* **21,** 983-989.
110. Bergamini, C. M., Gambetti, S., Dondi, A. & Cervellati, C. (2004) *Curr. Pharm. Des.* **10,** 1611-1626.
111. Yang, S., Zhu, H., Li, Y., Lin, H., Gabrielson, K., Trush, M. A. & Diehl, A. M. (2000) *Arch. Biochem. Biophys.* **378,** 259-268.
112. Seki, S., Kitada, T., Yamada, T., Sakaguchi, H., Nakatani, K. & Wakasa, K. (2002) *J. Hepatol.* **37,** 56-62.
113. Reddy, J. K. & Mannaerts, G. P. (1994) *Annu. Rev. Nutr.* **114,** 343-370.
114. Caldwell, S. H., Swerdlow, R. H., Khan, E. M., Iezzoni, J. C., Hespenheide, E. E., Parks, J. K. & Parker, W. D., Jr. (1999) *J. Hepatol.* **31,** 430-434.
115. Berson, A., De Beco, V., Letteron, P., Robin, M. A., Moreau, C., El Kahwaji, J., Verthier, N., Feldmann, G., Fromenty, B. & Pessayre, D. (1998) *Gastroenterology* **114,** 764-774.
116. Perez-Carreras, M., Del Hoyo, P., Martin, M. A., Rubio, J. C., Martin, A., Castellano, G., Colina, F., Arenas, J. & Solis-Herruzo, J. A. (2003) *Hepatology* **38,** 999-1007.
117. Cortez-Pinto, H., Zhi Lin, H., Qi Yang, S., Odwin Da Costa, S. & Diehl, A. M. (1999) *Gastroenterology* **116,** 1184-1193.
118. Johnson, E. F., Palmer, C. N., Griffin, K. J. & Hsu, M. H. (1996) *FASEB J* **10,** 1241-1248.
119. Kersten, S., Seydoux, J., Peters, J. M., Gonzalez, F. J., Desvergne, B. & Wahli, W. (1999) *J. Clin. Invest.* **103,** 1489-1498.
120. Tonsgard, J. H. & Getz, G. S. (1985) *J. Clin. Invest.* **76,** 816-825.
121. Hermesh, O., Kalderon, B. & Bar-Tana, J. (1998) *J. Biol. Chem.* **273,** 3937-3942.
122. Esterbauer, H., Schaur, R. J. & Zollner, H. (1991) *Free Radic. Biol. Med.* **11,** 81-128.
123. Gardner, H. W. (1989) *Free Radic. Biol. Med.* **7,** 65-86.
124. Yamauchi, T., Kamon, J., Waki, H., Imai, Y., Shimozawa, N., Hioki, K., Uchida, S., Ito, Y., Takakuwa, K., Matsui, J., Takata, M., Eto, K., Terauchi, Y., Komeda, K., Tsunoda, M., Murakami, K., Ohnishi, Y., Naitoh, T., Yamamura, K., Ueyama, Y., Froguel, P., Kimura, S., Nagai, R. & Kadowaki, T. (2003) *J. Biol. Chem.* **278,** 2461-2468.
125. Kern, P. A., Saghizadeh, M., Ong, J. M., Bosch, R. J., Deem, R. & Simsolo, R. B. (1995) *J. Clin. Invest.* **95,** 2111-2119.
126. Li, Z., Yang, S., Lin, H., Huang, J., Watkins, P. A., Moser, A. B., DeSimone, C., Song, X.-y. & Diehl, A. M. (2003) *Hepatology* **37,** 343-350.
127. Uysal, K. T., Wiesbrock, S. M., Marino, M. W. & Hotamisligil, G. S. (1997) *Nature* **389,** 610-614.
128. Cai, D., Yuan, M., Frantz, D. F., Melendez, P. A., Hansen, L., Lee, J. & Shoelson, S. E. (2005) *Nat. Med.* **11,** 183-190.
129. Arkan, M. C., Hevener, A. L., Greten, F. R., Maeda, S., Li, Z. W., Long, J. M., Wynshaw-Boris, A., Poli, G., Olefsky, J. & Karin, M. (2005) *Nat. Med.* **11,** 191-198. .

130. Bataller, R. & Brenner, D. A. (2005) *J. Clin. Invest.* **115,** 209-218.
131. Friedman, S. L. (2000) *J. Biol. Chem.* **275,** 2247-2250.
132. Sunil, G., Gordon, F. D. & Chopra, S. (1997) *Ann. Int. Med.* **126,** 137-145.
133. Falck-Ytter, Y., Younossi, Z. M., Marchesini, G. & McCullough, A. J. (2001) *Semin. Liver Dis.* **21,** 17-26.
134. Teli, M. R., James, O. F., Burt, A. D., Bennett, M. K. & Day, C. P. (1995) *Hepatology* **22,** 1714-1719.
135. Fassio, E., Alvarez, E., Dominguez, N., Landeira, G. & Longo, C. (2004) *Hepatology* **40,** 820-826.
136. Wang, R. T., Koretz, R. L. & Yee, J., Hal F. (2003) *Am. J. Med.* **115,** 554-559.
137. Dixon, J. B., Bhathal, P. S., Hughes, N. R. & O'Brien, P. E. (2004) *Hepatology* **39,** 1647-1654.
138. Kral, J. G., Thung, S. N., Biron, S., Hould, F.-S., Lebel, S., Marceau, S., Simard, S. & Marceau, P. (2004) *Surgery* **135,** 48-58.
139. Laurin, J., Lindor, K. D., Crippin, J. S., Gossard, A., Gores, G. J., Ludwig, J., Rakela, J. & McGill, D. B. (1996) *Hepatology* **23,** 1464-1467.
140. Lindor, K. D., Kowdley, K. V., Heathcote, E. J., Harrison, M. E., Jorgensen, R., Angulo, P., Lymp, J. F., Burgart, L. & Colin, P. (2004) *Hepatology* **39,** 770-778.
141. Basaranoglu, M., Acbay, O. & Sonsuz, A. (1999) *J. Hepatol.* **31,** 384.
142. Abdelmalek, M. F., Angulo, P., Jorgensen, R. A., Sylvestre, P. B. & Lindor, K. D. (2001) *Am. J. Gastroenterol.* **96,** 2711-2717.
143. Lavine, J. (2000) *J. Pediatr.* **136,** 734-738.
144. Kugelmas, M., Hill, D., Vivian, B., Marsano, L. & McClain, C. (2003) *Hepatology* **38,** 413-419.
145. Harrison, S. A., Torgerson, S., Hayashi, P., Ward, J. & Schenker, S. (2003) *Am. J. Gastroenterol.* **98,** 2485-2490.
146. Caldwell, S. H., Hespenheide, E. E., Redick, J. A., Iezzoni, J. C., Battle, E. H. & Sheppard, B. L. (2001) *Am. J. Gastroenterol.* **96,** 519-525.
147. Assy, N., Svalb, S. & Hussein, O. (2001) *Hepatology* **34,** 458A.
148. Marchesini, G., Brizi, M., Bianchi, G., Tomassetti, S., Zoli, M. & Melchionda, N. (2001) *Lancet* **358,** 893-894.
149. Nair, S., Diehl, A. M., Wiseman, M., Farr, G. H. & Perrillo, R. P. (2004) *Aliment. Pharmacol. Ther.* **20,** 23-28.
150. Neuschwander-Tetri, B. A., Brunt, E. M., Wehmeier, K. R., Oliver, D. & Bacon, B. R. (2003) *Hepatology* **38,** 1008-1017.
151. Promrat, K., Lutchman, G., Uwaifo, G. I., Freedman, R. J., Soza, A., Heller, T., Doo, E., Ghany, M., Premkumar, A., Park, Y., Liang, T. J., Yanovski, J. A., Kleiner, D. E. & Hoofnagle, J. H. (2004) *Hepatology* **39,** 188-96.
152. Rallidis, L. S., Drakoulis, C. K. & Parasi, A. S. (2004) *Atherosclerosis* **174,** 193-196.
153. Yokohama, S., Yoneda, M., Haneda, M., Okamoto, S., Okada, M., Aso, K., Hasegawa, T., Tokusashi, Y., Miyokawa, N. & Nakamura, K. (2004) *Hepatology* **40,** 1222-1225.
154. Merat, S., Malekzadeh, R., Sohrabi, M. R., Sotoudeh, M., Rakhshani, N., Sohrabpour, A. A. & Naserimoghadam, S. (2003) *J. Hepatol.* **38,** 414-418.
155. Adams, L. A., Zein, C. O., Angulo, P. & Lindor, K. D. (2004) *Am. J. Gastro.* **99,** 2365-2368.
156. Rodrigues, C. M., Fan, G., Ma, X., Kren, B. T. & Steer, C. J. (1998) *J. Clin. Invest.* **101,** 2790-2799.
157. Barak, A. J., Beckenhauer, H. C., Junnila, M. & Tuma, D. J. (1993) *Alcohol Clin. Exp. Res.* **17,** 552-555.
158. Lin, H. Z., Yang, S. Q., Chuckaree, C., Kuhajda, F., Ronnet, G. & Diehl, A. M. (2000) *Nat. Med.* **6,** 998-1003.