Nonalcoholic Fatty Liver (NAFL): Overview

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Summary. Nonalcoholic fatty liver (NAFL) or nonalcoholic fatty liver *disease* (NAFLD) as it is often called, is increasingly recognized as a common and potentially severe condition often associated with obesity, type 2 diabetes, and hyperlipidemia. The spectrum of disorders which fall under the term 'NAFL' include simple steatosis, steatosis with mild inflammation, and steatosis with inflammation and varying degrees of fibrosis. Most investigators reserve the term 'NASH' (nonalcoholic steatohepatitis) for those patients who have some degree of fibrosis, usually associated with increased numbers of ballooned hepatocytes. The prognosis varies, but it is now evident that a substantial portion of patients, especially those with NASH, will progress to cirrhosis with all of the attendant complications, and some may ultimately develop hepatocellular cancer. Well-developed cirrhosis may lose its fat content and appear as 'cryptogenic cirrhosis'. Lipid peroxidation of the excess oil in the liver appears to be the major pathogenic mechanism. The process appears to alter mitochondrial form and function. Initial treatment usually involves exercise and dietary modifications. Patients who fail this approach or who have more advanced conditions initially may be candidates for more aggressive measures, including drug therapy. Many agents have been reported in small series but all remain investigational.

Key words. Nonalcoholic fatty liver, Nonalcoholic steatohepatitis, Cryptogenic cirrhosis, Obesity, Type 2 diabetes

Introduction

The histologic findings in patients with NAFL vary widely in the degree of microscopic injury from none or minimal to cirrhosis. Although still somewhat controversial, the natural history and prognosis is variable between simple steatosis (sometimes referred to as 'pure' fatty liver), steatosis with inflammation, and steatosis with fibro-

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sis. These forms of NAFL have been united into a classification system [1]. Predictive factors of more severe histology have also been determined and, to a lesser extent, the prevalence of NAFL types within different high-risk groups (obesity, diabetes, hyperlipidemia) has come into focus. Insulin resistance (IR) appears to be a common condition in NAFL, but it appears not to be uniformly present. Recently, Cotrim et al. [2] in Brazil have described NASH in petrochemical workers without an elevated index of IR. In addition, it seems likely that some forms of NAFL involve primary problems with lipoprotein metabolism, raising concerns about the potential toxicity of 'statin' drugs, which has been very little investigated. Thus, NAFL probably exists as a spectrum of disorders, although that seen with obesity and type 2 diabetes is the most common form.

Historical Perspective

The association between obesity and liver injury has been known since the 1800s [3, 4]. Several papers in the mid-1900s further reported a relationship between steatosis, progressive liver injury, and obesity [5]. Later, the association between intestinal bypass and progressive steatohepatitis further raised awareness of this disease [6]. However, even after the publication of several landmark papers in the 1980s (including that of Ludwig et al. [7], which provided the disease with its most common appellation 'NASH'), it became commonly taught that 'fatty liver' was a benign condition warranting little concern. While these misconceptions have largely given way to a much broader concept of fatty liver, there remains a good deal of lingering doubt about the overall prognosis and treatment of this condition.

Defining Terms in NAFL

In spite of recent progress, the nomenclature in NAFL has remained controversial and a consensus on working terminology has emerged largely by default [8]. Although the term 'NAFLD' is commonly used, we have elected to use the term 'NAFL' or 'nonalcoholic fatty liver' rather than 'NAFLD' or 'nonalcoholic fatty liver *disease*' since the disease portion of the latter term is variable and is not yet established for all of the forms of NAFL. We have reserved the term 'NASH' or 'nonalcoholic steatohepatitis' for more severe forms of NAFL (type 3–4), as described below.

The diagnosis of NAFL depends on the presence of an abnormal amount of fat in the liver. Prior studies demonstrated that the normal liver contains less than 5% fat by weight [9]. Since weight measurements are clinically impractical and most imaging modalities lack sensitivity (see below), fatty liver is most accurately diagnosed by light microscopy of biopsy specimens, although sampling error remains a potential problem. The cutoff level for significant steatosis has varied in the literature, but steatosis can be said to be present when the percentage of fat-laden hepatocytes exceeds a minimum of 5%–10% of the hepatocytes. The other major criterion implied in the term 'NAFL' is the issue of what constitutes 'nonalcoholic'. Levels defined as insignificant are also variable in the literature. Recently, some measure of agreement has been reached, allowing approximately 20 g per day (14 units per week) as a cutoff below which steatosis is 'nonalcoholic' [10].

'Presumptive' NAFL

A number of studies have utilized noninvasive studies to estimate the prevalence of steatosis in large populations wherein biopsy is impractical [11–13]. Most of these studies have utilized ultrasound examination of the liver for exclusion of other known diseases to make the diagnosis of 'presumed' NAFL. The noninvasive approach provides a practical means of assessing the prevalence of hepatic steatosis in a population under investigation, has utility in detecting other lesions, and finds clinical use as a screening for patients and potential living liver donors in the field of transplantation [14]. In addition, imaging can provide relevant information about body fat distribution by measuring indices of central adiposity [15]. However, no imaging technique has emerged which is capable of determining the presence or absence of histological injury.

This limitation was clearly demonstrated in a study by Saadeh et al. [16], in which 25 patients underwent biopsy (8 had mild NAFL and 17 had NASH), ultrasound, computed tomographic (CT) and magnetic resonance (MR) imaging. Steatosis of at least 33% was optimal for noninvasive imaging and values less than this were associated with substantial loss of sensitivity. None of the imaging modalities were useful in detecting histological features of NASH, such as balloon degeneration, Mallory hyaline, or fibrosis. Thus, although radiologic criteria exist to help guide the noninvasive diagnosis of NAFL [17–20], biopsy remains the gold standard for grading and staging of the disease. A possible exception is the use of MR spectroscopy, which has been shown to detect a physiological defect in ATP homeostasis in patients with NAFL [21]. Whether this modality or some combination of standard imaging and surrogate markers of histological injury will emerge as viable substitutes for biopsy remains to be seen (see below).

Histological Classification of NAFL

In their original study of 132 patients with NAFL, Matteoni et al. [1] grouped the biopsies into four groups: type 1 NAFL or simple steatosis, type 2 or steatosis with inflammation, and types 3 and 4, which were characterized by steatosis, inflammation and fibrosis, balloon cells, or Mallory bodies. There is a high degree of correlation between types 3 and 4, such that these should probably be put together as one group representing 'NASH' with several different stages of fibrosis. With a median follow-up of 8 years, cirrhosis developed much more commonly in the combined type 3–4 group (25%) than in the combined type 1–2 group (3%), and the crude liver-related mortality rate was also higher in the combined type 3–4 group. Other studies, by Hilden et al. [22] and Teli et al. [23], have supported the long-term stability of milder (types 1, 2) forms of NAFL. However, a transition from simple steatosis to a more aggressive form of NASH has been observed [24].

A close association between NAFL and cryptogenic cirrhosis has also been observed in a number of studies [25–28]. Similar to the histologic classification of NAFL, cryptogenic cirrhosis can be classified into several histological categories, including NASH with cirrhosis, cirrhosis with features of NASH (steatosis and glycogenated nuclei), and bland cirrhosis [29, 30].

These broad categories of NAFL and cryptogenic cirrhosis provide an important conceptual framework and attain a greater degree of importance by providing a basis on which to interpret clinical investigations and therapeutic trials through the use of a common set of terms. However, this system is based largely on only a few retrospective clinical studies and remains to be validated in additional prospective studies. The distinction between the various types of NAFL is not always clear-cut. In our experience, it is not uncommon to find a few scattered balloon cells (characteristic of type 3–4 NAFL) in a specimen with no fibrosis and only mild inflammation suggesting type 2 NAFL [31]. These and similar issues of precise histological definition are coming into sharper focus with increasing experience and refinements in the histological criteria [32, 33]. Of perhaps greatest importance is that investigators carefully define their terms in all reports to facilitate communications, whether or not they are using this classification scheme.

Epidemiology

NAFL is one of the most common of all liver disorders, but is among the most likely of liver disorders to be overlooked by primary care physicians [34]. The prevalence of all forms of NAFL has been estimated to be as high as 15%–20% of the adult population in the United States, Japan, and Italy [35]. Only about 20%–30% of these are thought to have the more severe form (NASH) but this is still quite a large fraction of the population in developed countries. Among obese people in western countries, about 60% have simple steatosis, 20%–25% have NASH, and 2%–3% have cirrhosis (see also below under 'Physical Findings') [36]. From the same publication, it was noted that at least 70% of type 2 diabetic patients have some form of fatty liver, and it has also been estimated that about 60% of hyperlipidemic patients have this disorder, although detailed histologic data have not, to our knowledge, been reported in these high-risk groups [37]. Among adults with NAFL, the metabolic syndrome (defined as three or more markers, including waist circumference, fasting hyperglycemia, elevated high-density lipoprotein [HDL], elevated triglycerides, or hypertension) is present in 88% of NASH patients and 54% of simple steatosis patients (see reference [30]). These figures are of even greater concern when considering the pediatric patient, where it is now estimated that more than 1%–2% of adolescents in the United States have some form of fatty liver (see reference [31]). The spectrum of histologic disease in the pediatric population can be severe [38].

History, Symptoms, and Physical Findings

As noted above, a history of obesity, diabetes, and/or hyperlipidemia is common, although not invariable. A family history of NASH or cryptogenic cirrhosis is frequent and warrants concern for a more progressive course [39, 40]. Other features of the 'metabolic syndrome' may be seen, such as hypertension, hyperuricemia, and the polycystic ovary syndrome [insulin resistance, diabetes, obesity, hirsutism, dysmenorrhea] [41–44]. Although widely associated with obesity (defined as body mass index [BMI] $>30\,kg/m^2$ in people of primarily European or African descent) [45, 46], an increasing number of patients have been described with normal BMI, although most have central

adiposity and latent or overt insulin resistance [47–50]. Waist circumference or the hip-waist ratio is probably the best anthropometric indicator of this sort of central obesity [51, 52], although use of special calipers to measure 'abdominal height' has been advocated. Changes in body composition due to aging and cirrhosis may mask a history of prior, severe, and longstanding obesity [53]. Thus, inquiry regarding past weight should be incorporated into the clinical evaluation.

Among obese individuals, the prevalence of NAFL and the expected frequency of different degrees of histologic injury can be estimated from past studies. Although much has to be conjectured because of the lack of common terminology, it can be approximated from a number of series that about 60% of obese individuals have relatively stable 'simple steatosis' or at most type 2 NAFL, while about 20%–25% have type 3–4 NAFL or frank NASH with fibrosis [54–58]. Only about 5% of such individuals have normal histology and about 5% have previously unrecognized cirrhosis. Whether or not these prevalence figures also apply to diabetic and hyperlipidemic patients is unknown, but warrants additional study. It is known that a substantial fraction of type 2 diabetic patients and hyperlipidemic patients have fatty infiltration by noninvasive testing [59, 60].

Common symptoms include fatigue and right upper quadrant pain, although the disorder even in the presence of cirrhosis may be silent. Associated conditions warrant consideration, including signs or symptoms of mitochondrialopathy-associated neuromuscular disease [61], gut motility [62, 63], lipodystrophy (fat atrophy) [64], lipomatosis [65], and Weber-Christian disease (panniculitis) [66]. A history of possible aggravating factors should be sought, including use of certain medications such as methotrexate, amiodarone, and tamoxifen [67–69], and solvent exposure [70]. Whether these variables aggravate a preexisting condition or cause a separate form of steatohepatitis (secondary NAFL) remains uncertain [71], but increasingly there appears to be a separate form of NAFL related to toxin exposure. Rarely, previously unrecognized NASH with silent cirrhosis may present as subacute liver failure [72]. More often these patients are discovered incidentally during some other evaluation [73], while up to 50% present initially with a major complication of cirrhosis such as ascites, variceal bleeding, or hepatocellular carcinoma [74].

Laboratory Findings in NAFL

Serum aminotransferase elevation, usually less than 1.5× normal [75], is perhaps the most common cause for patient referral [76–79]. The pattern of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) elevation carries some additional prognostic significance (see below), but these values may be significantly altered in patients receiving medications for comorbidities such as diabetes and hyperlipidemia. NAFL is the most common cause of unexplained aminotransferase elevation in asymptomatic people [80]. Elevation appears to approximate the degree of inflammation, but less so for fibrosis. This is supported by early drug trials in NASH, which showed normalization of transaminases, reduced indices of inflammation, but no change in fibrosis with troglitazone therapy [81]. More recent studies using extended courses have, however, shown improvement in fibrosis scores (see below, under 'Current Therapy of NAFL: Antidiabetic and insulin sensitizing agents').

However, liver enzymes may be normal in spite of underlying disease [82]. This may represent the effects of 'burned-out' disease, the effects of drug therapy, as noted above, or an upward drift of the normal reference range over the past few years—a phenomenon, now being corrected, that is thought to have resulted from the unrecognized inclusion of NAFL and hepatitis C patients in the reference controls [83].

Other abnormalities include elevated iron indices, although usually without frank hemochromatosis [84–88], positive anti-nuclear antibody (ANA) in about 30% [89, 90], elevation of serum IgA [91, 92], and mild abnormalities of alkaline phosphatase and gamma glutamyl transpeptidase (GGTP). Among patients with positive ANA, there does not appear to be any worsening of histology [93]. Slightly low ceruloplasmin levels without frank Wilson's disease are seen in about 10% of patients (unpublished observation). Markers of insulin resistance and sensitivity based on fasting insulin and glucose levels (Homeostasis Model Assessment [HOMA] and Quantitative insulin-sensitivity check index [QUICKI] tests) are increasingly being utilized in the clinical setting to assess these patients and their response to therapy [94]. These tests are essentially mathematical models referenced to the more elaborate and complicated hyperinsulinemic euglycemic clamp test. Markers of exercise tolerance and conditioning, such as the lactate threshold, will also likely find a role in clinically evaluating these patients but remain to be more thoroughly investigated. Recent studies have also begun to more carefully tease out the lipid profiles associated with NAFL [95].

The Role of Biopsy in the Diagnosis of NAFL

The biopsy remains the gold standard and serves to confirm the diagnosis and to stage the extent of injury in NASH. It is used early when the clinical evaluation is suspicious for more advanced disease or when there is a question of medication-induced injury. For those in whom the disease is not suspected to have significantly progressed, the biopsy is often deferred and a conservative course of exercise and diet is prescribed. Predictors of biopsy findings have been studied, although these should be regarded cautiously due to a high number of exceptions. Age more than 40–50 years, the degree of obesity, the degree of diabetes or insulin resistance, hypertriglyceridemia, hypertension, family history of NASH or cryptogenic cirrhosis, complete abstinence from ethanol, transaminase elevation, and an AST/ALT ratio of more than 1 are all predictive of more advanced histology on the initial biopsy [96–101]. Scores have been developed which include several parameters, but these remain to be validated. Whether these variables also predict the longterm course remains to be proven but seems likely. Female sex has not been a consistent predictor; however, the increasing prevalence of females with increasing fibrosis scores [102], and the preponderance of older females in most series of cryptogenic cirrhosis leave the issue unresolved. The discovery of unexpected cirrhosis at biopsy for NAFL is of obvious importance, as it casts the overall management of the obese, diabetic patient into a new light. Important considerations emerge if cirrhosis is discovered, such as avoidance of certain medications, and surveillance issues for varices or hepatocellular cancer.

Imaging studies (with the possible exception of MR spectroscopy) provide only limited information on the underlying pathophysiology. Markers of collagen and extracellular matrix metabolism have not been adequately studied, but may provide

limited information [103]. Whether some combination of imaging such as contrast ultrasound [104] and collagen marker profiles [105] will emerge to replace some biopsies remains to be seen. Although biopsy is likely to remain the gold standard for establishing the diagnosis, advantages possessed by these noninvasive markers include a viable means of easily tracking treatment response and the potential avoidance of sampling error inherent with biopsy. The latter issue reflects the suspected existence of geographic variation within the NAFL—it is estimated that left and right lobe biopsies can vary in grade of activity in about 20% of patients [106]. Further propelling the field of noninvasive testing is the fact that liver biopsy is not without risk, and the lack of definitive therapy based on the results warrants caution in applying biopsy.

Fatal complications, usually hemorrhage, are estimated to occur in 1 : 10 000 biopsies. Predicting this result is difficult, as the major problem is transgression of a vessel and bleeding risk correlates poorly to tests of coagulation [107]. Ultrasound guidance does not alter the risk of bleeding, which depends to a greater extent on the needle type (increased with larger needles and the cutting style) and the number of passes made [108]. However, the use of narrower gauge needles results in substantially less specimen and may compromise interpretation of the biopsy. Pain requiring intravenous analgesia occurs in 30%–50% of patients regardless of the use of ultrasound guidance [109], while more severe pain occurs in 1%–5% [110]. The body habitus of the patients most often afflicted with NAFL poses additional problems. While those with more central adiposity generally pose no significant problems for a percutaneous biopsy, those with more peripheral adipose tissue may require ultrasound guidance.

Prognosis: General

Although it is one of the most common of all liver disorders [111, 112], the natural history of NAFL remains a subject of active study. A substantial fraction of these patients will ultimately develop severe liver injury, presenting with new-onset ascites or variceal bleeding many years after the diagnosis of 'fatty liver'. Furthermore, it has become common to see older patients with 'cryptogenic cirrhosis' in the setting of prior known fatty liver, longstanding obesity, and type 2 diabetes. Advanced liver disease may become the dominant clinical problem in these patients, overtaking diabetes-related vascular disease. Not uncommonly, these patients eventually develop hepatocellular cancer.

The Prognosis of NAFL Based on Initial Histologic Classification

As mentioned earlier, a prognosis-related classification of NAFL has been proposed, although it remains to be validated in additional studies [113]. Some additional discussion of this work is warranted. Matteoni et al. [1] ascertained 132 patients with long-term follow-up and whose baseline biopsy, performed between 1979 and 1987, revealed NAFL. The biopsies were grouped into classes as follows: type 1 NAFL or simple steatosis, type 2 or steatosis with inflammation, and types 3 and 4, characterized by steatosis, inflammation and fibrosis, balloon cells, or Mallory bodies. Another

recent paper has shown a high degree of correlation between types 3 and 4, such that these can be put together as one group representing 'NASH' [114]. The primary outcomes of cirrhosis, mortality, and liver-related mortality were determined with an average follow-up of 8 years.

The groups consisted of 49 type 1, 10 type 2, 19 type 3, and 54 type 4 subjects. Testing for hepatitis C virus (HCV) by polymerase chain reaction (PCR) in a subset of the biopsies excluded hepatitis C as a significant factor in most patients. No age or sex differences were noted between these groups. Combining types 1 and 2 and comparing these to the combined type 3 and 4 groups, the authors noted no difference in overall mortality, but a substantial difference in the frequency of cirrhosis was observed. Clinically defined cirrhosis developed much more commonly in the combined type 3–4 group (25%) than in the combined type 1–2 group (3%). In the combined type 3–4 group, the crude liver-related mortality rate was also higher than that in the combined type 1–2 group, and it was also substantially higher than the published crude death rate from United States census data.

The age of the patients with different types of NAFL warrants some additional comment. The similar age between the two major groups (type 1–2 versus type 3–4) suggests that these groups do not represent different stages in the evolution of NAFL, but rather, that they represent two distinct groups. In other words, it is unlikely that there is progression from type 1–2 over time to type 3–4. If there was such a progression, it can be reasoned that there would be either an age difference between the two groups (the more severely afflicted would be older) or there would be no detectable difference in the prognosis between the two groups. Thus, it is more likely that the individual who develops fatty infiltration of the liver, soon thereafter either controls the problem (through as yet inadequately understood mechanisms) and remains stable indefinitely, or the individual develops cellular injury manifested histologically as steatohepatitis.

Other studies support the validity of this classification scheme and its associated prognosis. Hilden et al. [115] reported on 58 patients with mild fatty liver followed for up 33 years. The study antedated the proposed classification scheme, but appears to have largely consisted of type 1 and 2 patients, since the presence of Mallory bodies was used as an exclusion criterion. Only one of these patients was known to have progressed to cirrhosis. In another retrospective study, Teli et al. [116] demonstrated similar results. They studied 40 patients with nonalcoholic steatosis and absent inflammation or fibrosis on the index biopsy (similar to type 1 NAFL). Although the inclusion of 6 patients with cancer-related cachexia and secondary steatosis limits the interpretation, the overall results were very similar to those noted above. None of the patients developed clinical cirrhosis. About one-half had persistent liver enzyme abnormalities, but among those undergoing repeat biopsy, only 1 showed the development of mild perivenular fibrosis after almost 10 years.

Lee [117] reported follow-up biopsy on 13 patients over an average of 3.5 years (1.2–6.9 years) after the baseline biopsy. Among these, 12 patients with features of NASH did not have cirrhosis at baseline. Follow-up histology revealed increased fibrosis in 5 and the development of cirrhosis in 2 patients. Similarly, Powell et al. [118] reported follow-up biopsy in 13 NASH patients with a median follow-up of 4.5 years. Repeat biopsy revealed worsening fibrosis in 3, progression to cirrhosis in 3, absent

change in 6, and decreased fibrosis in 1. Of note, this study also demonstrated the progression of NASH with fibrosis or cirrhosis to cryptogenic cirrhosis with loss of the histological hallmarks of steatohepatitis. Bacon et al. [119] reported serial biopsy in 2 patients studied over approximately 5 years. One of these developed cirrhosis. Finally, Ratziu [120] reported serial biopsy in 14 patients with NAFL. Four of these had NASH at baseline, while 10 had only steatosis with minimal or no necroinflammatory activity or fibrosis. Among the 4 with baseline NASH (necroinflammatory activity and some degree of fibrosis), 1 progressed to cirrhosis over approximately 5 years, whereas none of the 10 patients without fibrosis progressed to cirrhosis.

Recent work has questioned some of the findings of older studies and raised new concerns. Harrison et al. [121] reported on 22 patients with NAFL followed with repeat biopsy over a mean of 5.7 years. Two patients with baseline biopsies showing simple steatosis were noted to develop mild degrees of fibrosis in follow-up biopsies performed 3–15 years after the baseline. Another interesting point from this work was that some patients had a relatively rapid change of fibrosis score between biopsies only several years apart. This suggests the possibility that NASH can remain stable for a long period of time and then show relatively rapid progression. Similar to prior studies, these authors noted a doubling in the percentage of patients with baseline fibrosis scores of stage 3 or 4 (bridging or cirrhosis) from 9% at baseline to 19% on follow-up.

Overall, it can be estimated that a substantial portion of NASH patients will remain stable or even improve, but about 40% will have worsening histology over approximately 5–7 years. As many as 20% develop worsening fibrosis, and up to 15%–20% progress to cirrhosis. Infrequently, the time over which the disease progresses will be considerably shorter than what we typically think. Possible explanations include rapid weight loss or possibly dietary composition, changes of which could precipitate deleterious changes in the fat-laden hepatocyte.

Mortality in NAFL

Among people with major risks for NAFL such as obesity and type 2 diabetes, liver-related mortality has largely been overshadowed by the high rate of cardio- and cerebrovascular death [122]. Nonetheless, obesity is a risk factor for cirrhosis-related death and hospitalization [123] and cirrhosis has been shown to be a common cause of death among type 2 diabetics [124]. In the latter study, the authors reported on mortality in 1939 type 2 diabetic patients followed for over 9 years. Not unexpectedly, vascular disease was the most common cause of death, with heart disease accounting for 19% of deaths, cerebrovascular disease for 16%, and renal disease for 13%. Cirrhosis was determined to be the cause of death in 6% of these patients, but the observed/expected ratio was actually higher (O/E = 2.67) for cirrhosis than for cerebro-cardiovascular disease overall (O/E = 2.12). This indicates a substantial risk for liver-related mortality in these patients, and suggests that liver-related morbidity is also likely to be an under-recognized factor in the management of diabetes and obesity.

Silent Morbidity of Advanced NASH in the Metabolic Syndrome

Cirrhosis is commonly recognized during the evaluation of some other problem in patients with longstanding obesity, type 2 diabetes, or hyperlipidemia [125]. The surprise discovery may occur during gallbladder surgery, during the evaluation of thrombocytopenia, or during the evaluation of new-onset gastrointestinal (GI) bleeding or ascites. Because cirrhosis fundamentally changes the physiology of the individual to that of the low systemic resistance (hyperdynamic) state, medication response is potentially altered; thus, common interventions may have unexpected side-effects. For instance, angiotensin-converting enzyme (ACE) inhibitors can promote salt retention and ascites formation. In addition, aspirin and other anti-thrombotic medications can promote fluid retention and/or GI bleeding (often from gastric antral vascular ectasia or 'GAVE'). The silent development of cirrhosis may also provide an alternative and potentially treatable explanation for certain symptoms. For instance, fatigue in the obese, diabetic patient with occult cirrhosis may actually reflect subclinical encephalopathy, treatable with typical ammonia-lowering regimens. Gut dysmotility, either as a result of associated diabetes or as part of NAFL [126], may contribute to constipation, making these patients especially prone to bouts of encephalopathy. Dyspnea may reflect the development of hepatopulmonary syndrome rather than intrinsic lung disease.

Given the potential role of the mitochondria in NASH (see below) [127, 128], it is interesting to further speculate upon the potential role of systemic mitochondrial dysfunction in the manifestations of NAFL. The existence of variation in mitochondrial integrity in different tissues offers a possible explanation. Mitochondrial heteroplasmy [129] could explain the susceptibility of different organs (such as the liver) to oxidative stress. A little-described ocular gaze disorder (intermittent disconjugate gaze or 'IDG'—seen in about 15% of NASH patients) lends support to the hypothesis [130]. Vision impairment is typically absent in IDG, but simple examination demonstrates disconjugate left or right lateral gaze that fluctuates in severity and may at times be undetectable, suggesting easy muscle fatigue as the likely mechanism.

Disease Modifiers and Confounding Variables

There are a number of variables which may alter the natural course of NAFL and which likely play some role in patients encountered day to day with this condition. Anti-diabetic agents, anti-lipidemic agents, dietary plans, exercise, over-the-counter herbal remedies, and modest ethanol use (which may actually be protective) are variables which have not been very well studied. Some of these have a potential role as therapy, but this is incompletely known (see below). Other agents, such as tamoxifen for breast cancer, amiodarone for cardiac dysrythmias, or methotrexate for psoriasis may accelerate cellular injury, and require careful consideration of the risk-benefit ratio. Coexisting NASH with other liver disease further introduces uncertainty into the evaluation of the fatty liver. Hepatitis C, especially genotype 3, is associated with some fatty infiltration, but frank NASH usually indicates the existence of risk factors for NAFL such as obesity and probably accelerates fibrosis [131]. We have also noted the development of NAFL in patients receiving steroids for autoimmune hepatitis. Its

presence can be misleading, as it requires biopsy to differentiate from residual autoimmune inflammation.

Cryptogenic Cirrhosis

Cryptogenic cirrhosis—defined as cirrhosis of unknown cause after exhaustive diagnostic evaluation—remains a common problem, accounting for 5%–15% of cirrhosis patients in different series. While there are clearly a number of disease processes involved with the development of cryptogenic cirrhosis (including NASH, occult ethanol, subclinical autoimmune hepatitis, and non-B, non-C hepatitis), several studies have demonstrated a close association between NASH and cryptogenic cirrhosis. Based on the studies discussed below and a recent detailed histological analysis of explanted livers by Ayata et al. [132], it is estimated that NASH constitutes the underlying disease process in 30%–70% of cryptogenic cirrhosis patients.

The seminal observation linking cryptogenic cirrhosis and NASH was that of Elizabeth Powell and colleagues [133] in a 1990 report, in which serial biopsy of NASH patients demonstrated the loss of steatosis over years as the disease progressed from steatohepatitis with bridging fibrosis or cirrhosis to a stage of bland cirrhosis. The loss of steatosis in the regenerating nodules may result from altered blood flow from portosystemic shunting [134], capillarization of the sinusoids with loss of fenestrations, and secondary impairment of lipoprotein delivery, or a more fundamental alteration in hepatocyte fat metabolism.

Contos et al. [135] have published a useful descriptive scheme which divides patients with cryptogenic cirrhosis into two categories: those with inconclusive but suggestive features of NASH and those with 'bland' cirrhosis. Among 30 liver explants from [patients with] cryptogenic cirrhosis, 6 had absence of steatosis, but 24 had variable and patchy fatty infitration (mostly in the mild or 1+ range.) Twenty had Mallory hyaline and 21 had balloon cells. Seventeen of 30 had balloon degeneration, Mallory hyaline, and steatosis; 10 more had at least two of these features. Inflammatory changes were mild and mostly limited to the septae. Twenty-six of 30 had glycogenated nuclei (a finding considered corroborative of underlying and antecedent NASH.) The high prevalence of risk factors for NASH in these patients and the high recurrence rate following transplantation (nearly 100% by 5 years) supports the assertion that the majority of these cases represented progression of NASH.

We reported on a series of 70 consecutive patients with cryptogenic cirrhosis including both transplant and nontransplant candidates. Among these patients, 70% were female and 73% had a history of obesity and/or diabetes. These patients had an average age of 60 years, compared to 50 years for a control group of consecutive NASH patients, suggesting a 10-year interval of disease progression between NASH and cirrhosis. The prevalence of obesity and/or diabetes among the cirrhosis patients was not different from that in the NASH patients, but was significantly greater than that of age-matched patients with cirrhosis from hepatitis C or primary biliary cirrhosis (PBC). In many patients with cryptogenic cirrhosis, a past history of obesity may be hidden, due to weight loss associated with either aging or cirrhosis. A striking finding among the patients with cryptogenic cirrhosis was the fact that over half lacked major symptoms of portal hypertension; i.e., the cirrhosis was both cryptogenic and clini-

cally silent. We also noted the common presence among both NASH and cryptogenic cirrhosis patients of a family history of unexplained liver disease—an association further supported by two additional publications [136, 137]. It was further noted that serum IgA was commonly elevated out of proportion to IgG. Serum IgA elevation, possibly as a result of lipid peroxidation and neoantigen formation, has long been associated with alcohol-induced steatohepatitis. A histologic study has also demonstrated deposition of IgA in liver tissue of both nonalcoholic and alcohol-related steatohepatitis [138]. Further studies are underway to examine serum and liver IgA as a marker of prior NASH in patients with cryptogenic cirrhosis.

Poonwalla et al. [139] published a report of 65 consecutive patients with cryptogenic cirrhosis awaiting liver transplantation. Each patient was compared to two age-matched control subjects with advanced cirrhosis from other etiologies and awaiting transplantation. The prevalence of obesity (55% versus 24%) and diabetes (47% versus 22%) was twice as high in the cryptogenic group as in the control group. Interestingly, the authors found no difference in the prevalence of hypercholesterolemia between the groups. Ong et al. [140] also reported on a series of 51 cryptogenic cirrhosis patients undergoing orthotopic liver transplantation (OLT). Similar to other series, the patients were commonly overweight females, and one-third had diabetes. Among the 25 patients undergoing post-transplant biopsy, 13 developed NAFL. Of these, 5 developed type 1 NAFL (simple steatosis) and 8 developed type 3–4 NAFL (NASH). Predictors of more severe histology post-OLT included diabetes, hypertriglyceridemia, and greater BMI. Possible interactions include the promotion of hepatic steatosis by glucocorticoids and the effects of cyclosporin A on the mitochondrial permeability transition pore.

Another perspective on this issue was provided by a report from Nair et al. [141], which demonstrated cryptogenic cirrhosis as the second most common cause of cirrhosis (after hepatitis C) among obese patients awaiting transplantation. However, because NASH patients who progress to cirrhosis often are much older (the median age in our series was 63 years) and frequently have comorbid conditions due to obesity, diabetes, and hyperlipidemia, their candidacy for transplantation is likely to be compromised. Thus, assessment of the significance of cryptogenic cirrhosis based on transplant lists is probably an underestimation, since many such patients are not considered for this intervention.

Cryptogenic Cirrhosis: Prognosis

The prognosis of obesity-related cryptogenic cirrhosis remains somewhat uncertain. However, grounds for increased concern regarding the development of complications of portal hypertension and hepatocellular cancer are slowly emerging. Ratziu et al. [142] recently compared the course of 27 overweight patients with cryptogenic cirrhosis to that of 10 lean patients with cryptogenic cirrhosis and 391 patients with hepatitis C-related cirrhosis in a retrospective follow-up cohort study. The prevalences of diabetes and hyperlipidemia were significantly higher in the obese cryptogenic compared to the lean cryptogenic cirrhosis group and the hepatitis C group. This difference persisted even when controlling for body mass index in the hepatitis C group. The mean age of the obese cryptogenic cirrhosis group was 62 years compared to 45 years for the lean cryptogenic group.

Most striking in this report was the fact that 9 of 27 obese cryptogenic patients were initially diagnosed with cirrhosis at the time of a major complication of portal hypertension, and 3 more had hepatocellular cancer at or near the time of the initial diagnosis of cirrhosis. After a mean follow-up of 22 months, 2 of the 15 patients presenting only with abnormal liver tests developed major complications of portal hypertension and 5 developed hepatocellular cancer. The authors concluded that obesity-related cirrhosis often diverges from the slow, indolent process characteristic of NASH and it may behave as aggressively as hepatitis C-related cirrhosis. The explanation for this observation remains uncertain, since loss of an active steatohepatitis would, intuitively, suggest a slowing of the process. Older age or perhaps accelerated parenchymal extinction [143] (a microvascular process) may offer an explanation.

Hepatocellular Cancer and NAFLD

The incidence of hepatocellular cancer has been increasing in the United States [144]. Although much of this has been attributed to hepatitis C, several papers have recently been published linking NAFL, insulin resistance, cryptogenic cirrhosis, and hepatocellular cancer [145]. Obesity itself has been implicated as a risk for various neoplasms [146]. Insulin resistance, associated hepatocyte hyperplasia, and decreased apoptosis have been implicated as factors in the development of hepatocellular cancer in ob/ob mice [147]. Diabetes has also been implicated as a factor in patients with viral hepatitis or alcoholic liver disease [148]. The observations in two recent case reports indicating hepatocellular cancer as a possible natural progression of NASH-related cirrhosis have subsequently been supported by larger studies (in addition to that of Ratziu et al. [142], noted above) [149, 150]. These two case reports (of one male, age 62, and one female, age 58—both with obesity and diabetes) described the development of hepatocellular cancer 6–10 years after the diagnosis of NASH was established by serological evaluation and biopsy.

Bugianesi et al. [151] reported on 23 patients with cryptogenic cirrhosis and hepatocellular cancer and compared this cohort to 115 age-matched controls from a registry of 641 cirrhosis-related hepatocellular cancers. A history of obesity (BMI > 30) was significantly more common in the cryptogenic group (41% versus 16%), as was a history of diabetes (50% versus 20%). The authors did not detect a difference in the duration of disease, the prevalence of genetic markers for hemochromatosis, or the character of the tumor (whether multifocal or metastatic). Compared to the overall group of hepatocellular cancer patients ($n = 641$), the cryptogenic cirrhosis group was older. However, in contrast to past series of cryptogenic cirrhosis patients, but similar to the paper of Ratziu et al. [142], there was a preponderance of males, suggesting an increased risk of hepatocellular cancer in males with cryptogenic cirrhosis.

In another report, Marrero et al. [152] reported the results of a prospective study on cirrhosis-related hepatocellular cancer. Among 105 patients with cirrhosis and hepatocellular cancer, 51% had hepatitis C as the underlying disease, but cryptogenic cirrhosis was the second most common association, accounting for 29% of the cases. The majority (58%) of these had a history of obesity, and 6 (20%) had documented prior steatohepatitis by biopsy performed an average of 4.5 years before the diagnosis of hepatocellular cancer. In contrast to the patients noted in prior series, these

patients were mostly females, the tumors were often more advanced, and the patient was less likely to have undergone prior screening.

Pathogenesis of NAFL and NASH

Human fatty liver is a multifactorial disorder. Most likely, there are several different pathways by which one can arrive at a NASH, including the common metabolic syndrome patient with insulin resistance, toxin-induced fatty liver, such as Cotrim [2] has described in petrochemical workers, and possibly primary disorders of lipoprotein metabolism; for example, the fatty liver seen in kwashiorkor. Once fatty liver develops, there is some trigger that produces inflammation. Most likely this is lipid peroxidation, which serves as the 'second hit' proposed by James and Day [153]. However, further damage probably depends on additional variables, including genetic predisposition, nutritional status, exposure to toxins, exercise, and perhaps other less well-understood variables such as cold-exposure. Central to many of these variables is the functional status of the mitochondrion.

The Mitochondrion in NAFLD

Classically, mitochondrial disorders have included primarily neuromuscular diseases, many of which have been extensively characterized both clinically and physiologically [154]. Although cryptogenic cirrhosis has been described in patients with mitochondrial disease [155], liver disease in general or fatty liver in particular is rarely mentioned in the context of typical mitochondrial diseases. Nonetheless, a substantial body of literature has developed implicating mitochondrial dysfunction in various forms of hepatic steatosis [156]. The peculiar evolution of the mitochondrion from a free-living organism to an integral component of cell function may explain its relationship to cell death through apoptotic pathways [157]. Its role in fat metabolism and oxidative phosphorylation explains its role as both a source of free radicals and as a target for injury in oxidative stress [158].

The normal mitochondrion is a double-membrane organelle, numbering typically in the thousands per cell, with invaginations of the inner membrane which form cristae [159, 160]. The mitochondrial matrix contains enzymes responsible for the metabolism of amino acids (much of the AST elevation in steatohepatitis is mitochondrial in origin and presumably reflects injury to the organelle), as well as components of the tricarboxylic acid cycle, urea cycle, and heme synthesis pathway. In addition, the matrix contains 1–5 copies (up to 100 copies if replicating) of the mitochondrial genome organized into mitochondrial nuclei known as nucleoids [161]. Certain shapes are characteristic of different tissues—in liver, the tubular form is most common, while complex branching mitochondria are seen in skeletal muscle [162]. Variation in cristae (tubular in hepatocyte mitochondria, stacked in brown fat cells) and matrix density is seen between cell types.

The mitochondrial respiratory chain uses energy from the transport of electrons to generate an electrochemical gradient of protons (proton motive force, or PMF) across the inner mitochondrial membrane. Translocation of protons along the electrochemical gradient from the intermembrane space to the matrix through complex

V (ATP synthase) drives production of ATP. Uncoupling of oxidative phosphorylation, as in brown-fat mitochondria, results in the production of heat rather than ATP—an important function in thermogenesis. Opening of the mitochondrial transition pore also dissipates the electrochemical gradient, resulting in decreased ATP production. One percent to 2% of oxygen consumed during oxidative phosphorylation is only partially reduced, resulting in the formation of reactive oxygen species (ROS). These are primarily formed around ubiquinone of the respiratory chain (RC) and include superoxide anion and hydrogen peroxide (which can be converted to reactive hydroxyl ion). Mitochondrial superoxide dismutase and the selenium-dependant glutathione peroxidase serve to contain the ROS [163].

The Mitochondria in NAFL: Adaptation and Injury

Impaired oxidative phosphorylation and morphological changes of mitochondria in experimental steatosis were described roughly 50 years ago by Dianzani [164, 165]. Structural mitochondrial abnormalities characterized by enlargement and development of crystalline inclusions have been described in several forms of human fatty liver, including Wilson's disease [166], alcoholic steatohepatitis (where they were felt to be markers for early or less severe alcohol-related injury [167]), and nonalcoholic steatohepatitis (NASH) [168, 169]. Sanyal et al. [169] correlated the presence of these crystalline mitochondrial inclusions indirectly to oxidative stress. Once described as 'para'-crystalline, optical diffraction studies have demonstrated that the inclusions are in fact true crystals, although their composition remains uncertain [170]. From studies of NAFL patients in which we counted the number of megamitochondria in graded sections of electron microscope fields, we estimated that crystal-containing megamitochondria were seen in approximately 5%–15% of hepatocytes in NASH patients and in 5%–10% of the mitochondria within an afflicted cell [171]. The crystals occur as long parallel strands, each approximately 10 nm in diameter, with 20-nm spaces between strands. The 'sometimes seen' continuity with more normal-appearing cristae and the similarity of these structures to reversible lipid bilayers occurring in plants (etioplasts—precursors of chloroplasts) [172] and stress-induced structures in Amoeba mitochondria [173] suggests that these structures represent morphological or conformational changes in the cristae [174]. Similar structures containing bacterial DNA and a ferritin-like substance have also been observed in *Escherichia coli* exposed to a stress-induced protein (Dps) [175]. In spite of their pathological appearance, we were unable to demonstrate a relationship between the distribution of these structures and light microscopic changes, suggesting that they represent an adaptive response to oxidative injury [176].

These structural abnormalities are mirrored by disturbed ATP homeostasis, a likely result of impaired function of the mitochondrial electron transport chain. Dianzani [177] described decreased ATP and increased ADP in isolated mitochondria from rats with experimental fatty liver due to choline deficiency, CCL4 exposure, or white phosphorous exposure, and later demonstrated evidence of impaired oxidative-phosphorylation in experimental fatty liver. Rats fed choline-deficient diets developed fatty liver with increased mitochondrial lipid peroxides (Thiobarbituric reactive substances [TBARS] assay), decreased mitochondrial ATP, decreased ATP synthase

activity, and markedly increased susceptibility to hepatic injury from starvation due to decreased glutathione levels [178]. The ability of betaine, an antioxidant, to reduce mitochondrial changes in CCL4-induced steatosis and the ability of gene delivery of superoxide dismutase in experimental fatty liver transplantation to likewise reduce injury further point to an association between mitochondrial morphological abnormalities, impaired ATP synthesis, and lipid peroxidation [179, 180].

The susceptibility of fatty livers to injury from ischemia-reperfusion also points to impaired ATP homeostasis [181]. Dysfunction for all components of the respiratory chain in hepatic mitochondria was demonstrated in a study of 38 NASH patients by Perez-Carrera et al. [182]. Respiratory chain activity was reduced to roughly 60% compared to identically processed controls in liver tissue homogenates normalized for volume to citrate synthase activity. Using an in vivo approach, Cortez-Pinto et al. [183] showed delayed recovery of hepatic ATP in patients with NAFL compared to controls following an intravenous fructose challenge using MR spectroscopy. Fructose, unlike glucose, requires an initial energy investment in the form of ATP prior to its subsequent metabolism. The authors of this study postulated that the intravenous bolus of fructose uncovered an energy deficit in the liver of patients with hepatic steatosis.

The physiological abnormalities in fatty liver include the expression of uncoupling protein 2 (UCP-2), which has been demonstrated experimentally in obese mice [184]. Uncoupling of the mitochondrial electron transport chain (ETC) is characteristic of thermogenic brown fat and shifts the energy of the ETC from ATP production to heat production. It has been suggested that this process represents a physiologic adaptation which reduces the formation of ROS [185], although the importance of this relationship has been questioned in terms of disease progression [186]. Mitochondrial uncoupling may be a direct result of increased free fatty acids in the intermitochondrial space [187], but because UCP-2 is normally produced only in fat tissue, its expression in the steatotic liver suggests that the fat-laden hepatocyte may not only look microscopically like an adipocyte but may also function essentially as such. We have recently noted that these similarities extend to the ultrastructural level [188] and suggested that the ballooned cell in NAFL is not always pathological but may be a form of adaptation to overabundant fat stores. In support of this, a recent abstract from Japan showed that markers for apoptosis, while seen in cells with microdroplet fat, did not appear to be invariable, suggesting that some cells successfully make a transition to a more stable unilocular fat-storing cell [189].

Fatty infiltration of solid organs, including the heart and liver, does exist in nature to meet the special metabolic demands imposed by migration, hibernation, or torpor. The best example occurs in some species of migratory ducks and geese. These birds undertake long migrations in a fasting state, and accumulate abundant fat in their liver prior to migration. This trait has been exploited commercially by human beings for the production of foie gras. Marked changes in apolipoprotein metabolism (decreased secretion of very low density lipoprotein [VLDL]) are seen in species which develop more substantial steatosis [190]. Rodents such as deer mice (genus Peromyscus) or ground squirrels that undergo periods of hibernation or daily torpor also accumulate fat in both their livers and their hearts. The California Desert mouse, adapted to the stressful conditions of desert life, develops steatosis and steatohepatitis in conditions of overabundant food availability [191]. It is speculated that these

changes, adapted to feast and famine, provide both a ready source of metabolic fuel and a means of heat production—an essential function in cold-adapted homeothermic organisms, but potentially pathological in the overfed state [192].

Recently, a link between dietary thermogenesis, obesity, sympathetic nervous system activity, and cold exposure has been described [193, 194]. It is thought that the balance between dietary intake and cold exposure (or some other stress) influences both basal metabolic and facultative (in response to cold exposure) thermogenesis by acting through the sympathetic nervous system, which influences the expression of UCP in brown fat. The role of adrenergic stimulation in steatohepatitis in ObOb mice seems relevant to this process [195]. A similar process may be present in some rudimentary fashion in higher mammals, and could explain some of the familial [196, 197] and ethnic [198] variation described in human NAFL.

Variation in mitochondrial DNA susceptibility to oxidative stress could also play a role in variable tissue injury. Injury probably depends on a threshold effect depending on the energy needs of tissue [199–201]. The lack of substantial repair machinery in the mitochondria is thought to contribute to their increased susceptibility to oxidative injury and the accumulation of mtDNA mutations and/or deletions that eventually lead to impaired cellular respiration and cell death. In NASH patients, a 5-kb gene deletion (the 'common' deletion associated with Kearns-Sayer mitochondrialopathy) was detected in liver tissue of 1 in 5 patients tested in one series and in 10 of 14 patients in another series [203]. Another patient in the latter series expressed the 8344 mutation seen in the myoclonic epilepsy and ragged red fibers (MERRF) syndrome.

Hepatic steatosis and cryptogenic cirrhosis may be seen in specific mitochondrial gene mutations and deletions [204, 205]. Symmetrical lipomatosis, or Madelungs disease [206], which is characterized by obesity, lipomatosis, insulin resistance, and dyslipidemia has been associated with mitochondrial DNA mutation 8344, which also is evident in MERFF syndrome [207]. Another mitochondrial mutation (3243, seen also in MELAS syndrome—mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes) has been associated with MIDD (maternally inherited diabetes and deafness) [208]. Neither the prevalence of fatty liver in these patients nor the prevalence of these mutations in NASH are known. However, it has been estimated that 1%–2% of type 2 diabetes patients harbor the nt-3243 mutation [209].

Apoptotic bodies are occasionally seen in NASH at light microscopy and probably represent the result of mitochondrial injury and imbalance of pro- versus anti-apoptotic factors. Their infrequent presence by light microscopy, in comparison to their presence in alcohol-related liver injury [210], is consistent with the typically slower rate of clinical progression of NASH compared to alcoholic steatohepatitis (ASH). Mitochondrial swelling, outer membrane rupture, and extrusion of inner matrix material have been observed in mouse hepatocytes after stimulation of apoptosis by FAS activation [211]. Recently, it was shown that more subtle signs of apoptosis (evident by the terminal deoxynucleotidyl transferase (TdT)-mediated dUTP-biotin nick end labeling [TUNEL] assay) were increased in patients with NASH relative to control patients [212]. This was accompanied by increased activated caspase and FAS—both factors in the cascade leading to apoptosis. Agents thought to potentiate or stimulate the opening of the mitochondrial permeability transition (MPT) pore include reactive oxygen species in oxidative stress, Bax, tumor necrosis factor (TNF), and

ceramide. Both pro-apoptotic (Bax) and anti-apoptotic (Bcl-2, Bcl-xl) factors are increased in experimental models of fatty liver [213].

The association of partial lipodystrophy, a disorder of peripheral fat metabolism, and NAFL provides perhaps the most apparent clinical example of NAFL as a systemic disorder of fat metabolism [214]. Mitochondrial heteroplasmy (the uneven dispersion of weak and susceptible mitochondria in different tissues—see above) combined with environmental influences (such as dietary composition, exercise levels, and aging) and the metabolic demands of different tissues could explain the variable expression of a latent systemic disorder. Degonl et al. [215] have suggested that genetic variation in the trafficking of superoxide dismutase into the mitochondria may explain, to some extent, individual variation in susceptibility to oxidative injury in alcohol-related liver disease.

Skeletal muscle mitochondrial dysfunction was implicated, using MR spectroscopy, in the development of age-related insulin resistance [216]. The authors further speculated that mitochondrial injury could play a role in the development of islet cell failure associated with progression from silent hyperinsulinemia to frank type 2 diabetes in obese patients. Dietary fatty acid composition has also been implicated in skeletal muscle insulin sensitivity [217]. The lipotoxicity hypothesis may explain some aspects of cellular toxicity in conditions of excess body fat. Central to this hypothesis is the potential role of ceramide, an acylated sphingosine (derived from palmitic acid) and intermediate substance in the synthesis of sphingomyelin, which can activate mitochondrial-stimulated apoptosis. Recent gene chip microarray assays examining the hepatic expression of mRNA in NASH [218] indicated that potentially toxic fatty acids and weak or senescent mitochondria, coupled with deficient mitochondrial antioxidant systems (superoxide dismutase and catalase) could explain the development of cellular failure in many different organs, especially those with high basal metabolic rates.

Current Therapy of NAFL: General Considerations

Other than perhaps exercise and diet, there is no universally agreed upon treatment, and therapeutic strategies have been largely empirical because the pathogenesis remains uncertain in many respects. A number of papers have been published reviewing current therapy. Below, we provide a summary of some of the salient points regarding therapy [219–223]. Because NAFL is usually associated with the comorbid conditions of type II diabetes, obesity, and/or hypertryglyceridemia, treatment of these conditions is often a consideration. Other important general considerations include the issue of alcohol prohibition versus moderation to levels less than 20 g/day. Data indicating a protective effect of modest ethanol ingestion in both diabetes and NAFL support a moderate position on this [224, 225]. A pragmatic recommendation is to tailor the recommendation to the histology, with abstinence if significant fibrosis has already developed. The use of medications that may promote steatohepatitis (e.g., glucocorticoids, amiodarone, methotrexate, or tamoxifen) also requires consideration and the weighing of the risks and benefits of liver injury. Unfortunately, there are often no clear guidelines when making these recommendations. We frequently suggest the use of vitamin E, ursodiol, or ubiquinone in this setting, although data are

also lacking. Another increasingly common but little-explored issue is workplace exposure to solvents [226–228]. A part of treatment may necessitate measures to reduce or eliminate exposure, especially if fibrosis is evident on biopsy. Finally, if cirrhosis is proven or suspected in a NAFL patient, a review of medications with potential adverse effects in the cirrhotic patient (e.g., aspirin, other nonsteroidal anti-inflammatory drugs, and angiotensin-converting enzyme inhibitors) is warranted.

Exercise, Diet, and Weight Loss

Exercise Alone

Exercise and diet form a cornerstone of therapy [229]. Although it is difficult to clearly separate exercise from weight loss, the concept of the 'fit fat' individual (i.e., relatively well conditioned but obese) suggests a benefit to exercise in the absence of weight loss [230]. Further studies examining the lactate threshold and exercise oxygen consumption are needed to assess the role of 'conditioning' exercise.

Exercise, Diet, and Weight Loss

There are a number of small reports of combined diet and exercise therapies in both adults and children. These typically reveal improved biochemical parameters, but variable changes in histology [231–243]. Some of these diets included unrealistic and severe calorie restriction. It is also important to note that histological exacerbation has been observed with rapid weight loss [244]. This is thought to result from an increase in the flow of free fatty acids to the liver from the mesenteric fat stores, resulting in increased lipid peroxidation. Protein malnutrition with impaired export of apoprotein fat from the liver and micronutrient deficiency could also play a role. Recently, a more practical 15-month lifestyle modification plan, including dietary changes and exercise in 31 patients showed success in loss of weight in 68% [245]. Among these patients, the authors noted a significant relationship between decreased ALT and the degree of weight loss and reduction in fibrosis in 7 of 14 undergoing repeat biopsy.

Exercise alters substrate utilization in skeletal muscle and insulin sensitivity, although obese individuals may be resistant to these changes [246, 247], and only about one-third of patients achieve target levels of exercise [248–250]. Higher intensity exercise regimens are probably more effective in producing significant metabolic changes [251]. Intermittent exercise also has been found to be as effective as daily exercise in a randomized controlled study involving obese women [252]. Both aerobic and resistance exercise have been shown to have some benefit in reducing weight [253]. Another novel metabolic approach is the use of hyperthermia, which has been noted to alter hepatic steatosis in patients undergoing experimental treatment for hepatitis C [254]. A contrasting approach is that of cold exposure, popularly known as 'kniepering'. A physiologic basis for this approach may lie in experimental work showing altered thermogenesis in cold-exposed animals [255, 256].

Popular Diets

The effects on the fatty liver of many popular diets, such as the Zone or Atkins diet [257, 258], are not known, although a recent abstract suggested that reduced carbohydrate intake may be of benefit [259]. The metabolic changes induced by the low carbohydrate-high protein diet warrant some additional concern and perhaps precaution, as the fatty liver is prone to increased production of ketone bodies. A pragmatic approach is to recommend a reduced calorie, balanced diet such as that endorsed by the National Heart, Lung, and Blood Institute (NHLBI) and National Institute of Diabetes, Digestive and Kidney Disease [NIDDK] [260], the American Heart Association [261], the American Diabetes Association [262], or the low glycemic index diet which emphasizes dietary composition [263].

Dietary Supplements

The effects on the liver of dietary supplements have been well investigated. Increased polyunsaturated fatty acids (PUFA; fish, and flax seed oils) alter insulin sensitivity and prostaglandin metabolism, may increase UCP (uncoupling protein) expression, and may promote lipid peroxidation, but the net effect in steatohepatitis is not known [264–272]. Dietary fructans, but not cellulose, decreased triglyceride accumulation in the liver of obese Zucker rats [273], and, likewise, warrant further study. Appetite-suppressing agents have lost favor due to their side-effects [274, 275]. However, a potential role for orlistat (tetrahydrolipostatin), a lipase inhibitor, was noted in a study of ten patients with proven NASH who underwent 6 months of orlistat therapy. Normalization of transaminases in six patients, weight reduction, and mild but potentially significant improvement in steatosis and fibrosis were seen [276]. Malabsorption of vitamin E is a relevant concern with fat-malabsorbing agents. Phentermine and sibutramine, possibly effective in achieving weight loss, are of uncertain benefit in NAFL [277]. In light of the concept of the liver as a normal fat-storing organ (see above), agents that produce lipoatrophy may be of particular safety concern.

Surgical Weight Loss

Increasingly, patients who fail diet and exercise-based regimens are turning to surgical therapy. Several studies have reported beneficial effects of bariatric surgery [278–282]. Proximal gastric bypass has now largely replaced vertical-banded gastroplasty as well as the older jejunoileal bypass. Several of these studies have reported benefits in both hepatic histology and general parameters of the metabolic syndrome, and a recent report of post-transplantation NASH noted normalization of liver enzymes, lipids, and glucose, with histological regression of steatosis after weight loss from a Roux-en-Y gastric bypass [283]. Precipitous weight loss, however, has the potential to severely exacerbate steatohepatitis. Preoperative treatment with cytoprotective or anti-oxidant agents has not been tested but is sometimes recommended. Prior studies in the now discarded intestinal bypass procedure further support a role for antibiotics and amino-acid supplementation for patients who experience decompensation [284, 285].

Cytoprotective and Antioxidant Agents

Ursodeoxycholic acid (ursodiol or UDCA) has been studied extensively in other liver diseases and has shown promise in preliminary studies of its use in NAFLD [286–290]. Laurin et al. [291] compared UDCA to clofibrate in 40 patients over a year of therapy. They showed improvement of the liver enzymes as well as histological grade of steatosis in the UDCA group, but no change in fibrosis. The results of a randomized, multicenter trial (K.D. Lindor et al. [292]) are soon to be available. Preliminary results suggest that UDCA alone will not be sufficient to cause regression of NASH, although, based on its safety and the results of earlier studies, further investigation is warranted to assess its role as a co-factor with other agents such as vitamin E.

Early reports have shown that taurine, given to rats with ethanol-induced hepatic steatosis, normalized liver enzymes and improved steatosis [293–296]. The protective effects of taurine were attributed to the potential of bile acids, especially taurine-conjugated bile acids (taurocholic acid) to inhibit the activity of some microsomal enzymes (CYP2E1). A small study of ten children with fatty liver noted improved ALT and steatosis measured on CT [297]. Triacetyl uridine is a potentially important cytoprotective agent in experimental choline-deficient rats, but has not been tested in humans [298]. Lecithin increases plasma free choline and decreases hepatic steatosis in patients on long-term total parenteral nutrition, and other patients [299, 300].

Vitamin E has had positive effects in both experimental and human fatty liver [301–303]. Its excellent safety profile favors its use in NAFL, although caution is warranted in patients with prior coronary artery disease, in whom vitamin E is associated with blunted efficacy of statin drugs [304]. Less impressive results were seen in one study of combination antioxidants [305]. However, a recent controlled trial compared vitamin E, given for 1 year in 22 patients with various degrees of NAFL. Therapy was associated with reduction of ALT, modest improvement of steatosis and inflammation, and a significant reduction in the level of plasma transforming growth factor-β1, which has been implicated in the development of hepatic fibrosis [306]. Another recent randomized, placebo-controlled, double-blind study of vitamin E (1000 U) and vitamin C (1000 mg) demonstrated a significant reduction in fibrosis in the treated group [307].

Agents that potentiate the transmethylation pathways and augment the synthesis of glutathione (GSH) have undergone study. In animal studies with alcoholic-induced hepatic steatosis, S-adenosyl-methionine (SAM) replenished mitochondrial reduced GSH [308–310]. It was also shown that SAM significantly increased cytoslic and mitochondrial GSH in ischemic-reperfused steatotic rats [311–313]. Human studies with SAM in chronic liver disease have also been encouraging [314–316]. Betaine, a naturally occurring metabolite of choline, and a methyl donor in an alternative pathway for re-methylation of homocysteine to methionine, was studied in a small trial involving 10 NASH patients for 1 year [317, 318]. Transaminase normalized in 3, decreased in 3, and was unchanged in 1 of the 7 patients who completed the study. Improvement in the degree of steatosis and in necroinflammatory grade was noted. Miglio et al. [319] studied 191 patients with NAFL treated with an oral form of betaine glucuronate combined with diethanolamine glucuronate and nicotinamide ascorbate. They

noted improvement in hepatic steatosis, hepatomegaly, and aminotransferases. *N*-Acetylcysteine (NAC) has been studied in animal models with hepatic steatosis [320, 321]. A small human study of 11 NASH patients, managed initially with diet regulation followed by NAC therapy, showed improvement in aminotransferases [322].

Other miscellaneous agents with purported or suspected antioxidant effects include silymarin [323]; this is a milk thistle derivative commonly used by patients, although we are not aware of published studies of its use in NAFL. Others in this category include histamine, which possesses indirect antioxidant properties [324], and the 21-aminosteroids, a group of substances known as lazaroids, which may warrant pilot work [325]. Probiotics have shown promise in an animal model, in which Li et al. [326] showed that treatment with VSL#3 (a probiotic preparation) reduced hepatic total fatty content, and decreased serum ALT levels. The authors conjectured that these results supported the concept that intestinal bacteria induce signals that play a role in hepatic insulin resistance and NAFLD [326]. Less is known about the potential effects of selenium, β-carotene, ubiquinone (Co-Q), ethanolamines, niacinamide, glucosamine, chondroitin, vitamin B complexes, vitamin C, and lipoic acid, all agents with purported antioxidant effects.

Anti-Diabetic and Insulin-Sensitizing Agents

All of these agents represent a step up in the level of therapy above the mild side-effect profiles of the cytoprotective or antioxidant agents and thus will likely be restricted to patients with type 3–4 NAFL. Insulin, sometimes recommended early in the course of type II diabetes [327], and sulfonylureas have not been adequately addressed for their effects on the liver in patients with NAFL.

The newer class of agents known as thiazolidinediones (TZD) have been studied to some extent, and early work shows their promise as therapeutic agents in NAFL [328–334]. These agents stimulate nuclear transcription factors [335], alter skeletal muscle glucose uptake (through increased GLUT4 activity) [336], decrease central adiposity [337], promote adipocyte differentiation, alter mitochondrial mass [338], and alter thermogenesis [339]. The efficacy of troglitazone in lipodystrophy suggests a primary effect on lipid metabolism and basal metabolism, since the respiratory quotient (RQ) is altered [340]. We previously demonstrated a reduction in inflammatory and fatty infiltration after a short course (3–6 months) of troglitazone, with no change in apoptosis markers. This agent has been withdrawn due to rare but potentially fatal idiosyncratic hepatotoxicity. However, pioglitazone and rosiglitazone, in two controlled studies, by Sanyal [341] and Tetri [342], have also shown reduction in inflammation and steatosis, as well as fibrosis. The effect on adipocyte differentiation probably explains the shift in adiposity from central solid-organ deposition (including liver) to peripheral fat stores in all human studies to date. Although hepatic effects subside after therapy is stopped, the effect on peripheral fat stores appears to be a lasting effect, as weight gain during therapy appears to persist after discontinuation of the medication [343]. This is of obvious concern and will limit the overall utility of these agents.

Metformin has undergone limited but promising studies in experimental and human NAFL [344–347]. It appears to divert fatty acids from triglyceride production

to mitochondrial beta oxidation [348]. Data regarding histological response in human NAFL is very limited, but one report, by Ahmet Uygen et al. (personal communication) has shown histological improvement in a necroinflammatory index, although details of this report are not yet available. Other candidate agents include acarbose [an α-glucosidase inhibitor) [349], acipimox (inhibits lipolysis) [350], and d-chiro-inositol [351].

Anti-Hyperlipidemic Agents

Fibrates altered lipoprotein activity through the PPAR-α receptor, but had no histological effects in an early report by Laurin et al. [352]. However, bezafibrate has since shown benefit in tamoxifen-associated steatohepatitis in one very small pilot study [353]. In addition, Basaranoglu et al. [354] showed improvement in liver enzymes in a study of gemfibrozil, but histology was not measured. The hydroxymethylglutaryl-Co-enzyme A (HMG-CoA) reductase inhibitors, commonly known as statins, are becoming increasingly popular agents for the treatment of hypercholesterolemia, but their risk-benefit profile in fatty liver remains ill-defined [355]. A pilot study has shown improvement in biochemical and histological parameters in a small sample of patients treated with atorvastatin, and another recent report showed no significant histologic differences between controls and patients using various statin drugs [356, 357]. However, a report of these drugs, subclinical skeletal muscle toxicity, characterized by the formation of ragged red fibers and mediated by mitochondrial injury [358], is justifiable cause for concern for their longterm use in NAFL and raises concern for silent liver injury. The potential injurious effects of 'statin' drugs is emphasized by the potential role of apoprotein metabolism in NAFL [359, 360]. Other lipid-lowering agents such as colesevelam or other resin-binding agents have not been investigated. Dietary changes, such as supplementation with polyunsaturated fats, are discussed above.

Treatment of Secondary Causes of Hepatic Steatosis

Abnormal iron indices without frank hemochromatosis have been reported (see above). Iron deposition could be detrimental in NAFL due to the pro-oxidant effects of iron. One study has shown improvement in liver enzymes and insulin sensitivity in a group of *HFE* gene-negative patients treated with serial phlebotomy for iron reduction [361]. Whether this form of therapy has general applicability or is better reserved for patients with evidence of iron overload remains to be seen. Hepatitis C has been associated with steatosis, especially in patients with genotype 3a [362–367]. Recent studies have shown improvement of steatosis in chronic hepatitis C patients after successful antiviral therapy [368–370]. On the other hand, Hickman et al. [371] showed improvement of liver histology in chronic hepatitis C patients after weight reduction alone. This remains to be resolved. A secondary form of NASH may be seen in steroid-treated patients—it can be confused with persistent autoimmune hepatitis. This has resolved after the withdrawal of the medication. Other secondary forms of NAFL, such as that associated with tamoxifen, may be ameliorated by the addition of anti-oxidants or cytoprotective agents, as noted above, but this remains conjectural.

Liver Transplantation

NAFL patients who progress to cirrhosis are often poor candidates for transplantation, due to comorbid conditions. However, in the United States, firm guidelines do not exist to exclude these patients from transplantation, although it is known that the results of transplantation in obese patients are not as good as those in relatively lean patients [372]. Both the *recurrence* of NASH, in patients with previously established NASH leading to cirrhosis [373–376], and the *occurrence* of NASH, following transplantation for cryptogenic cirrhosis [377, 378], have been reported. Progression to steatohepatitis and subsequently to cirrhosis in the graft may be seen, although predictive factors and treatment have not been well defined. Immunosuppression could play a role, due to the promotion of fatty liver and diabetes with corticosteroid use, and could have more direct effects, such as the effect of cyclosporine on the mitochondrion [379].

References

1. Matteoni CA, Younossi ZM, Gramlich T, Boparai N, Liu YC, McCullough AJ (1999) Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. Gastroenterology 116: 1413–1419
2. Cotrim HP, De Freitas LA, Freitas C, Braga L, Sousa R, Carvalho F, Parana R, Santos-Jesus R, Andrade NASH in workers exposed to chemicals with or without associated metabolic conditions. Liver Int 2004
3. Morgan W (1877) The Liver and its Diseases, Both Functional and Organic. Their History, Anatomy, Chemistry, Pathology, Physiology, and Treatment. Homoeopathic, London, University of Virginia Historical Collection, p 144
4. Tumen HJ, Cohn EM (1946) Cirrhosis. In: Bockus HL (ed) Gastroenterology, vol. III. Saunders, Philadelphia, pp 385–392
5. Zelman S (1958) The liver in obesity. Arch Intern Med 90: 141–156
6. Faloon WW (1988) Hepatobiliary effects of obesity and weight-reducing surgery. Semin Liver Dis 8: 229–236
7. Ludwig J, Viggiano TR, McGill DB, Ott BJ (1980) Nonalcoholic steatohepatitis. Mayo Clin Proc 55: 434–438
8. Sanyal AJ (2002) AGA technical review on nonalcoholic fatty liver disease. Gastroenterology 123: 1705–1725
9. Cairns SR, Peters TJ (1983) Biochemical analysis of hepatic lipid in alcoholic and diabetic and control subjects. Clin Sci 65: 645–652
10. James OFW (2002) NASH/NAFLD Management. AASLD Single Topic Conference on NASH Syllabus, Atlanta, Georgia
11. Bellentani S, Saccoccio G, Masutti F, Croce LS, Brandi G, Sasso F, Cristanini G, Tiribelli C (2000) Prevalence of and risk factors for hepatic steatosis in Northern Italy. Ann Intern Med 132: 112–117
12. Clark JM, Brancati FL, Diehl AM (2001) Nonalcoholic fatty liver disease: the most common cause of abnormal liver enzymes in the US population (abstract). Gastroenterology 120: 65
13. Nomura H, Kashiwagi S, Hayashai J, Kujiyama W, Tani S, Goto M (1988) Prevalence of fatty liver in a general population of Okinawa, Japan. Jpn J Med 27: 142–149
14. Siegelman ES, Rosen MA (2001) Imaging of steatosis. Semin Liver Dis 21: 71–80
15. Van der Kooy K, Seidell JC (1993) Techniques for the measurements of visceral fat: a practical guide. Int J Obes 17: 187–196

16. Saadeh S, Younossi ZM, Remer EM, Gramlish T, Ong JP, Hurley M, Mullen KD, Cooper JN, Sheridan MJ (2002) The utility of radiological imaging in nonalcoholic fatty liver disease. Gastroenterology 123: 745–750
17. Celle G, Savarino V, Picciotto A, Magnolia MR, Scalabrini P, Dodero M (1988) Is hepatic ultrasonography a valid alternative tool to liver biopsy? Report on 507 cases studied with both techniques. Dig Dis Sci 33: 467–471
18. Quinn SF, Gosink BB (1985) Characteristic sonographic signs of hepatic fatty infiltration. AJR Am J Roentgenol 145: 753–755
19. Jacobs JE, Birnbaum BA, Shapiro MA, Langlotz CP, Slosman F, Rubesin SE, Horii SC (1998) Diagnostic criteria for fatty infiltration of the liver on contrast enhanced helical CT. AJR Am J Roentgenol 171: 659–664
20. Levenson H, Greensite F, Hoefs J, Friloux L, Applegate G, Silva E, Kanel G, Buxton R (1991) Fatty infiltration of the liver: quantification with phase-contrast MR imaging at 1.5 T vs biopsy. AJR Am J Roentgenol 156: 307–312
21. Cortez-Pinto H, Chatham J, Chacko VP, Arnold C, Rashid A, Diehl AM (1999) Alterations in liver ATP homeostasis in human nonalcoholic steatohepatitis: a pilot study. JAMA 282: 1659–1664
22. Hilden M, Juhl E, Thomsen AC, Christoffersen P (1973) Fatty liver persisting for up to 33 years. Acta Med Scand 194: 485–489
23. Teli MR, James OFW, Burt AD, Bennett MK, Day CP (1995) The natural history of nonalcoholic fatty liver: a followup study. Hepatology 22: 1714–1719
24. Harrison SA, Torgerson S, Hayashi PH (2003) The natural history of nonalcoholic fatty liver disease: a clinical histopathological study. Am J Gastroenterol 98: 2042–2047
25. Powell EE, Cooksley WG, Hanson R, Searll J, Halliday JW, Powell LW (1990) The natural history of nonalcoholic steatohepatitis: a follow-up study of 42 patients for up to 21 years. Hepatology 11: 74–80
26. Caldwell SH, Oelsner DH, Iezzoni JC, Hespenheide EE, Battle EH, Driscoll CJ (1999) Cryptogenic cirrhosis: clinical characterization and risk factors for underlying disease. Hepatology 29: 664–669
27. Poonawala A, Nair SP, Thuluvath PJ (2000) Prevalence of obesity and diabetes in patients with cryptogenic cirrhosis: a case-control study. Hepatology 32: 689–692
28. Mendiola AE, Gish RG (2001) Risk factors for NASH in patients with cryptogenic cirrhosis (abstract). Gastroenterology 120: 545
29. Contos MJ, Cales W, Sterling RK, Lukeric VA, Shiffman ML, Mills AS, Fisher RA, Ham J, Sanyal AJ (2001) Development of nonalcoholic fatty liver disease after orthotopic liver transplantation for cryptogenic cirrhosis. Liver Transpl 7: 363–373
30. Ayata G, Gordon FD, Lewis WD, Pomfret E, Pomposelli JJ, Jenkins RL, Khettry U (2002) Cryptogenic cirrhosis: clinicopathologic findings at and after liver transplantation. Hum Pathol 33: 1098–1104
31. Basaranoglu M, Ozbay G, Caldwell SH, Sonsuz A (2003) Refinement of a clinicopathological typing system for NAFLD (abstract). Hepatology 34: 505A
32. Brunt EM, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR (1999) Nonalcoholic steatohepatitis: a proposal for grading and staging the histologic lesions. Am J Gastroenterol 94: 2467–2474
33. Mendler MH, Yashar B, Govindarajan S, Kanel G (2002) A novel semi-quantitative histological scoring system for non-alcoholic fatty liver disease: evaluation and clinical correlations (abstract). Hepatology 36: 407
34. Navarro VJ, St Louis T, Bell BZ, Sofair AN (2003) Chronic liver disease in the primary care practices of Waterbury, Connecticut. Hepatology 38: 1062–1063
35. Marchesini G, Bugianesi E, Forlani G, Cerrelli F, Lenzi M, Manini R, Natale S, Vanni E, Villanova N, Melchionda N, Rizzetto M (2003) Nonalcoholic fatty liver steatohepatitis, and the metabolic syndrome. Hepatology 37: 917–923
36. Neuschwander-Tetri BA, Caldwell SH (2003) Nonalcoholic steatohepatitis: summary of an AASLD single topic conference. Hepatology 37: 1202–1219

37. Assy N, Kaita K, Mymin D, Levy C, Rosser B, Minuk G (2000) Fatty infiltration of liver in hyperlipidemic patients. Dig Dis Sciences 45: 1929–1934
38. Rashid M, Roberts EA (2000) Nonalcoholic steatohepatitis in children. J Pediatr Gastroenterol Nutr 30: 48–53
39. Struben VMD, Hespenheide EE, Caldwell SH (2000) Familial patterns of non-alcoholic steatohepatitis and cryptogenic cirrhosis. Am J Med 108: 9–13
40. Willner IR, Waters B, Patil SR, Reuben A, Morelli J, Riely CA (2001) Ninety patients with nonalcoholic steatohepatitis: insulin resistance, familial tendency and severity of disease. Am J Gastroenterol 96: 2957–2961
41. Cortez-Pinto H, Camilo ME, Baptista A, de Oliveira AG, De Moura MC (1999) Non-alcoholic fatty liver: another feature of the metabolic syndrome. Clin Nutr 18: 353–358
42. Knobler H, Schatter A, Zhornicki T, Malnick SD, Keter D, Sokolovsaya N, Lurie Y, Bass DD (19999) Fatty liver—an additional and treatable feature of the insulin resistance syndrome. QJM 92: 73–79
43. Ikai E, Ishizaki M, Suzuki Y, Ishida M, Noborizaka Y, Yamada Y (1995) Association between hepatic steatosis, insulin resistance, and hyperinsulinemia as related to hypertension in alcohol consumers and obese people. J Hum Hypertens 9: 101–105
44. Lobo RA, Carmino E (2000) The importance of diagnosing the polycystic ovary syndrome. Ann Intern Med 132: 989–993
45. Kuczmarski RJ, Carroll MD, Flegal KM, Troiano RP (1997) Varying body mass index cutoff points to describe overweight prevalence among US adults: NHANES III (1988 to 1994). Obesity Res 5: 542–548
46. NHLBI Obesity Task Force (1998) Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults—the evidence report. Obesity Res 6(Suppl 2): 51S–209S
47. Lee JH, Rhee PL, Lee JK, Lee KT, Kim JJ, Koh KC, Paik SW, Rhee JC, Choi KW (1998) Role of hyperinsulinemia and glucose intolerance in the pathogenesis of nonalcoholic fatty liver in patients with normal body weight. Korean J Intern Med 13: 12–14
48. Banerji MA, Faridi N, Atluri R, Chaiken RL, Lebovitz HE (1999) Body composition, visceral fat, leptin, and insulin resistance in Asian Indian men. J Clin Endocrinol Metab 84: 137–144
49. Pagano G, Pacini G, Musso G, Gambino R, Mecca F, Depetris N, Cassader M, David E, Cavallo-Perin P, Rizzetto M (2002) Nonalcoholic steatohepatitis, insulin resistance and metabolic syndrome: further evidence for an etiologic association. Hepatology 35: 367–372
50. Chitturi S, Abeygunasekera S, Farrell GC, Holmes-Walker J, Hui JM, Fung C, Karim R, Lin R, Samarasinghe D, Liddle C, Weltman M, George J (2002) NASH and insulin resistance: insulin hypersecretion and specific association with the insulin resistance syndrome. Hepatology 35: 373–379
51. Pouliot MC, Despres JP, Lemieux S, Moorjani S, Bouchard C, Tremblay A, Nadeau A, Lupien PJ (1994) Waist circumference and abdominal sagittal diameter: best simple anthropometric indices of abdominal visceral adipose tissue accumulation and related cardiovascular risk in men and women. Am J Cardiol 73: 460–468
52. Ong JP, Hazem E, Younoszai A, Goodman Z, Grant G, Christensen A, Chandhoke V, Cooper J, Bopari N, Younossi ZM (2002) Predictors of non-alcoholic steatohepatitis and fibrosis in non-alcoholic fatty liver disease (abstract). Hepatology 36: 407
53. Kuczmarski RJ (1989) Need for body composition information in elderly subjects. Am J Clin Nutr 50: 1150–1157
54. Andersen T, Gluud C (1984) Liver morphology in morbid obesity: a literature study. Int J Obes 8: 97–106
55. Andersen T, Christoffersen P, Gluud C (1984) The liver in consecutive patients with morbid obesity: a clinical, morphological and biochemical study. Int J Obes 8: 107–115

56. Ratziu V, Giral P, Charlotte F, Bruckert E, Thibault V, Theodorou I, Khalil L, Turpin G, Opolon P, Poynard T (2000) Liver fibrosis in overweight patients. Gastroenterology 118: 1117–1123
57. Garcia-Monzon C, Martin-Perez E, Iacono OL, Fernandez-Bermejo M, Majano PL, Apolinario A, Larranaga E, Moreno-Otero R (2000) Characterization of pathogenic and prognostic factors of nonalcoholic steatohepatitis associated with obesity. J Hepatol 33: 716–724
58. Braillon A, Capron JP, Herve MA, Degott C, Quenum C (1985) Liver in obesity. Gut 26: 133–139
59. Marchesini G, Brizi M, Morselli-Labate AM, Bianchi G, Bugianesi E, McCullough AJ, Forlani G, Melchionda N (1999) Association of nonalcoholic fatty liver disease with insulin resistance. Am J Med 107: 450–455
60. Assy N, Kaita K, Mymin D, Levy C, Rosser B, Minuk G (2000) Fatty infiltration of liver in hyperlipidemic patients. Dig Dis Sci 45: 1929–1934
61. Caldwell SH, Swerdlow RH, Khan EM, Iezzoni JC, Hespenheide EE, Parks JK, Parker WD (1999) Mitochondrial abnormalities in non-alcoholic steatohepatitis. J Hepatol 31: 430–434
62. Sozo A, Arrese M, Glasinovic JC (2001) Evidence of intestinal bacterial overgrowth in patients with NASH (abstract). Gastroenterology 120: 118
63. Gamez J, Ferreiro BS, Accarino ML, Guarner L, Tadesse S, Marti RA, Andreu AL, Raguer N, Cervera C, Hirano M (2002) Phenotypic variation in a Spanish family with MNGIE. Neurology 59: 455–457
64. Powell EE, Searle J, Mortimer R (1989) Steatohepatitis associated with limb lipodystrophy. Gastroenterology 97: 1022–1024
65. Feliciani C, Amerio P (1999) Madelung's disease: inherited from an ancient Mediterranean population? N Engl J Med 340: 1481
66. Wasserman JM, Thung SN, Berman R, Bodenheimer HC, Sigal SH (2001) Hepatic Weber-Christian disease. Semin Liver Dis 21: 115–118
67. Dahl MG, Gregory MM, Scheuer PJ (1971) Liver damage due to methotrexate in patients with psoriasis. BMJ 1: 625–630
68. Pirovino M, Muller O, Zysset T, Honegger U (1988) Amiodarone-induced hepatic phospholipidosis: correlation of morphological and biochemical findings in an animal model. Hepatology 8: 591–598
69. Pinto HC, Baptista A, Camilo ME, de Costa EB, Valente A, de Moura MC (1995) Tamoxifen-associated steatohepatitis—report of three cases. J Hepatol 23: 95–97
70. Cotrim HP, Andrade ZA, Parana R, Portugal M, Lyra LG, Freitas LA (1999) Non-alcoholic steatohepatitis: a toxic liver disease in industrial workers. Liver 19: 263–264
71. Chitturi S, Farrell GC (2001) Etiopathogenesis of nonalcoholic steatohepatitis. Semin Liver Dis 21: 27–41
72. Caldwell SH, Hespenheide EE (2002) Subacute liver failure in obese females. Am J Gastroenterol 97: 2058–2062
73. Caldwell SH, Oelsner DH, Iezzoni JC, Hespenheide EE, Battle EH, Driscoll CJ (1999) Cryptogenic cirrhosis: clinical characterization and risk factors for underlying disease. Hepatology 29: 664–669
74. Ratziu V, Bonyhay L, Di Martino V, Charlotte F, Cavallaro L, Sayegh-Tainturier MH, Giral P, Grimaldi A, Opolon P, Poynard T (2002) Survival, liver failure, and hepatocellular carcinoma in obesity-related cryptogenic cirrhosis. Hepatology 35: 1485–1493
75. Garcia-Monzon C, Martin-Perez E, Iacono OL, Fernandez-Bermejo M, Majano PL, Apolinario A, Larranaga E, Moreno-Otero R (2000) Characterization of pathogenic and prognostic factors of nonalcoholic steatohepatitis associated with obesity. J Hepatol 33: 716–724

76. Mathieson UL, Franzen LE, Fryden A, Foberg U, Bodemar G (1999) The clinical significance of slightly to moderately elevated liver transaminase values in asymptomatic patients. Scand J Gastroenterol 34: 85–91
77. Patt CH, Yoo HY, Dibadj K, Flynn J, Thuluvath PJ (2003) Prevalence of transaminase abnormalities in asymptomatic, healthy subjects participating in an executive health-screening program. Digestive Diseases & Sciences 48(4): 797–801
78. Sorbi D, McGill DB, Thistle JL, Therneau TM, Henry J, Lindor KD (2000) An assessment of the role of liver biopsies in asymptomatic patients with chronic liver test abnormalities. Am J Gastroenterol 95: 3206–3210
79. Hay JE, Czaja AJ, Rakela J, Ludwig J (1989) The nature of unexplained chronic aminotransferase elevations of a mild to moderate degree in asymptomatic patients. Hepatology 9: 193–197
80. Daniel S, Ben-Menachem T, Vasudevan G, Ma CK, Blumenkehl M (1999) Prospective evaluation of unexplained chronic liver transaminase abnormalities in asymptomatic and symptomatic patients. Am J Gastroenterol 94: 3010–3014
81. Caldwell SH, Hespenheide EE, Redick JA, Iezzoni JC, Battle EH, Sheppard BL (2001) A pilot study of a thiazolidinedione, troglitazone, in nonalcoholic steatohepatitis. Am J Gastroenterol 96: 519–525
82. Mofrad P, Contos MJ, Haque M, Sargeant C, Fisher RA, Luketic VA, Sterling RK, Shiffman ML, Stravitz RT, Sanyal AJ (2003) Clinical and histologic spectrum of NAFLD associated with normal ALT values. Hepatology 37: 1286–1292
83. Prati D, Taioli E, Zanella A, Della Torre E, Butelli S, Del Vecchio E, Vianello L, Zanuso F, Mozzi F, Milani S, Conte D, Colombo M, Sirchia G (2002) Updated definitions of healthy ranges for serum alanine aminotransferase levels. Ann Intern Med 137: 1–10
84. Shan Y, Bigelow C, Bonkovsky HL (2002) Prevalence of liver disease in the US population and association with iron homeostasis: analysis of data from NHANES III (abstract). Hepatology 36: 408
85. Bonkovsky H, Jawaid Q, Tortorelli K, LeClair P, Cobb J, Lambrecht R, Banner B (1999) Non-alcoholic steatohepatitis and iron: increased prevalence of mutations of the HFE gene in non-alcoholic steatohepatitis. J Hepatol 31: 421–429
86. George D, Goldwurm S, MacDonald G, Cowley LL, Walker NI, Ward PJ, Jazwinska EC, Powell LW (1998) Increased hepatic iron concentration in nonalcoholic steatohepatitis is associated with increased fibrosis. Gastroenterology 114: 311–318
87. Angulo P, Keach JC, Batts KP, Lindor KD (1999) Independent predictors of liver fibrosis in patients with nonalcoholic steatohepatitis. Hepatology 30: 1356–1362
88. Younossi ZM, Gramlich T, Bacon BR, Matteoni CA, Boparai N, O'Neill R, McCullough AJ (1999) Hepatic iron and nonalcoholic fatty liver disease. Hepatology 30: 847–850
89. Tumiel M, Whitcomb BJ, Krawitt EL (1994) Circulating antinuclear antibodies in patients with nonalcoholic steatohepatitis (abstract). Hepatology 20: 409
90. Tajiri K, Takenawa H, Yamaoka K (1997) Nonalcoholic steatohepatitis masquerading as autoimmune hepatitis. J Clin Gastrroenterol 25: 538–540
91. Nagore N, Scheuer PJ (1988) Does a linear pattern of sinusoidal IgA deposition distinguish between alcoholic and diabetic liver disease? Liver 8: 281–286
92. Caldwell SH, Hespenheide EE (2001) Obesity and cryptogenic cirrhosis. In: Leuschner U, James O, Dancygier H (eds) Steatohepatitis (ASH and NASH). Falk Symposium 121. Kluwer Academic, Norwell, MA, p 151
93. Kanjii K, Jakate S, Keshavarzian A, Jensen DM, Cotler SJ (2003) Prevalence and clinical features of associated autoantibodies in NASH. Hepatology 34: 506A
94. Rosado-Carrion B, Lindor K, Talwalker J, Miles J, Rizza R, Gorman B, Charlton M (2003) Continuous glucose monitoring and metabolic evidence of subclinical glucose intolerance in NAFLD: looking beyond the tip of the insulin resistance iceberg. Hepatology 34: 508A
95. Adams LA, Keach J, Lindor KD, Angulo P (2003) Lipid profiles in NAFLD. Hepatology 34: 512A

96. Angulo P, Keach JC, Batts KP, Lindor KD (1999) Independent predictors of liver fibrosis in patients with nonalcoholic steatohepatitis. Hepatology 30: 1356–1362
97. Nanji AA, French SW, Freeman JB (1986) Serum alanine aminotransferase to aspartate aminotransferase ratio and degree of fatty liver in morbidly obese patients. Enzyme 36: 266–269
98. Sorbi D, McGill DB, Thistle JL, Therneau TM, Henry J, Lindor KD (2000) An assessment of the role of liver biopsies in asymptomatic patients with chronic liver test abnormalities. Am J Gastroenterol 95: 3206–3210
99. Garcia-Monzon C, Martin-Perez E, Iacono OL, Fernandez-Bermejo M, Majano PL, Apolinario A, Larranaga E, Moreno-Otero R (2000) Characterization of pathogenic and prognostic factors of nonalcoholic steatohepatitis associated with obesity. J Hepatol 33: 716–724
100. Struben VMD, Hespenheide EE, Caldwell SH (2000) Familial patterns of nonalcoholic steatohepatitis and cryptogenic cirrhosis. Am J Med 108: 9–13
101. Dixon JB, Bathal PS, O'Brien PE (2001) Nonalcoholic fatty liver disease: predictors of nonalcoholic steatohepatitis and liver fibrosis in the severely obese. Gastroenterology 121: 91–100
102. Harrison SA, Hayashi P (2002) Clinical factors associated with fibrosis in 102 patients with non-alcoholic steatohepatitis (abstract). Hepatology 36: 412
103. Costanzo M, Miele L, Di Rocco P, Forgione A, Alfei B, Pompili M, Rapacini GL, Grieco A, Gasbarrini G (2002) Serum markers of fibrogenesis and fibrosis in nonalcoholic steatohepatitis (abstract). Hepatology 36: 412
104. Iijima H, Moriyasu F, Tsuchiya K, Sasaki S, Suzuki S (2003) Diagnosis of NASH using Kupffer imaging of contrast enhanced ultrasound. Hepatology 34: 514A
105. Ratziu V, Le Calvez S, Imber-Bismut F, Messous D, Charlotte F, Bonyhay L, Munteanu M, Poynard T (2003) Diagnostic value of biochemical markers (Fibrotest) for the prediction of liver fibrosis in patients with NAFLD. Hepatology 34: 510A
106. Merriman RB, Ferrell LD, Patti MG, Ostroff JW, Bagetelos K, Aouizerat BE, Bass NB (2003) Histologic correlation of paired right and left lobe biopsies in morbidly obese individuals with suspected NAFLD. Hepatology 34: 232A
107. Ewe K (1981) Bleeding after liver biopsy does not correlate with indices of peripheral coagulation. Dig Dis Sci 26: 388–393
108. Piccinino F, Sagnelli E, Pasquale G, Giusti G (1986) Complication following percutaneous liver biopsy. A multicenter retrospective study on 68 276 biopsies. J Hepatol 2: 165–173
109. Caldwell SH (2001) Controlling pain in liver biopsy. Am J Gastroenterol 96: 1327–1329
110. Janes CH, Lindor KD (1993) Outcome of patients hospitalized for complications after outpatient liver biopsy. Ann Intern Med 118: 96–98
111. Byron D, Minuk GY (1996) Profile of an urban hospital-based practice. Hepatology 24: 813–815
112. Mathieson UL, Franzen LE, Fryden A, Foberg U, Bodemar G (1999) The clinical significance of slightly to moderately elevated liver transaminase values in asymptomatic patients. Scand J Gastroenterol 34: 85–91
113. Matteoni CA, Younossi ZM, Gramlich T, Boparai N, Liu YC, McCullough AJ (1999) Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. Gastroenterology 116: 1413–1419
114. Saadeh S, Younossi ZM, Remer EM, Gramlish T, Ong JP, Hurley M, Mullen KD, Cooper JN, Sheridan MJ (2002) The utility of radiological imaging in nonalcoholic fatty liver disease. Gastroenterology 123: 745–750
115. Hilden M, Juhl E, Thomsen AC, Christoffersen P (1973) Fatty liver persisting for up to 33 years. Acta Med Scand 194: 485–489
116. Teli MR, James OFW, Burt AD, Bennett MK, Day CP (1995) The natural history of nonalcoholic fatty liver: a followup study. Hepatology 22: 1714–1719

117. Lee RG (1989) Nonalcoholic steatohepatitis: a study of 49 patients. Hum Pathol 20: 594–598
118. Powell EE, Cooksley WG, Hanson R, Searll J, Halliday JW, Powell LW (1990) The natural history of nonalcoholic steatohepatitis: a follow-up study of 42 patients for up to 21 years. Hepatology 11: 74–80
119. Bacon BR, Farahvish MJ, Janney CG, Neuschwander-Tetri BA (1994) Non-alcoholic steatohepatitis: an expanded clinical entity. Gastroenterology 107: 1103–1109
120. Ratziu V, Giral P, Charlotte F, Bruckert E, Thibault V, Theodorou I et al (2000) Liver fibrosis in overweight patients. Gastroenterology 118: 1117–1123
121. Harrison SA, Torgerson S, Hayashi PH (2003) The natural history of NAFLD: a clinical histopathological study. Am J Gastroenterol 98: 2042–2047
122. Gaede P, Vedel P, Larsen N, Jenesn GVH, Parving H-H, Pendersen O (2003) Multifactorial intervention and cardiovascular disease in patients with type 2 diabetes. N Engl J Med 348: 383–393
123. Ioannou GN, Weiss NS, Kowdley KV, Dominitz JA (2003) Is obesity a risk factor for cirrhosis-related death or hospitalization? A population based cohort study. Gastroenterology 125: 1053–1059
124. Sasaki A, Horiuchi N, Hasegawa K, Uehara M (1989) Mortality and causes of death in type 2 diabetic patients. Diabetes Res Clin Pract 7: 33–40
125. Caldwell SH, Han K, Hess CE (1997) Thrombocytopenia and unrecognized cirrhosis. Ann Intern Med 127: 572–573
126. Sozo A, Arrese M, Glasinovic JC (2001) Evidence of intestinal bacterial overgrowth in patients with NASH. Gastroenterology 120: A-118
127. Caldwell SH, Swerdlow RH, Khan EM, Iezzoni JC, Hespenheide EE, Parks JK, Parker WD (1999) Mitochondrial abnormalities in non-alcoholic steatohepatitis. J Hepatol 31: 430–434
128. Sanyal AJ, Campbell-Sargent C, Mirshahi F, Rizzo WB, Contos MJ, Sterling RK, Luketic VA, Shiffman ML, Clore JN (2001) Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities. Gastroenterology 120: 1183–1192
129. Johns DR (1995) Mitochondrial DNA and disease. N Engl J Med 333: 638–644
130. Al-Osaimi A, Berg CL, Caldwell SH (2002) Intermittent disconjugate gaze: a novel finding in NASH and cryptogenic cirrhosis (abstract). Hepatology 36: 408A
131. Sanyal AJ, Contos MJ, Sterling RK, Luketic VA, Shiffman ML, Stravitz RT, Mills AS (2003) Nonalcoholic fatty liver disease in patients with hepatitis C is associated with features of the metabolic syndrome. Am J Gastroenterol 98: 2064–2071
132. Ayata G, Gordon FD, Lewis WD, Pomfret E, Pomposelli JJ, Jenkins RL, Khettry U (2002) Cryptogenic cirrhosis: clinicopathologic findings at and after liver transplantation. Hum Pathol 33: 1098–1104
133. Powell EE, Cooksley WG, Hanson R, Searll J, Halliday JW, Powell LW (1990) The natural history of nonalcoholic steatohepatitis: a follow-up study of 42 patients for up to 21 years. Hepatology 11: 74–80
134. Nosadini R, Avogaro A, Mollo F, Marescotti C, Tiengo A, Duner E, Merkel C, Gatta A, Zuin R, de Kreutzenberg S et al (1984) Carbohydrate and lipid metabolism in cirrhosis. Evidence that hepatic uptake of gluconeogenic precursors and of free fatty acids depends on effective hepatic flow. J Clin Endocrinol Metab 58: 1125–1132
135. Contos MJ, Cales W, Sterling RK, Lukeric VA, Shiffman ML, Mills AS, Fisher RA, Ham J, Sanyal AJ (2001) Development of nonalcoholic fatty liver disease after orthotopic liver transplantation for cryptogenic cirrhosis. Liver Transpl 7: 363–373
136. Struben VMD, Hespenheide EE, Caldwell SH (2000) Familial patterns of nonalcoholic steatohepatitis (NASH) and cryptogenic cirrhosis. Am J Med 108: 9–13
137. Willner IR, Waters B, Patil SR, Reuben A, Morelli J, Riely CA (2001) Ninety patients with nonalcoholic steatohepatitis: insulin resistance, familial tendency and severity of disease. American Journal of Gastroenterology 96(10): 2957–2961

138. Nagore N, Scheuer PJ (1988) Does a linear pattern of sinusoidal IgA deposition distinguish between alcoholic and diabetic liver disease. Liver 8: 281–286
139. Poonawala A, Nair SP, Thuluvath PJ (2000) Prevalence of obesity and diabetes in patients with cryptogenic cirrhosis: a case-control study. Hepatology 32: 689–692
140. Ong J, Younossi ZM, Reddy V, Price LL, Gramlich T, Mayes J, Boparis N (2001) Cryptogenic cirrhosis and post-transplantation non-alcoholic fatty liver disease. Liver Transplantation 7: 797–801
141. Nair S, Mason A, Eason J, Loss G, Perillo RP (2002) Is obesity an independent risk factor for hepatocellular carcinoma in cirrhosis? Hepatology 36: 150–155
142. Ratziu V, Bonyhay L, Di Martino V, Charlotte F, Cavallaro L, Sayegh-Tainturier MH, Giral P, Grimaldi A, Opolon P, Poynard T (2002) Survival, liver failure, and hepatocellular carcinoma in obesity-related cryptogenic cirrhosis. Hepatology 35: 1485–1493
143. Wanless IR, Wong F, Blendis LM, Greig P, Heathcote EJ, Levy G (1995) Hepatic and portal vein thrombosis in cirrhosis: possible role in the development of parenchymal extinction and portal hypertension. Hepatology 21(5): 1238–1247
144. El-Serag H, Davila JA, Petersen NJ, McGlynn KA (2003) The continuing increase in the incidence of hepatocellular carcinoma in the US: an update. Ann Intern Med 139: 817–823
145. Ong JP, Younossi ZM (2002) Is hepatocellular carcinoma part of the natural history of nonalcoholic steatohepatitis? Gastroenterology 123: 375–378
146. Murphy TK, Calle EE, Rodriquez C, Khan HS, Thun MJ (2000) Body mass index and colon cancer mortality in a large prospective study. Am J Epidemiol 152: 847–854
147. Yang S, Lin HZ, Hwang J, Chacko VP, Diehl AM (2001) Hepatocyte hyperplasia in noncirrhotic fatty livers: is obesity-related hepatic steatosis a premalignant condition? Cancer Res 61: 5016–5023
148. El-Sarag HB, Richardson PA, Everhart JE (2001) The role of diabetes in hepatocellular carcinoma: a case control study among United States veterans. Am J Gastroenterol 96: 2462–2467
149. Cotrim HP, Parana R, Braga E, Lyra L (2000) Nonalcoholic steatohepatitis and hepatocellular carcinoma: natural history? Am J Gastroenterol 95: 3018–3019
150. Zen Y, Katayanagi K, Tsuneyama K, Harada K, Araki I, Nakanuma Y (2001) Hepatocellular carcinoma arising in non-alcoholic steatohepatitis. Pathol Int 51: 127–131
151. Bugianesi E, Leone N, Vanni E, Marchesini G, Brunello F, Carucci P, Musso A, de Paolis P, Capussotti L, Salizzoni M, Rizzetto M (2002) Expanding the natural history of nonalcoholic steatohepatitis from cryptogenic cirrhosis to hepatocellular carcinoma. Gastroenterology 123: 134–140
152. Marrero JA, Fontana RJ, Su GL, Conjeevarum HS, Emick DM, Lok AS (2002) NAFLD may be a common underlying liver disease in patients with hepatocellular carcinoma in the US. Hepatology 36: 1349–1354
153. Day CP, James OFW (1998) Steatohepatitis: A tale of two hits. Gastroenterology 114: 842–845
154. DiMauro S, Schon EA (2003) Mitochondrial respiratory-chain diseases. N Engl J Med 348: 2656–2668
155. Carrozzo R, Hirano M, Fromenty B, Casali C, Santorelli FM, Bonilla E, DiMauro S, Schon EA, Miranda AF (1998) Multiple mtDNA deletions features in autosomal dominant and recessive diseases suggest distinct pathogeneses. Neurology 50: 99–106
156. Hoek JB, Cahill A, Pastorino JG (2002) Alcohol and mitochondria: a dysfunctional relationship. Gastroenterology 122: 2049–2063
157. Green DR, Reed JC (1998) Mitochondria and apoptosis. Science 281: 1309–1312
158. Caldwell SH, Chang C (2004) Mitochondria in NAFL. Clin Liver Dis Ed. AJ Sanyal, in press

159. Bereiter-Hahn J, Voth M (1994) Dynamics of mitochondria in living cells: shape changes, dislocations, fusion and fission of mitochondria. Microsc Res Tech 27: 198–219
160. Hepatocyte Ultrastructure Text—University of Virginia library
161. Kuroiwa T, Ohta T, Kuroiwa H, Shigeyuki K (1994) Molecular and cellular mechanisms of mitochondrial nuclear division and mitochondriokinesis. Microsc Res Tech 27: 220–232
162. Lea PJ, Temkin RJ, Freeman KB, Mitchell GA, Robinson BH (1994) Variations in mitochondrial ultrastructure and dynamics observed by high resolution scanning electron microscopy (HRSEM). Microsc Res Tech 27: 269–277
163. Lehmann TG, Wheeler MD, Schwabe RF, Connor HD, Schoonhoven R, Bunzendahl H, Samulski RJ, Thurman RG (2000) Gene delivery of Cu/Zn-superoxide dismutase improves graft function after transplantation of fatty livers in the rat. Hepatology 32: 1255–1264
164. Dianzani MU (1954) Uncoupling of oxidative phosphorylation in mitochondria from fatty livers. Biochim Biophys Acta 14: 514–532
165. Dianzani MU (1957) The content of adenosine polyphosphates in fatty livers. Biochem J 65: 116–124
166. Sternlieb I (1968) Mitochondrial and fatty changes in hepatocytes of patients with Wilson's disease. Gastroenterology 55: 354–362
167. Chedid A, Mendenhall CL, Tosch T, Chen T, Rabin L, Garcia-Pont P, Goldberg SJ, Kiernan T, Seeff LB, Sorrell M et al (1986) Significance of megamitochondria in alcoholic liver disease. Gastroenterology 90: 1858–1864
168. Caldwell SH, Swerdlow RH, Khan EM, Iezzoni JC, Hespenheide EE, Parks JK, Parker WD Jr. (1999) Mitochondrial abnormalities in NASH. J Hepatol 31: 430–434
169. Sanyal AJ, Campbell-Sargent C, Mirshani F, Rizzo WB, Contos MJ, Sterling RK, Luketic VA, Shiffman ML, Clore JN (2001) Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities. Gastroenterology 120: 1183–1192
170. Sternlieb I, Berger JE (1969) Optical diffraction studies of crystalline structures in electron micrographs. J Cell Biol 43: 448–455
171. Caldwell SH, Hespenheide EE, Redick JA, Iezzoni JC, Battle EH, Sheppard BL (2001) A pilot study of a thiazolidinedione, troglitazone, in nonalcoholic steatohepatitis. Am J Gastroenterol 96: 519–525
172. Williams WP, Selstam E, Brain T (1998) X-ray diffraction studies of the structural organization of prolamellar bodies isolated from Zea mays. FEBS Lett 422: 252–254
173. Deng Y, Kohlwein SD, Manella CA (2002) Fasting induces cyanide-resistant respiration and oxidative stress in the amoeba *Chaos carolinensis*: implications for the cubic structural transition in mitochondrial membranes. Protoplasm 219: 160–167
174. Spycher MA, Ruttner JR (1968) Kristalloide Einschlusse in menschlichen Lebermitochondrien. Virchows Arch B Cell Pathol 1: 211–221
175. Wolf SG, Frenkiel D, Arad T, Finkel SE, Kolter R, Minsky A (1999) DNA protection by stress-induced biocrystallization. Nature 400: 83–85
176. Le TH, Caldwell SH, Redick JA, Sheppard BL, Davis CA, Arseneau KO, Iezzoni JC, Hespenheide EE, Peterson T (2004) The lobular distribution of megamitochondria with crystalline inclusions in type 3–4 NAFL
177. Dianzani MU, Scuro S (1956) The effects of some inhibitors of oxidative phosphorylation on the morphology and enzymatic activities of mitochondria. Biochem J 62: 205–215
178. Vendemiale G, Grattagliano I, Caraceni P, Caraccio G, Domenicali M, Dall'Agata M, Trevisani F, Guerrieri F, Bernardi M, Altomare E (2001) Mitochondrial oxidative injury and energy metabolism alteration in rat fatty liver: effect of the nutritional status. Hepatology 33: 808–815
179. Junnila M, Rahko T, Sukura A, Lindberg LA (2000) Reduction of carbon tetrachloride induced hepatotoxic effects by oral administration of betaine in male Han-Wistar rats: a morphometric histologic study. Vet Med 37: 231–238

180. Lehmann TG, Wheeler MD, Schwabe RF, Connor HD, Schoonhoven R, Bunzendahl H, Brenner DA, Jude Samulski R, Zhong Z, Thurman RG (2000) Gene delivery of Cu/Zn-superoxide dismutase improves graft function after transplantation of fatty liver in the rat. Hepatology 32: 1255–1264
181. Trevasani F, Colantoni A, Caraceni P, Van Thiel DH (1996) The use of donor fatty livers for liver transplantation: a challenge or a quagmire. J Hepatol 22: 114–121
182. Perez-Carreras M, Del Hoyo P, Martin MA, Rubio JC, Martin A, Castellano G, Colina F, Arenas J, Solis-Herruzo JA (2003) Defective hepatic mitochondrial respiratory chain in patients with nonalcoholic steatohepatitis. Hepatology 38(4): 999–1007
183. Cortez-Pinto H, Chatham J, Chako VP, Arnold C, Rasid A, Diehl AM (1999) Alterations in liver ATP homeostasis in human nonalcoholic steatohepatitis: a pilot study. JAMA 282: 1659–1664
184. Chavin KD, Yang SQ, Lin HZ, Chatham J, Chakko VP, Hoek JB, Walajtys-Rode E, Rashid A, Chen CH, Huang CC, Wu TC, Lane MD, Diehl AM (1999) Obesity induces expression of uncoupling protein-2 in hepatocytes and promotes liver ATP depletion. J Biol Chem 274: 5692–5700
185. Diehl AM, Hoek JB (1999) Mitochondrial uncoupling: role of uncoupling protein anion carriers and relationship to thermogenesis and weight control, 'the benefits of losing control'. J Bioenerg Biomembr 31: 493–506
186. Baffy G, Zhang CY, Glickman JN, Lowell BB (2002) Obesity-related fatty liver is unchanged in mice deficient for mitochondrial UCP-2. Hepatology 35: 753–761
187. Pessayre D, Mansouri A, Fromenty B (2002) Nonalcoholic steatosis and steatohepatitis V. Mitochondrial dysfunction in steatohepatitis. Am J Physiol Gastrointest Liver Physiol 282: G193–G199
188. Caldwell S, Chang C, Redick J, Davis C, Krugner-Higby L, Al-Osaimi A (2004) The ballooned hepatocyte in NAFL: degenerative or adaptive? Submitted to EASL
189. Ikura Y, Ohsasa M, Ogawa Y, Kaneda K, Hai E, Kayo S, Yoshima N, Shirai N, Sugama Y, Fujino H, Itabe H, Ueda M (2003) Localization of oxidized phosphatidylcholine in steatotic livers: its relation to oxidative injury in fatty liver disorders. Hepatology 34: 507A
190. Fournier E, Peresson R, Guy G, Hermier D (1997) Relationship between storage and secretion of hepatic lipids in two breeds of geese with different susceptibility to liver steatosis. Poultry Sci 76: 599–607
191. Krugner-Higby L, Attie A, Schueler K, Thuren T, Shelness G, Wendland A. Hyperlipidemia in California mice (*Peromyscus californicus*) due to increased hepatic secretion of VLDL
192. Silva JE (2003) The thermogenic effect of thyroid hormone and its clinical implications. Ann Intern Med 139: 205–213
193. Dulloo AG (2002) A sympathetic defense against obesity. Science 297: 780–781
194. Bachman ES, Dhillon H, Zhang CY, Cinti S, Bianco AC, Kobilka BK, Lowell BB (2002) betaAR signaling required for diet-induced thermogenesis and obesity resistance. Science 297: 843–845
195. Li Z, Oben JA, Yang S, Lin H, Diehl AM (2003) Norepinephrin mediates effects of leptin deficiency on hepatic innate immune system in a mouse model of NASH. Hepatology 34: 192A
196. Struben VMD, Hespenheide EE, Caldwell SH (2000) Familial patterns of nonalcoholic steatohepatitis (NASH) and cryptogenic cirrhosis. Am J Med 108: 9–13
197. Willner IR, Waters B, Patil SR, Reuben A, Morelli J, Riely CA (2001) Ninety patients with nonalcoholic steatohepatitis: insulin resistance, familial tendency and severity of disease. Am J Gastroenterol 96: 2957–2961
198. Caldwell SH, Harris DM, Patrie J, Hespenheide EE (2002) Are NASH and cryptogenic cirrhosis under diagnosed in African Americans? Am J Gastroenterol 97: 1496–1500
199. Schapira AHV (1997) Mitochondrial disorders: an overview. J Bioenerg Biomembr 29: 105–107

200. Schon EA, Bonilla E, DiMauro S (1997) Mitochondrial DNA mutations and pathogenesis. J Bioenerg Biomembr 29: 131–149
201. Johns DR (1995) Mitochondrial DNA and disease. N Engl J Med 333: 638–644
202. Wallace DC (1999) Mitochondrial diseases in mouse and man. Science 283: 1482–1488
203. Bohan A, Droogan O, Nolan N, Mayne P, Bonham J, Thornton P, Farrell MA, Hegarty JE (2000) Mitochondrial DNA abnormalities without significant deficiency of intramitochondrial fatty acid beta oxidation enzymes in a well defined subgroup of patients with nonalcoholic steatohepatitis (abstract). Hepatology 32: 387A
204. Carrozzo R, Hirano M, Fromenty B, Casali C, Santorelli FM, Bonilla E, DiMauro S, Schon EA, Miranda AF (1998) Multiple mtDNA deletions features in autosomal dominant and recessive diseases suggest distinct pathogeneses. Neurology 50: 99–106
205. Hinokio Y, Suzuki S, Komatu K, Ohtomo M, Onoda M, Matsumoto M, Hirai S, Sato Y, Akai H, Ae K, Miyabayasi S, Abe R, Toyota T (1995) A new mitochondrial DNA deletion associated with diabetic amyotrophy, diabetic myoatrophy, and diabetic fatty liver. Muscle Nerve 3: S142–149
206. Feliciani C, Amerio P (1999) Madelung's disease: inherited from an ancient Mediterranean population? N Engl J Medicine 340: 1481
207. Vila MR, Gamez J, Solano A, Playan A, Schwartz S, Santorelli FM, Cervera C, Casali C, Montoya J, Villarroya F (2000) Uncoupling protein-1 m RNA expression in lipomas from patients bearing pathogenic mitochondrial DNA mutations. Biochem Biophys Res Commun 278: 800–802
208. Guillausseau PJ, Massin P, Dubois-LaForgue D, Timsit J, Virally M, Gin H, Bertin E, Blickle JF, Bouhanick B, Cahen J, Caillat-Zucman S, Charpentier G, Chedin P, Derrien C, Ducluzeau PH, Grimaldi A, Guerci B, Kaloustian E, Murat A, Olivier F, Paques M, Paquis-Flucklinger V, Porokhov B, Samuel-Lajeunesse J, Vialettes B (2001) Maternally inherited diabetes and deafness: a multicenter study. Ann Intern Med 134: 721–728
209. Kadowaki H, Tobe K, Mori Y, Sakura H, Sakuta R, Nonaka I, Hagura R, Yazaki Y, Akanuma Y, Kadowaki T (1993) Lancet 341: 893–894
210. Minana JB, Gomez-Cambronero L, Lloret A, Pallardo FV, Del Olmo J, Escudero A, Rodrigo JM, Pelliin A, Vina JR, Vina J, Sastre J (2002) Mitochondrial oxidative stress and CD95 ligand: a dual mechanism for hepatocyte apoptosis in chronic alcoholism. Hepatology 35: 1205–1214
211. Feldman G, Haouzi D, Moreau A, Durang-Schneider A-M, Bringuier A, Berson A, Mansouri A, Fau D, Pessayre D (2000) Opening of the mitochondrial permeability transition pore causes matrix expansion and outer membrane rupture in FAS-mediated hepatic apoptosis in mice. Hepatology 31: 674–683
212. Feldsteine A, Canbay A, Angulo P, Taniai M, Burgart LJ, Lindor KD (2003) Hepatocyte apoptosis in patients with non-alcoholic steatohepatitis. Quantification and clinical relevance. Gastroenterology 124: 703A
213. Rashid A, Wu T-C, Huang CC, Chen CH, Lin HZ, Yang SQ, Lee FY, Diehl AM (1999) Mitochondrial proteins that regulate apoptosis and necrosis are induced in mouse fatty liver. Hepatology 29: 1131–1138
214. Powell EE, Searle J, Mortimer R (1989) Steatohepatitis associated with limb lipodystrophy. Gastroenterology 97: 1022–1024
215. Degoul F, Sutton A, Mansouri A, Cepanec C, Degott C, Fromenty B, Beaugrand M, Valla D, Pessayre D (1995) Homozygosity for alanine in the mitochondrial targeting sequence of manganese superoxide dismutase and risk for severe alcoholic liver disease. Gastroenterology 108: 193–200
216. Petersen KF, Befroy D, Dufour S, Dziura J, Ariyan C, Rothman DL, DiPietro L, Cline GW, Shulman GI (2003) Mitochondrial dysfunction in the elderly: possible role in insulin resistance. Science 300: 1140–1142

217. Borkman M, Storlien LH, Pan DA, Jenkins AB, Chisholm DJ, Campbell LV (1993) The relationship between insulin sensitivity and the fatty-acid composition of skeletal muscle phospholipids. N Engl J Med 328: 238–244
218. Sreekumar R, Rosado B, Rasmussen D, Charlton M (2003) Hepatic gene expression in histologically progressive nonalcoholic steatohepatitis. Hepatology 38: 244–251
219. Angulo P (2002) Nonalcoholic fatty liver disease. N Engl J Med 346: 1221–1231
220. Tokar JL, Berg CL (2002) Therapeutic options in nonalcoholic fatty liver disease. Curr Treat Opt Gastroenterol 5: 425–436
221. Agrawal S, Bonkovsky HL (2002) Management of nonalcoholic steatohepatitis. J Clin Gastroenterol 35: 253–261
222. Younossi ZM, Diehl AM, Ong J (2002) Nonalcoholic fatty liver disease: an agenda for clinical research. Hepatology 35: 746–752
223. McCullough AJ (2002) Update on nonalcoholic fatty liver disease. J Clin Gastroenterol 34: 255–262
224. Davies MJ, Baer DJ, Judd JT, Brown ED, Campbell WS, Taylor PR (2003) Effects of moderate alcohol intake on fasting insulin and glucose concentrations and insulin sensitivity in postmenopausal women. JAMA 287: 2559–2562
225. Dixon JB, Bathal PS, O'Brien PE (2001) Nonalcoholic fatty liver disease: predictors of nonalcoholic steatohepatitis and liver fibrosis in the severely obese. Gastroenterology 121: 91–100
226. Cotrim HP, Andrade ZA, Parana R, Portugal M, Lyra LG, Freitas LA (1999) Nonalcoholic steatohepatitis: a toxic liver disease in industrial workers. Liver 19: 263–264
227. Brodkin CA, Daniell W, Checkoway H, Echeverria D, Johnson J, Wang K, Sohaey R, Green D, Redlich C, Gretch D, Rosenstock L (1995) Hepatic ultrasonic changes in workers exposed to perchloroethylene. Occup Environ Med 52: 679–685
228. Redlich CA, Cullen MR (1997) Nonalcoholic steatohepatitis (letter). Ann Intern Med 127: 410
229. Saksena S, Johnson J, Ouiff SP, Elias E (1999) Diet and exercise: important first steps in therapy of NASH (abstract). Hepatology 30: 436
230. Bertram SR, Venter I, Stewart RI (1990) Weight loss in obese women—exercise versus dietary education. S Afr Med J 78: 15–18
231. Eriksson S, Eriksson KF, Bondesson L (1986) Nonalcoholic steatohepatitis in obesity: a reversible condition. Acta Med Scand 220: 83–88
232. Ueno T, Sugawara H, Sujaku K, Hashimoto O, Tsuji R, Tamaki S, Torimura T, Inuzuka S, Sata M, Tanikawa K (1997) Therapeutic effects of restricted diet and exercise in obese patients with fatty liver. J Hepatol 27: 103–107
233. Keeffe EB, Adesman PW, Stenzel P, Palmer RM (1987) Steatosis and cirrhosis in an obese diabetic resolution of fatty liver by fasting. Dig Dis Sci 32: 441–445
234. Palmer M, Schaffner F (1990) Effect of weight reduction on hepatic abnormalities in overweight patients. Gastroenterology 99: 1408–1412
235. Rozental P, Biava C, Spencer H, Zimmerman HJ (1967) Liver morphology and function tests in obesity and during total starvation. Am J Dig Dis 12: 198–208
236. Drenick EJ, Simmons F, Murphy J (1970) Effect on hepatic morphology of treatment of obesity by fasting, reducing diets and small-bowel bypass. N Engl J Med 282: 829–834
237. Andersen T, Gluud C, Franzmann M-B, Christoffersen P (1991) Hepatic effects of dietary weight loss in morbidly obese subjects. J Hepatol 12: 224–229
238. Vajro P, Fontanella A, Perna C, Orso G, Tedesco M, de Vincenzo A (1994) Persistent hypertransaminasemia resolving after weight reduction in obese children. J Pediatr 125: 239–241
239. Vajro P, Franzese A, Valerio G, Iannucci MP, Aragione N (2000) Lack of efficacy of ursodeoxycholic acid for the treatment of liver abnormalities in obese children. J Pediatr 136: 739–743

240. Park HS, Kim MW, Shin ES (1995) Effect of weight control on hepatic abnormalities in obese patients with fatty liver. J Korean Med Sci 10414–10421
241. Eriksson J, Tuominen J, Valle T, Sundberg S, Sovijarvi A, Lindholm H, Tuomilehto J, Koivisto V (1998) Aerobic endurance exercise or circuit-type resistance training for individuals with impaired glucose tolerance? Horm Metab Res 30: 37–41
242. Franzese A, Vajro P, Argenziano A, Puzziello A, Iannucci MP, Saviano MC, Brunetti F, Rubino A (1997) Liver involvement in obese children: ultrasonography and liver enzyme levels at diagnosis and during follow-up in an Italian population. Dig Dis Sci 42: 1428–1432
243. Okita M, Hayashi M, Sasagawa T, Takagi K, Suzuki K, Kinoyama S, Ito T, Yamada G (2001) Effect of a moderately energy-restricted diet on obese patients with fatty liver. Nutrition 17: 542–547
244. Capron JP, Delamarre J, Dupas JL, Braillon A, Degott C, Quenum C (1982) Fasting in obesity: another cause of liver injury with alcoholic hyaline? Dig Dis Sci 54: 374–377
245. Hickman IJ, Jonsson JR, Prins JB, Ash S, Purdie DM, Clouston AD, Powell EE (2003) Benefit of sustained weight loss and exercise in overweight patients with liver disease (abstract). Hepatology 34: 504A
246. van Baak MA (1999) Exercise training and substrate utilization in obesity. Int J Obes 23 (Suppl 3): S11–S17
247. Hoppeler H (1999) Skeletal muscle substrate metabolism. Int J Obes 23 (Suppl 3): S7–S10
248. Eden KB, Orleans T, Mulrow CD, Pender NJ, Teutsch SM (2002) Does counseling by clinicians improve physical activity? A summary of the evidence for the US preventive services task force. Ann Intern Med 137: 208–215
249. Tuomilehto J, Lindstrom J, Eriksson JG, Valle TT, Hamalainen H, Ilanne-Parikka P, Keinanen-Kiukaanniemi S, Laakso M, Louheranta A, Rastas M, Salminen V, Uusitupa M (2001) Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. N Engl J Med 344: 1343–1350
250. Epstein FH (2000) Exercise limitations in health and disease. N Engl J Med 343: 632–640
251. Kraus WE, Houmard JA, Duscha BD, Knetzger KJ, Wharton MB, McCartney JS, Bales CW, Henes S, Samsa GP, Otvos JD, Kulkarni KR, Slentz CA (2002) Effects of the amount and intensity of exercise on plasma lipoproteins. N Engl J Med 347: 1483–1492
252. Jackicic JM, Winters C, Lang W, Wing RR (1999) Effects of intermittent exercise and use of home exercise equipment adherence, weight loss, and fitness in overweight women: a randomized trial. JAMA 282: 1554–1560
253. Sothern MS, Loftin JM, Udall JN, Suskind RM, Ewing TL, Tang SC, Blecker U (2000) Safety, feasibility, and efficacy of a resistance training program in preadolescent obese children. Am J Med 319: 370–375
254. Schipper MEI, van Soest H, van Hattum J (2002) Improvement of steatosis after extracorporeal whole body hyperthermia in patients with chronic hepatitis C virus infection. Single Topic Conference on NASH Syllabus, Atlanta, Georgia, 2002
255. Dulloo AG (2002) Biomedicine. A sympathetic defense against obesity. Science 297: 780–781
256. Bachman ES, Dhillon H, Zhang CY, Cinti S, Bianco AC, Kobilka BK, Lowell BB (2002) betaAR signaling required for diet-induced thermogenesis and obesity resistance. Science 297: 843–845
257. Cheuvront SN (2000) The Zone diet and athletic performance. Sports Med 27: 213–228
258. Landers P, Wolfe MM, Glore S, Guild R, Phillips L (2002) Effect of weight loss plans on body composition and diet duration. J Oklah State Med Assoc 95: 329–331
259. Amir R, Solga SF, Clark JM, Torbenson M, Cohen A, Diehl AM, Magnuson TH (2003) Dietary composition and fatty liver disease. Hepatology 34: 513A

260. Anonymous. (1998) Executive summary of the clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults. Arch Intern Med 158: 1855–1867
261. Krauss RM, Eckel RH, Howard B, Appel LJ, Daniels SR, Deckelbaum RJ, Erdman JW Jr, Kris-Etherton P, Goldberg IJ, Kotchen TA, Lichtenstein AH, Mitch WE, Mullis R, Robinson K, Wylie-Rosett J, St Jeor S, Suttie J, Tribble DL, Bazzarre TL (2000) AHA dietary guidelines: revision 2000. A statement for healthcare professionals from the nutrition committee of the American Heart Association. Stroke 31: 2751–2766
262. Clark MJ, Sterrett JJ, Carson DS (2000) Diabetes guidelines: a summary and comparison of the recommendations of the American Diabetes Association, Veterans Health Administration, and American Association of Clinical Endocrinologists. Clin Ther 22: 899–910
263. Spieth LE, Harnish JD, Lenders CM, Raezer LB, Pereira MA, Hangen SJ, Ludwig DS (2000) A low glycemic index diet in the treatment of pediatric obesity. Arch Pediatr Adolesc Med 154: 947–951
264. Vessby B (2000) Dietary fat and insulin action in humans. Br J Nutr 83 (Suppl 1): S91–S96
265. Kurihara T, Adachi Y, Yamagata M, Abe K, Akimoto M, Hashimoto H, Ishiguro H, Niimi A, Maeda A, Shigemoto M, et al (1994) Role of eicosapentanoic acid in lipid metabolism in the liver with special reference to experimental fatty liver. Clin Ther 16: 830–837
266. Nanji AA, Sadrzadeh SMH, Yang EK, Fogt F, Meydani M, Dannenberg AJ (1995) Dietary saturated fatty acids: a novel treatment for alcoholic liver disease. Gastroenterology 109: 547–554
267. Lokesh B, LiCari J, Kinsella JE (1992) Effect of different dietary triglycerides on liver fatty acids and prostaglandin synthesis by mouse peritoneal cells. J Parenter Enteral Nutr 16: 316–321
268. Lanza-Jacoby S, Smythe C, Phetteplace H, Tabares A (1992) Adaptation to a fish oil diet before inducing sepsis in rats prevents fatty infiltration of the liver. J Parenter Enteral Nutr 16: 353–358
269. Grattagliano I, Palmieri VO, Palasciano G (2002) Hepatotoxicity of polyunsaturated fatty acids in alcohol abuser. J Hepatol 37: 291–292
270. Luo J, Rizkalla SW, Boillot J, Alamowitch C, Chaib H, Bruzzo F, Desplanque N, Dalix AM, Durand G, Slama G (1996) Dietary (n-3) polyunsaturated fatty acids improve adipocyte insulin action and glucose metabolism in insulin-resistant rats: relation to membrane fatty acids. J Nutr 126: 1951–1958
271. Clarke SD, Baillie R, Jump DB, Nakamura MT (1997) Fatty acid regulation of gene expression. Its role in fuel partitioning and insulin resistance. Ann N Y Acad Sci 927: 178–187
272. Montagne O, Vedel I, Durand-Zalaski I (1999) Assessment of the impact of fibrates and diet on survival and their cost-effectiveness: evidence from randomized, controlled trials in coronary heart disease and health economics evaluations. Clin Ther 21: 2027–2035
273. Daubioul C, Rousseau N, Demeure R, Gallez B, Taper H, Declerck B, Delzenne N (2002) Dietary fructans, but not cellulose, decrease triglyceride accumulation in the liver of obese Zucker fa/fa rats. J Nutr 132: 967–973
274. Kolanowski J (1999) A risk-benefit assessment of anti-obesity drugs. Drug Safety 20: 119–131
275. Favreaux JT, Ryu ML, Braunstein G, Orshansky G, Park SS, Coody GL, Love LA, Fong T-L (2002) Severe hepatotoxicity associated with the dietary supplement LipoKinetix. Ann Intern Med 136: 590–595
276. Harrison SA, Fincke C, Helinski D, Torgerson S (2002) Orlistat treatment in obese, non-alcoholic steatohepatitis patients: a pilot study (abstract). Hepatology 36: 406

277. Yanovsky SZ, Yanovsky JA (2002) Obesity. N Engl J Med 346: 591–602
278. Mun EC, Blackburn GL, Matthews JB (2001) Current status of medical and surgical therapy for obesity. Gastroenterology 120: 669–681
279. Luyckx FH, Desaive C, Thiry A, Dewe W, Scheen AJ, Gielen JE, Lefebvre PJ (1998) Liver abnormalities in severely obese subjects: effect of drastic weight loss after gastroplasty. Int J Obes Relat Metab Disord 22: 222–226
280. Silverman EM, Sapala JA, Appelman HD (1995) Regression of hepatic steatosis in morbidly obese persons after gastric bypass. Am J Clin Pathol 104: 23–31
281. Ranlov I, Hardt F (1990) Regression of liver steatosis following gastroplasty or gastric bypass for morbid obesity. Digestion 47: 208–214
282. Busetto L, Tregnaghi A, De Marchi F, Segato G, Foletto M, Sergi G, Favretti F, Lise M, Enzi G (2002) Liver volume and visceral obesity in women with hepatic steatosis undergoing gastric banding. Obes Res 10: 408–411
283. Duchini A, Brunson ME (2001) Roux-en-Y gastric bypass for recurrent nonalcoholic steatohepatitis in liver transplant recipients with morbid obesity. Transplantation 72: 156–171
284. Ackerman NB (1979) Protein supplementation in the management of degenerating liver function after jejunoileal bypass. Surg Gynecol Obstet 149: 8–14
285. Drenick EJ, Fisler J, Johnson D (1982) Hepatic steatosis after intestinal bypass. Prevention and reversal by metronidazole, irrespective of protein-calorie malnutrition. Gastroenterology 82: 535–548
286. Laurin J, Lindor KD, Crippen JS, Gossard A, Gores GJ, Ludwig J, Rakela J, McGill DB (1996) Ursodeoxycholic acid or clofibrate in the treatment of non-alcohol-induced steatohepatitis: a pilot study. Hepatology 23: 1464–1467
287. Guma G, Viola L, Thome M, Galdame O, Albarez E (1997) Ursodeoxycholic acid in the treatment of nonalcoholic steatohepatitis: results of a prospective clinical controlled trial (abstract). Hepatology 26: 387
288. Ceriani R, Brunati S, Morini L, Sacchi E, Colombo G (1998) Effect of ursodeoxycholic acid plus diet in patients with nonalcoholic steatohepatitis (abstract). Hepatology 28: 386
289. Mendez-Sanchez N, Gonzalez V, Pichardo-Bahena R, Uribe M (2002) Weight reduction and ursodeoxycholic acid in subjects with nonalcoholic fatty liver disease: a randomized, double-blind, placebo-controlled trial (abstract). Hepatology 36: 412
290. Okan A, Astarcioglu H, Tankurt E, Sagul O, Altekin E, Astarcioglu I, Gonen O (2002) Effect of ursodeoxycholic acid on hepatic steatosis in rats. Dig Dis Sci 47: 2389–2397
291. Laurin J, Lindor KD, Crippin JS, Gossard A, Gores GJ, Ludwig J, Rakela J, McGill DB (1996) Ursodeoxcholic acid or clofibrate in the treatment of non-alcoholic steatohepatitis: A pilot study. Hepatology 23: 1464–1467
292. Lindor KD, Kowdley KV, Heathcote EJ, Harrison ME, Jorgensen R, Angulo P, Lymp JF, Burgart L, Colin P (2004) Ursodeoxycholic acid for treatment of nonalcoholic steatohepatitis: results of a randomized trial. Hepatology 39(3): 770–778
293. Kerai MD, Waterfield CJ, Kenyon SH, Asker DS, Timbrell JA (2001) The effect of taurine depletion by β-alanine treatment on the susceptibility to ethanol-induced hepatic dysfunction in rats. Alcohol Alcohol 36: 29–38
294. Kerai MD, Waterfield CJ, Kenyon SH, Asker DS, Timbrell JA (1999) Reversal of ethanol-induced hepatic steatosis and lipid peroxidation by taurine: a study in rats. Alcohol Alcohol 34: 529–541
295. Zaman N, Tam YK, Jewell LD, Coutts RT (1996) Effects of taurine supplementation in parenteral nutrition-associated hepatosteatosis and lidocaine metabolism. A study using isolated rat liver perfusion. Drug Metab Dispos Biol Fate Chem 24: 534–541
296. Kerai MD, Waterfield CJ, Kenyon SH, Asker DS, Timbrell JA (1998) Taurine: protective properties against ethanol-induced hepatic steatosis and lipid peroxidation during chronic ethanol consumption in rats. Amino Acids 15: 53–76

297. Obinata K, Maruyama T, Hayashi M, Watanabe T, Nittono H (1996) Effect of taurine on the fatty liver of children with simple obesity. Adv Exp Med Biol 403: 607–613
298. Simon JB, Scheig R, Klatskin G (1968) Protection by orotic acid against the renal necrosis and fatty liver of choline deficiency. Proc Soc Exp Biol Med 129: 874–877
299. Buchman AL, Dubin M, Jenden D, Moukarzel A, Roch MH, Rice K, Gornbein J, Ament ME, Eckhert CD (1992) Lecithin increases plasma free choline and decreases hepatic steatosis in long-term total parenteral nutrition patients. Gastroenterology 102: 1363–1370
300. Demetriou AA (1992) Lecithin increases plasma free choline and decreases hepatic steatosis in long-term total parenteral nutrition patients. J Parenter Enteral Nutr 16: 487–488
301. Antisiewicz J, Nishizawa Y, Liu X, Usukura J, Wakabayashi T (1994) Suppression of the hydrazine-induced formation of megamitochondria in the rat liver by α-tocopherol. Exp Mol Pathol 60: 173–187
302. Soltys K, Dikdan G, Koneru B (2001) Oxidative stress in fatty liver of obese Zucker rats: rapid amelioration and improved tolerance to warm ischemia with tocopherol. Hepatology 34: 13–18
303. Lavine JE (2000) Vitamin E treatment of nonalcoholic steatohepatitis in children: a pilot study. J Pediatr 136: 711–713
304. Brown BG, Zhao XQ, Chait A, Fisher LD, Cheung MC, Morse JS, Dowdy AA, Marino EK, Bolson EL, Alaupovic P, Frohlich J, Albers JJ (2001) Simvastatin and niacin, antioxidant vitamins, or the combination for the prevention of coronary disease. N Engl J Med 345: 1583–1592
305. Fu CS, Esrason K, Alshak NS, Conteas CN, Simmons VJ (1998) Dietary lecithin, antioxidant and vitamin B complex (LAB) decrease hepatic steatosis in patients with NASH (abstract). Gastroenterology 114: 1243
306. Hasegawa T, Yoneda M, Nakamura K, Makino I, Terano A (2001) Plasma transforming growth factor-β1 level and efficacy of α-tocopherol in patients with non-alcoholic steatohepatitis: a pilot study. Aliment Pharmacol Ther 15: 1667–1672
307. Harrison SA, Torgerson S, Hayashi P, Ward J, Schenker S (2003) Vitamin E and vitamin C treatment improves fibrosis in patients with NASH. Am J Gastroenterol 98: 2485–2490
308. Alvaro D, Gigliozzi A, Piat C, Carli L, Bini A, La Rosa T, Furfaro S, Capocaccia L (1995) Effect of S-adenosyl-L-methionine on ethanol cholestasis and hepatotoxicity in isolated perfused rat liver. Dig Dis Sci 40: 1592–1600
309. Lu S (1998) Methionine adenosyltransferase and liver disease: it's all about SAM. Gastroenterology 114: 403–407
310. Colell A, Garcia-Ruiz C, Morales A, Ballesta A, Ookhtens M, Rodes J, Kaplowitz N, Fernandez-Checa JC (1997) Transport of reduced glutathione in hepatic mitochondria and mitoplasts from ethanol-treated rats: effect of membrane physical properties and S-adenosyl-methionine. Hepatology 26: 699–708
311. Lieber CS (2002) *S*-Adenosyl-L-methionine and alcoholic liver disease in animal models: implications for early intervention in human beings. Alcohol 27: 173–177
312. Nakano H, Yamaguchi M, Kaneshiro Y, Yoshida K, Kigawa G, Nagasaki H, Fujiwara Y, Matsumoto F, Kitamura N, Sasaki J, Kuzume M, Takeuchi S, Kumada K (1998) *S*-Adenosyl-L-methionine attenuates ischemia-reperfusion injury of steatotic rats. Transplant Proc 30: 3735–3736
313. Avila MA, Carretero MV, Rodriguez EN, Mato JM (1998) Regulation by hypoxia of methionine adenosyltransferase activity and gene expression in rat hepatocytes. Gastroenterology 114: 364–371
314. Mazzanti R, Arcangeli A, Salvadori G, Smorlesi C, Di Perri T, Auteri A, Boggiano CA, Pippi L, Toti M, Boncompagni P, Angioli D, Caremani M, Magnolfi F, Camarri E, Motta R, Forconi A, Tommasi AC, Lomi M, Soldi A (1978) The anti-steatotic effect of sulfo-

adenosylmethionine (SAMe) in various forms of chronic hepatopathy. Multicentric research. Minerva Med 69: 3283–3292
315. Labo G, Gasbarrini GB (1975) Therapeutic action of S-adenosylmethionine in some chronic hepatopathies. Minerva Med 66: 1563–1570
316. Labo G, Gasbarrini G, Miglio F (1972) Various effects of SAM dependent transmethylations in hepatology. Minerva Med 63: 2007–2017
317. Neuschwander-Tetri BA (2001) Betaine: an old therapy for a new scourge. Am J Gastroenterol 96: 2534–2546
318. Abdelmalek MF, Angulo P, Jorgensen RA, Sylvestre PB, Lindor KD (2001) Betaine, a promising new agent for patients with nonalcoholic steatohepatitis: results of a pilot study. Am J Gastroenterol 96: 2711–2717
319. Miglio F, Rovati LC, Santoro A, Setnikar I (2000) Efficacy and safety of oral betaine glucuronate in non-alcoholic steatohepatitis. A double-blind, randomized, parallel-group, placebo-controlled prospective clinical study. Arzneimittelforschung 50: 722–727
320. Nakano H, Nagasaki H, Yoshida K, Kigawa G, Fujiwara Y, Kitamura N, Kuzume M, Takeuchi S, Sasaki J, Shimura H, Yamaguchi M, Kumada K (1998) *N*-Acetylcysteine and anti-ICAM-1 monoclonal antibody reduce ischemia-reperfusion injury of the steatotic rat liver. Transplant Proc 30: 3763
321. Nakano H, Nagasaki H, Barama A, Boudjema K, Jaeck D, Kumada K, Tatsuno M, Baek Y, Kitamura N, Suzuki T, Yamaguchi M (1997) The effect of *N*-acetylcysteine and anti-intercellular adhesion molecule-1 monoclonal antibody against ischemia-reperfusion injury of the rat steatotic liver produced by a choline-methionine-deficient diet. Hepatology 26: 670–678
322. Gulbahar O, Karasu ZA, Ersoz G, Akarca US, Musoglu A (2000) Treatment of nonalcoholic steatohepatitis with N-acetylcysteine (abstract). Gastroenterology 118: 1444
323. Venkataramanan R, Ramachandran V, Komoroski BJ, Zhang S, Schiff PL, Strom SC (2000) Milk thistle, a herbal supplement, decreases the activity of CYP3A4 and uridine diphosphoglucuronosyl transferase in human hepatocyte cultures. Drug Metab Dispos Biol Fate Chem 28: 1270–1273
324. Azuma Y, Shinohara M, Wang PL, Hidaka A, Ohura K (2001) Histamine inhibits chemotaxis, phagocytosis, superoxide anion production, and the production of TNF-α and IL-12 by macrophages via H2-receptors. Int J Immunopharmacol 1: 1867–1875
325. Villa RF, Gorini A (1997) Pharmacology of lazaroids and brain energy metabolism: a review. Pharmacol Rev 49: 99–136
326. Li Z, Yang S, Lin H, Huang J, Watkins PA, Moser AB, DeSimone C, Song X, Diehl AM (2003) Probiotics and antibodies to TNF inhibit inflammatory activity and improve nonalcoholic fatty liver disease. Hepatology 37: 343–350
327. DeFronzo RA (1999) Pharmacologic therapy for type 2 diabetes mellitus. Ann Intern Med 131: 281–303
328. Caldwell SH, Hespenheide EE, Redick JA, Iezzoni JC, Battle EH, Sheppard BL (2001) A pilot study of a thiazolidinedione, troglitazone, in nonalcoholic steatohepatitis. Am J Gastroenterol 96: 519–525
329. Katoh S, Hata S, Matsushima M, Ikemoto S, Inoue Y, Yokoyama J, Tajima N (2001) Troglitazone prevents the rise in visceral adiposity and improves fatty liver associated with sulfonylurea therapy—a randomized controlled trial. Metabolism 50: 414–417
330. Acosta RC, Molina EG, O'Brien CB, Cobo MC, Amaro R, Neff GW, Schiff ER (2001) The use of pioglitazone in nonalcoholic steatohepatitis (abstract). Gastroenterology 120: 546
331. Neuschwander-Tetri BA, Brunt EM, Bacon BR, Sponseller C, Wehmeier KR, Hampton K (2002) Histologic improvement in NASH following increased insulin sensitivity

with the PPAR-γ ligand rosiglitazone for 48 weeks (abstract). Hepatology 36: 379
332. Galli A, Crabb DW, Ceni E, Salzano R, Melo T, Svegliati-Baroni G, Ridolfi F, Trozzi L, Surrenti C, Casini A (2002) Antidiabetic thiazolidinediones inhibit collagen synthesis and hepatic stellate cell activation in vivo and in vitro. Gastroenterology 122: 1924–1940
333. Azuma T, Tomita K, Kato S, Adachi M, Inokuchi N, Kitamura N, Nishimura T, Ishii H (2002) A pilot study of a thiazolidinedione, pioglitazone, in nonalcoholic steatohepatitis (abstract). Hepatology 36: 406
334. Sanyal AJ, Contos MJ, Sargeant C, Stravitz RT, Luketic VA, Sterling RK, Shiffman ML (2002) A randomized controlled pilot study of pioglitazone and vitamin E versus vitamin E for non-alcoholic steatohepatitis (abstract). Hepatology 36: 382
335. Vamecq J, Latruffe N (1999) Medical significance of peroxisome proliferator-activated receptors. Lancet 354: 141–148
336. Ribon V, Johnson JH, Camp HS, Saltiel AR (1998) Thiazolidinediones and insulin resistance: peroxisome proliferator-activated receptor γ activation stimulates expression of the CAP gene. Proc Natl Acad Sci U S A 95: 14751–1476
337. Kelly IE, Han TS, Walsh K, Lean ME (1999) Effects of a thiazolidinedione compound on body fat and fat distribution of patients with type 2 diabetes. Diabetes Care 22: 288–293
338. Lenhard JM, Kliewer SA, Paulik MA, Plunket KD, Lehmann JM, Weiel JE (1997) Effects of troglitazone and metformin on glucose and lipid metabolism. Biochem Pharmacol 54: 801–808
339. Aubert J, Champigny O, Saint-Marc P, Negrel R, Collins S, Ricquier D, Ailhaud G (1997) Up-regulation of UCP-2 gene expression by PPAR agonists in preadipose and adipose cells. Biochem Biophys Res Commun 238: 606–611
340. Arioglu E, Duncan-Morin J, Sebring N, Rother KI, Gottlieb N, Lieberman J, Herion D, Kleiner DE, Reynolds J, Premkumar A, Sumner AE, Hoofnagle J, Reitmen ML, Taylor SI (2000) Efficacy and safety of troglitazone in the treatment of lipodystrophy syndromes. Ann Intern Med 133: 263–274
341. Sanyal AJ, Contos MJ, Sargeant C, Stravitz RT, Luketic VA, Sterling RK, Shiffman ML (2002) A randomized controlled pilot study of pioglitazone and vitamin E versus vitamin E for non-alcoholic steatohepatitis. Hepatology 36: 382
342. Neuschwander-Tetri BA, Brunt EM, Wehmier KR, Sponseller CA, Hampton K, Bacon BR (2003) Interim results of a pilot study demonstrating the early effects of the PPAR-gamma ligand rosiglitazone on insulin sensitivity, aminotransferases, hepatic steatosis and body weight in patients with non-alcoholic steatohepatitis (2003) Hepatology 38(4): 434–440
343. Lutchman GA, Promrat K, Soza A, Heller T, Mi L, Ghany M, Park Y, Freedman R, Yanovsky J, Kleiner D, Liang TJ, Hoofnagle J (2003) Biochemical relapse and continued weight gain in subjects with NASH after discontinuation of pioglitazone. Hepatology 34: 510A
344. Lin HZ, Yang SQ, Chuckaree C, Kuhajda F, Ronnet G, Diehl AM (2000) Metformin reverses fatty liver disease in obese leptin-deficient mice. Nat Med 6: 998–1003
345. Coyle WJ, Delaney N, Yoshihashi A, Lawson P (1999) Metformin treatment in patients with nonalcoholic steatohepatitis (abstract). Gastroenterology 116: 1198
346. Urso R, Visco-Comandini U (2002) Metformin in non-alcoholic steatohepatitis. Lancet 359: 355–356
347. Marchesini G, Brizi M, Bianchi G, Tomassetti S, Zoli M, Melchionda N (2001) Metformin in non-alcoholic steatohepatitis. Lancet 358: 893–894
348. Zhou G, Myers R, Li Y, Chen Y, Shen X, Fenyk-Melody J, Wu M, Ventre J, Doebber T, Fujii N, Musi N, Hirshman MF, Goodyear LJ, Moller DE (2001) Role of AMP-activated protein kinase in mechanism of metformin action. J Clin Invest 108: 1167–1174

349. Chiasson JL, Josse RG, Gomis R, Hanefeld M, Karasik A, Laakso M (2002) Acarbose for prevention of type 2 diabetes mellitus: the STOP-NIDDM randomized trial. Lancet 359: 2072–2077
350. Santomauro ATMG, Boden G, Sliva MER, Rocha DM, Santos RF, Ursich MJM, Strassmann PG, Wajchenberg BL (1999) Overnight lowering of free fatty acids with acipimox improves insulin resistance and glucose tolerance in obese diabetic and nondiabetic subjects. Diabetes 48: 1836–1841
351. Nestler JE, Jakubowicz DJ, Iuorno MJ (2000) Role of inositolphosphoglycan mediators of insulin action in the polycystic ovary syndrome. J Pediatr Endocrinol 13 (Suppl 5): 1295–1298
352. Laurin J, Lindor KD, Crippin JS, Gossard A, Gores GJ, Ladwig J, Rakela J, McGill DB (1996) Ursodeoxcholic acid or clofibrate in the treatment of non-alcoholic steatohepatitis: A pilot study. Hepatology 23: 1464–1467
353. Saibara T, Onishi S, Ogawa Y, Yoshida S, Enzan H (1999) Bezafibrate for tamoxiphen-induced non-alcoholic steatohepatitis. Lancet 353: 1802
354. Basaranoglu M, Acbay O, Sonsuz A (1999) A controlled trial of gemfibrozil in the treatment of patients with nonalcoholic steatohepatitis. J Hepatol 31: 384
355. Caldwell SH, Zaidman JS, Hespenheide EE (2003) The liver and statin drug therapy: uncertain navigation in the sea of risk-benefit (editorial). Pharmacoepidemiology Drug Safety 12(4): 303–306
356. Horlander JC, Kwo PY, Cummings OW, Koukoulis G (2001) Atorvastatin for the treatment of NASH (abstract). Gastroenterology 120: 544
357. Nair S, Wiseman M (2002) HMG-CoA reductase inhibitors in nonalcoholic fatty liver disease: is their potential hepatotoxicity an issue in these patients? A case control study based on histology (abstract). Hepatology 36: 409
358. Phillips PS, Haas RH, Bannykh S, Hathaway S, Gray NL, Kimura BJ, Vladutiu GD, England JD (2002) Statin-associated myopathy with normal creatine kinase levels. Ann Intern Med 137: 581–585
359. Mensenkamp AR, Havekes LM, Romijn JA, Kuipers F (2001) Hepatic steatosis and very low density lipoprotein secretion: the involvement of apolipoprotein E. J Hepatol 35: 816–822
360. Charlton M, Sreekumar R, Rasmussen D, Lindor K, Nair KS (2002) Apolipoprotein synthesis in nonalcoholic steatohepatitis. Hepatology 35: 898–904
361. Facchini FS, Hua NW, Stoohs RA (2002) Effect of iron depletion in carbohydrate-intolerant patients with clinical evidence of nonalcoholic fatty liver disease. Gastroenterology 122: 931–939
362. Pawlotsky J-M (2003) Hepatitis C virus genetic variability: pathogenic and clinical implications. Clin Liver Dis 7: 45–66
363. Adinolfi LE, Utili R, Andreana A, Tripodi M-F, Marracino M, Gambardella M, Giordano M, Ruggiero G (2001) Serum HCV RNA levels correlate with histological liver damage and concur with steatosis in progression of chronic hepatitis C. Dig Dis Sci 46: 1677–1683
364. Monto A, Alonzo J, Watson JJ, Grunfeld C, Wright TL (2002) Steatosis in chronic hepatitis C: relative contributions of obesity, diabetes mellitus, and alcohol. J Hepatol 36: 729–736
365. Mihm S, Fayyazi A, Hartmann H, Ramadori G (1997) Analysis of histopathological manifestations of chronic hepatitis C virus infection with respect to virus genotype. Hepatology 25: 735–739
366. Rubbia-Brandt L, Leandro G, Spahr L, Giostra E, Quadri R, Male PJ, Negro F (2001) Liver steatosis in chronic hepatitis C: a morphological sign suggesting infection with HCV genotype 3. Histopathology 39: 119–124
367. Adinolfi LE, Gambardella M, Andreana A, Tripodi M-F, Utili R, Ruggiero G (2001) Steatosis accelerates the progression of liver damage of chronic hepatitis C patients

and correlates with specific HCV genotype and visceral obesity. Hepatology 33: 1358–1364
368. Rubbia-Brandt L, Quadri R, Abid K, Giostra E, Male PJ, Mentha G, Spahr L, Zarski J-P, Borisch B, Hadengue A, Negro F (2000) Hepatocyte steatosis is a cytopathic effect of hepatitis C virus genotype 3. J Hepatol 33: 106–115
369. Rubbia-Brandt L, Giostra E, Mentha G, Quadri R, Negro F (2001) Expression of liver steatosis in hepatitis C virus infection and pattern of response to α-interferon. J Hepatol 35: 307
370. Mendler MH, Panesar J, Govindarajan S, Milstein S, Lindsay K (2002) Changes in fatty infiltration of the liver in patients treated for chronic hepatitis C: a viral or host effect (abstract)? Hepatology 36: 404
371. Hickman IJ, Clouston AD, Macdonald GA, Purdie DM, Prins JB, Ash S, Jonsson JR, Powell EE (2002) Effect of weight reduction on liver histology and biochemistry in patients with chronic hepatitis C. Gut 53(3): 413–419
372. Nair S, Verma S, Thuluvath PJ (2002) Obesity and its effect on survival in patients undergoing orthotopic liver transplantation in the United States. Hepatology 35: 105–109
373. Czaja AJ (1997) Recurrence of nonalcoholic steatohepatitis after liver transplantation. Liver Transpl Surg 3: 185–186
374. Molloy RM, Komorowski R, Varma RR (1997) Recurrent nonalcoholic steatohepatitis and cirrhosis after liver transplantation. Liver Transpl Surg 3: 177–178
375. Carson K, Washington MK, Treem WR, Clavien PA, Hunt CM (1997) Recurrence of nonalcoholic steatohepatitis in a liver transplant recipient. Liver Transpl Surg 3: 174–176
376. Kim WR, Poterucha JJ, Porayko MK, Dickerson ER, Steers JL, Weisner RH (1996) Recurrence of nonalcoholic steatohepatitis following liver transplantation. Transplantation 62: 1802–1805
377. Contos MJ, Cales W, Sterling RK, Luketic VA, Shiffman ML, Mills AS, Fisher RA, Ham J, Sanyal AJ (2001) Development of nonalcoholic fatty liver disease after orthotopic liver transplantation for cryptogenic cirrhosis. Liver Transplantation 7: 363–373
378. Ong J, Younossi ZM, Reddy V, Price LL, Gramlich T, Mayes J, Boparis N (2001) Cryptogenic cirrhosis and post-transplantation non-alcoholic fatty liver disease. Liver Transplantation 7: 797–801
379. Cassarino DS, Swerdlow RH, Parks JK, Parker WD, Bennett JP (1998) Cyclosporin A increases resting mitochondrial membrane potential in SY5Y cells and reverses the depressed mitochondrial membrane potential of Alzheimer's disease cybrids. Biochem Biophys Res Commun 248: 168–173