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SPECIAL REPORTS AND REVIEWS

Steatosis and Hepatitis C Virus: Mechanisms and Significance for Hepatic and Extrahepatic Disease

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Nonalcoholic fatty liver disease (NAFLD) and hepatitis C virus (HCV)-related liver disease are common in the general population, but their concurrence is 2- to 3-fold higher than would be expected by chance alone. In patients with chronic HCV infection, steatosis is attributable to a variable combination of the mechanisms considered to play a role in the pathogenesis of NAFLD—insulin resistance in the obese and in the lean subject—along with a direct effect of HCV on hepatic lipid metabolism that leads to triglyceride accumulation through inhibition of export proteins that are required for very low density lipoprotein (VLDL) assembly and secretion. Accumulating evidence suggests that steatosis contributes to the progression of fibrosis in HCV-related disease in a pattern similar to that observed in NAFLD. Potential mechanisms of this effect include the increased sensitivity of steatotic livers to oxidative stress and cytokine-mediated injury. Steatosis-related hepatic insulin resistance may also play a role through the profibrogenic effects of the compensatory hyperinsulinemia and provides a potential explanation for the association between HCV and type 2 diabetes mellitus. Indeed, an appreciation of the importance of fat in HCV has recently led to trials of adjuvant therapy for HCV directed at steatosis-associated disease mechanisms, with encouraging results reported for various modalities, including weight loss and antioxidants. Future therapy should be aimed at exploiting the interactions of HCV with host insulin and lipid metabolism, particularly in nonresponders to standard antiviral schedules.

The term "nonalcoholic steatohepatitis" (NASH) was first coined by Ludwig et al. in 1980.[1] NASH is now recognized to be part of a larger spectrum of disease known as nonalcoholic fatty liver disease (NAFLD) that encompasses all of the classical histologic lesions of alcoholic liver disease (steatosis, steatohepatitis with or without fibrosis and cirrhosis) but is observed in subjects who deny drinking alcohol to excess.[2] The clinical importance of NAFLD appears to have been appreciated even before Ludwig's seminal paper,[3,4] whereas its close association with the metabolic syndrome dates back to 1992.[5] Only recently, however, has this condition been recognized to be a chronic liver disease that affects a substantial proportion of the world's population.[6,7] This seems likely to be due, in part, to an epidemic explosion in the conditions associated with NAFLD (type 2 diabetes mellitus [T2DM], obesity, and dyslipidemia) as well as to an increased awareness of the condition.[8] Although asymptomatic in most cases, NAFLD may be the precursor lesion of cryptogenic cirrhosis, hepatocellular carcinoma, and end-stage liver failure necessitating transplantation.[9–14]

Similar to NAFLD, hepatitis C virus (HCV)-related liver disease is a common and clinically significant condition. HCV is an RNA virus belonging to the flaviviridae family that is transmitted parenterally and is currently considered to be one of the most common chronic viral infections worldwide. Following exposure to HCV, chronic hepatitis develops in 60%–85% of cases, and up to 20% may progress to cirrhosis.[15] HCV is a common cause of hepatocellular carcinoma and of end-stage liver disease, necessitating liver transplantation.[16] In contrast to chronic hepatitis B viral infection,[17] steatosis is a common histologic feature of chronic infection with HCV.[18] This raises 3 important questions. First, is the presence of steatosis in chronic HCV infection a chance association of 2 common conditions, or does HCV infection contribute to the development of steatosis? Second,

Abbreviations used in this paper: HCV, hepatitis C virus; MTP, microsomal triglyceride transfer protein; NAFLD, Nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; T2DM, type 2 diabetes mellitus.

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does the steatosis observed in HCV play any role in the progression of chronic HCV-related disease? Third, how can this knowledge be exploited to improve current and future treatment of HCV-related chronic liver disease? This review aims to answer these questions by analyzing the pathogenesis and potential consequences of steatosis in chronic HCV infection in light of our increasing understanding of the epidemiologic profile, risk factors, and pathogenic and clinical features of NAFLD.

Epidemiology

Is the concurrence of HCV infection and steatosis a chance or causal association? The precise prevalence of NAFLD in the general population is unknown. Epidemiologic studies using ultrasonography both from Japan[19,20] and Italy[21,22] have reported that the prevalence of "fatty liver syndromes" (fatty liver and steatohepatitis of alcoholic and nonalcoholic etiology) in the general population is around 20% (reviewed in Loria et al.[23]). An ad hoc analysis of the NHANES III database suggests that the prevalence of NAFLD in the United States may be as high as 14%–31% of men and 11%–16% of women.[2] The prevalence of anti-HCV positivity in the United States is 1.8%, corresponding to an estimated 3.9 million people with chronic HCV infection.[24] In Northern Italy, the prevalence of HCV chronic infection increases as a function of the age, averaging 3.2% in the general population.[22] This is markedly lower than data from central and some areas of southern Italy, where figures range from 8%–25%, respectively.[25,26]

Although NAFLD and HCV-related liver disease are common conditions and would therefore be expected to coexist by chance in some patients, circumstantial evidence suggests that their concurrence is greater than predicted by chance alone. The prevalence of HCV infection in Chinese patients with NAFLD is higher than in the general population.[27] Conversely, the prevalence of steatosis in liver biopsy specimens from patients with chronic HCV infection has been reported at around 50%, with a range of 30%–70%.[28–30] From the data on prevalence of NAFLD and chronic HCV infection quoted above, it can be calculated that the frequency of a chance concurrence of HCV and steatosis should be 0.36% of the general population or 20% of all cases of HCV infections. Therefore, the observed value of steatosis in HCV-infected patients is 2.5-fold higher than would be expected to occur by chance alone. This suggests either that chronic HCV infection directly causes steatosis and/or that the presence of steatosis favors the progression of HCV-related liver disease, resulting in its higher than expected frequency in HCV patients subjected to liver biopsy.

Mechanisms and Factors Associated With Steatosis in HCV

Insulin Resistance

It has recently been shown that the presence of steatosis in some patients with chronic HCV infection is associated with risk factors for NASH rather than with alcohol consumption.[31] This finding implies that steatosis in the context of HCV chronic infection may represent a host-related reaction and enables us to anticipate that conditions associated with NAFLD, principally insulin resistance and its clinical correlates, including obesity, diabetes, and arterial hypertension, will also be associated with steatosis in HCV. Obesity is the best established risk factor, both for the occurrence of NAFLD and for its progression to fibrosis and cirrhosis (reviewed in Loria et al.[23]). The association between obesity, particularly with a central-type body fat distribution,[32] and steatosis is now thought to be due to the associated insulin resistance and secondary hyperinsulinemia which, in turn, lead to an increased supply of free fatty acids to the liver and to inhibition of hepatic lipid oxidation and export.[33–35] The association between obesity, insulin resistance, and fibrosis in NAFLD may be due to steatosis per se being involved in fibrogenesis, or alternatively, to high levels of insulin and glucose stimulating the release of the fibrogenic growth factor or connective tissue growth factor (CTGF) from hepatic stellate cells[36] and/or to a fibrogenic effect of the adipose tissue–derived hormone leptin (reviewed in Anania[37]). It is perhaps not surprising, therefore, that an increased body mass index (BMI) and T2DM have both been associated with the development of steatosis and fibrosis in patients with chronic HCV infection.[38–42] More recent studies have shown that the steatosis in patients with chronic HCV is strictly correlated with abdominal fat mass,[43] suggesting that, as in NAFLD, it is insulin resistance and an increased supply of fatty acids to the liver that is important in the pathogenesis of obesity-related steatosis in chronic HCV infection.

A Direct Steatogenic Effect of HCV

Some of this article's authors (L.E.A., G.R.) have recently demonstrated that, although obesity plays a role in the development of steatosis in patients with HCV genotypes 1 and perhaps 2a/c, it is not essential in patients infected with genotype 3a.[43] In this latter group,[44,45] and in patients infected with HCV of unknown genotype,[46] steatosis correlates with levels of in-

trahepatic viral replication, suggesting that HCV exerts a direct steatogenic effect. This is further supported by a recent report that steatosis resolves following successful antiviral treatment in patients with chronic hepatitis C infected with genotype 3 but not genotype 1.[47] Consistent with a direct steatogenic effect of HCV, it has been shown that the HCV core protein induces accumulation of intracytoplasmic triglyceride-rich droplets in transgenic mice and transfected cells,[48,49] with the effect most prominent in (although not restricted to) cells transfected with constructs derived from genotype 3 isolates.[50] The core protein expression alters the double membrane structure within mitochondria and causes an impairment of lipid oxidation, which contributes to the development of steatosis.[51] More recently, it has been shown in the HCV core protein transgenic mouse model of HCV-related steatosis that the HCV core protein targets microsomal triglyceride transfer protein (MTP) activity, thus interfering with the hepatic assembly and secretion of apolipoprotein (apo) B-containing very low density lipoproteins (VLDL).[52] Consistent with this model, studies in humans have demonstrated that the severity of steatosis in patients with chronic HCV correlates with hypobetalipoproteinemia,[53] mirroring the situation in congenital abetalipoproteinemia in which steatosis is due to mutations in the MTP gene leading to impaired VLDL assembly.[54] This is in marked contrast to the situation in "normal" NAFLD in which hyperlipidemia is common[55] and serum levels of triglycerides, cholesterol, and apoB are independent predictors of an ultrasonographic "bright liver."[21] Here, as in T2DM, hypertriglyceridemia is the most common finding and probably results from steatosis-associated insulin resistance attenuating the down-regulating effect of hyperinsulinemia on hepatic apoB-100 synthesis and accordingly VLDL secretion.[56] The development and persistence of steatosis in these patients is presumably due to hepatic apoB synthesis remaining low relative to triglyceride synthesis despite the presence of hepatic insulin resistance.[33,34,57]

Steatosis and Progression of Chronic Hepatitis C

Given that steatosis is common in chronic HCV, what is the evidence that it plays a role in disease progression? Similar to studies in NAFLD,[58,59] indirect evidence supporting a role for steatosis in progression of chronic HCV has come from several studies showing that the severity of steatosis on index biopsy or its worsening are independent predictors of both the severity and the progression of fibrosis in HCV-positive patients with and without genotype 3.[40,43,60–63] Further evidence that steatosis is involved in the pathogenesis of fibrosis in HCV comes from the striking similarity between risk factors for disease progression in chronic HCV and NAFLD. Several studies have shown that alcohol intake, BMI, T2DM, age, and male gender are associated with fibrosis in HCV-related liver disease,[40–42,64] and all of these factors have also been associated with an increased risk of fibrosis in patients with NAFLD.[64–68] Alcohol intake, BMI, T2DM, and, most recently reported, increasing age[69] would all be expected to increase the severity of steatosis as at least one potential profibrotic mechanism. Perhaps the best evidence that steatosis plays a role in the HCV-related fibrosis thus far comes from the recent finding that weight reduction in chronic HCV-related liver disease leads not only to a decrease in steatosis and in abnormal liver enzymes but also to an improvement in fibrosis despite persistence of the virus.[70] Importantly, steatosis may also adversely affect the response to α-interferon therapy,[71] offering an explanation for reports that elevated serum γ-GT, a common finding in steatosis,[43,72] predicts a low chance of response to interferon therapy.[73–76] These observations have led to the as yet untested, hypothesis that weight reduction may improve the response to interferon in chronic HCV infection through a reduction in steatosis.[43,77]

Mechanisms of Fibrosis Associated With Steatosis in HCV

If, as seems likely, steatosis is involved in the fibrotic progression of HCV, what are the potential mechanisms of this effect? A recent study has reported that steatohepatitis-type lesions, sinusoidal fibrosis, and ballooning degeneration are present in 16% and 19% of patients with chronic HCV, respectively, and, along with age and the presence of T2DM, correlate with the severity of fibrosis.[42] A second study has confirmed that the pattern of fibrosis in HCV (subsinusoidal and central vein) is similar to that observed in NAFLD.[61] These reports suggest that the mechanisms of fibrosis in HCV-associated steatosis are likely to share similarities with those involved in NAFLD. A recent consensus has emerged that steatosis—the first "hit"—requires a second "hit" or insult for its progression to inflammation and fibrosis.[8,78] The strongest candidates for this second hit in NAFLD are oxidative stress leading to lipid peroxidation—the products of which are both proinflammatory and profibrotic—and cytokines. Certainly, lipid peroxidation has been detected in liver biopsy specimens from patients with chronic HCV, and its magnitude and location correlate with the stage and site of fibrosis,

respectively.[79] Further support for a role of oxidative stress in the progression of chronic HCV has come from a pilot study showing that antioxidant therapy with vitamin E abrogates the fibrogenesis cascade in patients with chronic HCV refractory to interferon therapy.[80] The oxidant stimulus for lipid peroxidation in HCV is unclear at present; however, as in NAFLD, increased fatty acid oxidation by mitochondria and microsomal CYP2E1 secondary to steatosis-induced hepatic insulin resistance seem likely to play a role in that both generate reactive oxygen species.[81–83] However, the fact that fibrosis appears to be more common in HCV-related steatosis than in NAFLD despite a greater amount of fat in the latter condition suggests that the second hits are of greater magnitude in HCV-related disease. The most obvious candidate for the "extra" second hit in chronic HCV infection is the antiviral inflammatory response, which will produce a source of free radicals as well as proinflammatory and profibrotic cytokines. In support, the study by Clouston et al. showed that the degree of sinusoidal fibrosis, in addition to steatosis, correlated with the severity of both portal and lobular inflammation,[61] and steatotic livers have an increased sensitivity to cytokine-mediated inflammation in an animal model of NAFLD.[84] More recently, it has been shown, both in vitro and in vivo, that HCV core protein may induce oxidative stress directly through an effect on mitochondrial electron transport.[85] In addition to a role in fibrosis, this induction of oxidative stress by HCV may contribute to HCV-related hepatocarcinogenesis.[86] In addition to the direct profibrogenic effect of steatosis acting as the "first hit," the potential contribution of hepatic steatosis to insulin resistance discussed below may contribute indirectly to NAFLD and HCV-related fibrosis via hyperinsulinemia and hyperglycemia, increasing the expression of CTGF in the liver.[36]

Role of Steatosis in the Association Between HCV and Type 2 Diabetes

Type 2 Diabetes Mellitus and NAFLD

Previous studies have reported that between 21% and 55% of patients with NAFLD have either overt T2DM or hyperglycemia.[87] More recent studies have reported that insulin resistance is present in almost all patients with NAFLD irrespective of the coexistence of impaired glucose tolerance or obesity,[35,81,88,89] leading to the suggestion that NAFLD/NASH is the liver manifestation of the insulin resistance or metabolic syndrome.[88] Contrary to previous reports, these studies showed that the hyperinsulinemia was not due to impaired hepatic insulin extraction but to enhanced insulin secretion compensating for insulin resistance.[35,89] Accordingly, NAFLD has been considered to be an *effect* of peripheral insulin resistance because of an increased supply of fatty acids from unopposed adipose tissue lipolysis along with the steatogenic effects of the secondary hyperinsulinemia.[90] However, as alluded to above, a growing body of evidence has recently shown that the accumulation of fat in nonadipose tissue, including muscle and liver, is associated with insulin resistance as part of the syndrome of lipotoxicity.[91] Support for this hypothesis comes from observations in patients with lipodystrophy syndromes that have a severe deficiency/absence of peripheral adipose tissue. Possibly as a result of the limitation in triglyceride deposition in adipose tissue storage depots (which may divert triglycerides for accumulation in other tissues such as the liver), along with leptin deficiency,[92] these patients develop marked hepatic steatosis, which can be associated with NASH, and are severely insulin resistant.[93–95] This suggests that, in NAFLD, steatosis may be a contributory *cause* as well as an effect of insulin resistance and T2DM. In support, 2 studies have demonstrated impaired insulin-mediated suppression of hepatic glucose production in patients with NAFLD compared with controls.[81,88] A third study has reported that hepatic steatosis is characterized by insulin resistance in normal weight and moderately overweight subjects independent of body mass index and intra-abdominal and overall obesity.[96] The mechanism of the anti-insulin effect of steatosis is unclear but may involve an increased concentration of fatty acids or their metabolites that have been postulated to activate a serine kinase cascade leading to defects in insulin signaling.[91] Polyunsaturated fatty acids, particularly those of the n-3 family, have been shown to suppress directly the up-regulation of lipogenic and glycolytic enzymes by insulin,[97] and free fatty acids also directly impair the ability of insulin to suppress hepatic glucose output.[98] As discussed above, once established, hyperglycemia, along with insulin, up-regulates the expression of CTGF[36] providing a further explanation for the associations between steatosis severity, the presence of T2DM/hyperinsulinemia, and fibrosis in NAFLD and probably in chronic HCV infection.

Type 2 Diabetes and HCV

The association between chronic HCV and T2DM has been reported by several groups working in different geographic areas,[99–104] with only one study reporting a lack of any association.[104] A large case-control study showed that chronic HCV infection and age were the only significant independent predictors for T2DM,[100] and a more recent study has confirmed the association

with age, showing that T2DM is more common in patients with HCV over the age of 40 years.[103] In this latter study, the prevalence of HCV genotype 2a was significantly higher in HCV patients with T2DM (29%) than in HCV patients without T2DM (3%). Interestingly, genotype 2a has also been reported to be associated with mixed cryoglobulinemia and monoclonal gammopathy in patients with chronic HCV infection.[106–108] The T2DM seen in patients with chronic HCV is associated with worsening insulin sensitivity and an impaired first phase insulin response[101] and is independent of the presence of cirrhosis.[101,102] It has, therefore, been suggested that HCV is diabetogenic through an extrahepatic effect, which might be either direct, through the HCV core protein,[104] or immune-mediated.[109] Alternatively, in light of the above discussion, it would appear logical to suggest that HCV infection causes T2DM through the induction of hepatic steatosis and associated hepatic insulin resistance, which, if prolonged, will lead to the development of glucose intolerance in predisposed individuals. In this model, the increased incidence of T2DM with age in HCV is explained by the recently reported age-related increase in liver and muscle lipid content and associated insulin resistance that has been attributed to a decline in mitochondrial oxidative and phosphorylation function.[69] In support of this hypothesis, a recent study in 103 HCV patients has reported that only 25% with T2DM had no steatosis compared with 50% without T2DM.[110] If this hypothesis were true, one would expect that the same HCV genotype(s) that induce steatosis would also be associated with T2DM. Patients infected with genotype 3 have the highest prevalence of steatosis[43–45,111–113] followed by type 2a/c and type 1,[43,112] whereas only genotype 2a has been associated with T2DM.[100] It is important to note, however, that patients infected with genotype 3a are significantly younger that those infected with genotype 2a/c,[43,111,113] and because the prevalence of T2DM increases with age, this may account for the lack of association between genotype 3a and T2DM at present.

Steatosis, HCV, and Atherosclerosis

The observation that arterial hypertension is highly prevalent in subjects with ultrasonographic evidence of steatosis[114,115] further supports the notion that NAFLD is part of the metabolic syndrome. More unexpectedly, hypertension has been shown to be an independent predictor of advanced fibrosis in obesity-associated NAFLD, attributed by the authors of the study to a potential fibrogenic effect of angiotensin II.[68] Hypertension, along with other features of the metabolic syndrome, insulin-resistance, and dyslipidemia (high triglyceride, low HDL-cholesterol), plays a significant role in the pathogenesis of atherosclerosis. Because steatosis can contribute to the development of insulin resistance and subsequent dyslipidemia, we have hypothesized that it could trigger, worsen, or perpetuate these cardiovascular risk factors and play a central role in atherogenesis in patients with NAFLD.[34,57] The prevalence and significance, if any, of arterial hypertension in patients with chronic HCV infection remain to be ascertained. However, an association between HCV infection and the risk of atherosclerosis has recently been reported in a series of 4784 subjects from Japan. HCV seropositivity was associated with carotid artery plaque formation and carotid intima-media thickening, which was independent of the classical risk factors for atherosclerosis.[116] The role of steatosis in development of atheroma in HCV seems worthy of further study.

Therapeutic Approaches: Present and the Future

Current Therapy for NAFLD

If it is accepted that the steatosis occurring in association with chronic HCV infection is important in disease progression and possibly some of the extrahepatic manifestations, can this be used to inform treatment strategies for this growing number of patients? Optimal therapy for NAFLD has not been established but traditionally includes dietary intervention and correction of comorbid risk factors.[6,7,13,117,118] Weight loss achieved by a moderately restricted diet, with or without exercise and optimal treatment of T2DM and dyslipidemia, has been consistently shown to result in biochemical, ultrasonographic, and even histologic improvement in some patients.[119–125] No pharmacologic treatments for NAFLD have been tested in randomized clinical trials; however, a number of encouraging pilot studies have been reported with treatments directed at either oxidant stress or insulin resistance. Thus, treatment with the weak antioxidant ursodeoxycholic acid (UDCA) resulted in a significant improvement in liver biochemistry but not in hepatic histology.[125] Vitamin E (α-tocopherol) normalized liver biochemistry in children with NASH[127] and improved biochemistry and histology in adults, and betaine, another antioxidant agent, also leads to a significant improvement in serum aminotransferase levels and histology in 10 adults with NASH.[128] Metformin and the thiazolidinediones troglitazone and rosiglitazone that improve insulin sensitivity, therefore reducing hyperinsulinemia, have been successfully used in NASH in 3

human pilot studies and a mouse model of NAFLD.[129–132] Phlebotomy to near iron depletion has also recently been reported to normalize aminotransferase and plasma glucose levels in NAFLD patients with impaired glucose tolerance.[133] Whether this reflects a beneficial effect on insulin clearance,[134] hepatic insulin sensitivity,[135] or an antioxidant effect is unclear at present.

Therapy for HCV-Related Liver Disease Directed at Steatosis

Treatment for patients with chronic hepatitis C is currently based on antiviral therapy with a combination of interferon and ribavirin. Unfortunately, this leads to a sustained clearance of virus in only 30%–60% of patients,[136,137] indicating a need for alternative or adjuvant therapy. Evidence that steatosis may be involved in the progression of chronic HCV and may impair the response to interferon[71] has recently led several investigators to explore adjuvant therapy for HCV that is directed either at steatosis—the first "hit"—or at oxidative stress—an important second "hit" in the progression of NAFLD. Moreover, the beneficial effects of some other adjuvant treatments may be explained by their effects on steatosis-related mechanisms. Considering the first hit, a recent pilot study has shown that weight loss in patients with chronic HCV is associated with a reduction in steatosis and an improvement in liver biochemistry and fibrosis in the absence of any antiviral effects.[70] If confirmed by further studies, this would suggest that weight reduction and/or other lifestyle/pharmacologic therapies aimed at improving insulin sensitivity should be important adjuvant treatment strategies for patients with chronic HCV. This appears to be the case for nonsteroidal antiinflammatory drugs, which have also been used as adjuvant therapy in chronic HCV on the basis of their ability to enhance the activity of interferon, with a pilot study reporting that some interferon nonresponders can benefit from a combination of interferon and ketoprofen.[138] Recent evidence that nonsteroidal antiinflammatory drugs ameliorate insulin resistance by inhibiting IκB kinase β[139,140] suggests that their beneficial effect may also be due to an improvement in peripheral and hepatic insulin resistance in HCV patients with steatosis.

With respect to the second hit (oxidative stress), several studies have demonstrated that UDCA, alone or in combination with interferon, may reduce disease severity in patients with chronic HCV,[141,142] and 2 pilot studies have reported beneficial effects of vitamin E either as monotherapy[80] or as an adjuvant to interferon therapy.[143] As in NAFLD, the reported beneficial effect of iron depletion in chronic HCV, either by menstruation[144] or by phlebotomy,[145–150] may also be due to improved hepatic insulin sensitivity and lowered insulin levels,[151] in addition to any antioxidant effect or a direct effect on viral replication.[152,153] In support of a role for iron in the progression of HCV-related liver disease are 2 recent studies that have used multivariate regression analysis to control for confounding factors and have reported that both hemochromatosis-associated *HFE* gene mutations are associated with iron loading and advanced fibrosis in HCV-infected individuals.[154,155] This contrasts with the situation in NAFLD in which there is, as yet, no convincing and reproducible evidence that hepatic iron excess or the presence of *HFE* gene mutations are associated with disease severity.[156,157] On the basis of the current data, we suggest that there is now sufficient evidence to justify pilot studies of established[129–132] and novel[158] insulin-sensitizing drugs in patients with HCV infection and steatosis, particularly in nonresponders to standard antiviral therapy.

Therapy for HCV and Host Lipid Metabolism

In addition to its effects on hepatic lipid metabolism that contribute directly to the development of steatosis,[48,52,159] other interactions between HCV and host lipid metabolism have important implications both for the treatment of any associated hyperlipidemia and potentially for the treatment of HCV per se. HCV core protein is localized around cytoplasmic lipid droplets in vitro in HCV core expressing cells and binds to apolipoprotein A2 (apoA2).[52,160] The HCV nonstructural protein NS5A colocalizes with core protein on these lipid droplets in hepatocyte cell lines and associates with apoA1 as well as with apoA2.[161] The biologic significance of these interactions between structural and nonstructural HCV proteins and apoA proteins, the major protein components of high-density lipoprotein (HDL) particles, is presently unknown, although some evidence suggests that the interaction may favor the secretion of viral proteins from the cell.[160] In addition to these HCV-lipid interactions within the hepatocyte, HCV circulates in association with β-lipoproteins (low-density lipoprotein [LDL] and VLDL) in the sera of infected patients.[162] This led Monazahian et al. to propose a model whereby HCV infects hepatocytes via the LDL receptor.[163] This model anticipates that an increase in serum β-lipoproteins would interfere with the rate of HCV infection of liver cells, resulting in decreased HCV replication in the liver and, accordingly, decreased HCV antigen in the sera. In support, Andre et al.[164] reported that HCV RNA circulates in large spherical particles (100 nm) containing triglycerides, apoB, and core protein. These particles bind to and enter hepatocyte cell lines in a competitive way with VLDL and LDL. Anti-apoB antibodies de-

crease, and LDL receptor up-regulation increases, their internalization into cells. This model provides a potential teleological explanation for the inhibition of MTP by HCV. The inhibition of VLDL secretion by hepatocytes and the resulting lowered serum concentration of VLDL and LDL will favor the entry of HCV into hepatocytes by reducing competition for the LDL receptor. Steatosis can therefore be viewed as an epiphenomenon of this prosurvival effect of HCV. In view of the inhibitory effect of HCV on MTP activity and VLDL secretion,[52,53] hyperlipidemia is uncommon in patients with chronic HCV. Nevertheless, this model has potential implications for the use of lipid-lowering drugs in these patients. Treatment of hypercholesterolemia with statins, which increase the expression of LDL receptors,[165] might facilitate the entry of HCV into hepatocytes and exacerbate chronic HCV infection, whereas treatment of hypertriglyceridemia with fibrates, which induce the expression of apoA2 in hepatocyte cell lines,[166] may modulate the secretion of HCV core protein.[161] Of greater interest, however, is the potential of using the interaction between HCV and host lipid metabolism to reduce hepatic HCV replication. For example, lifestyle changes or drug treatments that induce a decrease in the number of LDL receptors will theoretically decrease HCV entry into hepatocytes and subsequent replication.

Summary

Steatosis is common in patients with chronic HCV infection because of a combination of the usual risk factors for NAFLD and to a direct steatogenic effect of some genotypes of the virus. The capacity of HCV to induce steatosis directly through interference with lipid metabolism may teleologically represent a mechanism favoring the entry of HCV into hepatocytes with a subsequent increase in viral replication. A growing body of evidence supports the view that steatosis plays a role in the progression of HCV to fibrosis providing the substrate or "first hit" for HCV-related oxidative stress and cytokine release—the "second hits"—to induce necroinflammation, apoptosis, and fibrosis. Steatosis may also be involved in some of the extrahepatic manifestations of HCV, including type 2 diabetes and atherosclerosis.

Future Research Needs

Although a considerable body of work has been performed on the mechanisms and significance of steatosis occurring in patients with chronic HCV infection, a number of outstanding questions remain unanswered that clearly warrant further study. First, with respect to mechanisms of steatosis, more direct evidence of a role for insulin resistance in the pathogenesis of HCV-related steatosis should be sought by correlating steatosis severity with measures of hepatic, muscle, and adipose tissue insulin sensitivity, derived from formal clamp studies combined with isotopic tracer studies.[81] It will be important to determine whether any associations observed between tissue-specific insulin sensitivity and steatosis differ according to viral genotype. With respect to the direct steatogenic effect of HCV, more studies on transfected cells and transgenic mice using genotype-specific constructs are required to increase our understanding of the mechanistic basis for the apparent between-genotype differences in steatogenic potential. Second, with respect to mechanisms of fibrosis, the recently reported association between a "low activity" promoter polymorphism in the MTP gene and steatosis and fibrosis severity in NAFLD[167,168] provides an excellent opportunity to look for genetic evidence of a link between steatosis and fibrosis in HCV. Whether the profibrogenic effect of steatosis is via sensitizing the liver to the putative second "hits" or by contributing to insulin resistance will be difficult to determine; however, indirect evidence that the latter mechanism plays a role should be sought by looking for a correlation between fibrosis severity in HCV and fasting levels of insulin and glucose as has been reported in both NAFLD[68] and alcoholic liver disease.[169]

The apparent ability of HCV genotype 3a to induce steatosis in lean patients[43] provides an opportunity to test the hypothesis that steatosis per se is capable of inducing insulin resistance in the absence of concomitant obesity and thereby provides more direct evidence of a role for steatosis in the pathogenesis of HCV and non-HCV-related type 2 diabetes. If this can be confirmed, then studies examining the frequency of other features of the metabolic syndrome in patients with HCV appear warranted, as do studies assessing the prevalence of ischemic vascular disease and its association with steatosis. Finally, with respect to treatment of HCV, there now appears to be sufficient scientific rationale, along with some encouraging pilot data, to justify formal randomized controlled trials of pharmacologic and nonpharmacological therapies directed at steatosis and associated insulin resistance in patients who are nonresponders to antiviral therapy. In addition, the development of antiviral therapies based on exploiting the interaction between HCV and host lipid metabolism appears to be a particularly fertile area for future research efforts.

References

1. Ludwig J, Viggiano TR, McGill DB, Oh BJ. Nonalcoholic steatohepatitis: Mayo Clinic experiences with a hitherto unnamed disease. Mayo Clin Proc 1980;55:434–438.

2. Sanyal A. AGA technical review on nonalcoholic fatty liver diseases. Gastroenterology 2002;123:1705–1725.
3. Massarrat S, Asholt K, Schmitz-Moormann P. Should nonalcoholic fatty liver be considered precirrhotic? Verh Dtsch Inn Med 1977;83:543–546.
4. Itoh S, Igarashi M, Tsukada Y, Ichinoe A. Nonalcoholic fatty liver with alcoholic hyalin after long-term glucocorticoid therapy. Acta Hepatogastroenterol 1977;24:415–418.
5. Huth K, Burkard M, Goebel T. Dyslipoproteinemia and diabetes mellitus in a metabolic syndrome. Fortschr Med 1992;110: 200–204.
6. Angulo P. Nonalcoholic fatty liver disease. N Engl J Med 2002; 346:1221–1231.
7. McCullough AJ. Update on nonalcoholic fatty liver disease. J Clin Gastroenterol 2002;34:255–262.
8. Day CP. Non-alcoholic steatohepatitis (NASH): where are we now and where are we going? Gut 2002;50:585–588.
9. Poonawala A, Nair SP, Thuluvath PJ. Prevalence of obesity and diabetes in patients with cryptogenic cirrhosis: a case-control study. Hepatology 2000;32:689–692.
10. Caldwell SH, Oelsner DH, Iezzoni JC, Hespenheide EE, Battle EH, Driscoll CJ. Cryptogenic cirrhosis: clinical characterization and risk factors for underlying disease. Hepatology 1999;29: 664–669.
11. Keefe EB. Liver transplantation: current status and novel approaches to liver replacement. Gastroenterology 2001;120: 749–762.
12. Czaja AJ. Recurrence of nonalcoholic steatohepatitis after liver transplantation. Liver Transpl Surg 1997;3:185–186.
13. Marchesini G, Forlani G. NASH: from liver diseases to metabolic disorders and back to clinical hepatology. Hepatology 2002;35: 497–499.
14. Bugianesi E, Leone N, Vanni E, Marchesini G, Brunello F, Carucci P, Musso A, De Paolis P, Capussotti L, Salizzoni M, Rizzetto M. Expanding the natural history of nonalcoholic steatohepatitis: from cryptogenic cirrhosis to hepatocellular carcinoma. Gastroenterology 2002;123:134–140.
15. Seeff LB. Natural history of chronic hepatitis C. Hepatology 2002;36:S35–S46.
16. El-Serag HB. Hepatocellular carcinoma and hepatitis C in the United States. Hepatology 2002;36:S74–S83.
17. Goodman ZD, Ishak KG. Histopathology of hepatitis C virus infection. Semin Liver Dis 1995;15:70–81.
18. Giusti G, Pasquale G, Galante D, Russo M, Sardaro C, Gallo C, Gaeta GB. Clinical and histological aspects of chronic HCV infection and cirrhosis. Hepatogastroenterology 1993;40:365–369.
19. Matsuura K, Tobe K, Tsuji T. Fatty liver and obesity in university students. Nippon Shokakibyo Gakkai Zasshi 1995;92:1743–1745.
20. Nomura H, Kashiwagi S, Hayashi J, Kajiyama W, Tani S, Goto M. Prevalence of fatty liver in a general population of Okinawa, Japan. Jpn J Med 1988;27:142–149.
21. Lonardo A, Bellini M, Tartoni M, Tondelli E. The bright liver syndrome. Prevalence and determinants of a "bright" liver echopattern. Ital J Gastroenterol Hepatol 1997;29:351–356.
22. Bellentani S, Tiribelli C. The spectrum of liver disease in the general population: lessons from the Dionysos study. J Hepatol 2001;35:531–537.
23. Loria P, Lonardo A, Carulli N. NASH (nonalcoholic steatohepatitis): aspetti diagnostici ed epidemiologici. In: Gentilini P, ed. Aggiornamenti di fistopatologia epatica 4. Firenze: Scientific Press, 2001;231–247.
24. Alter MJ, Kruszon-Moran D, Nainan OV, McQuillan GM, Gao F, Moyer LA, Kaslow LA, Margolis HS. The prevalence of hepatitis C virus infection in the United States, 1988 through 1994. N Engl J Med 1999;341:556–562.
25. Stroffolini T, Menchinelli M, Taliani G, Dambruoso V, Poliandri G, Bozza A, Lecce R, Clementi C, Menniti Ippolito F, Compagnoni A, De Bac C. High prevalence of hepatitis C virus infection in a small central Italian town: lack of evidence of parenteral exposure. Ital J Gastroenterol 1995;27:235–238.
26. Osella AR, Misciagna G, Leone A, Di Leo A, Fiore G. Epidemiology of hepatitis C virus infection in an area of Southern Italy. J Hepatol 1997;27:30–35.
27. Lin SC, Shih SC, Kao CR, Chou SY. Prevalence of antibodies to hepatitis C virus in patients with nonalcoholic fatty liver. Chuang Hua I Hsue Tsa Chih 1995;56:80–85.
28. Scheuer PJ, Ashrafzadeh P, Sherlock S, Brown D, Dusheiko GM. The pathology of hepatitis C. Hepatology 1992;15:567–571.
29. Bach N, Thung SN, Schaffner F. The histological features of chronic hepatitis C and autoimmune chronic hepatitis C: a comparative analysis. Hepatology 1992;15:572–577.
30. Lefkowitch JH, Schiff ER, Davis GL, Perrillo RP, Lindsay K, Bodenheimer HC Jr, Balart LA, Ortego TJ, Payne J, Dienstag JL. Pathological diagnosis of chronic hepatitis C: a multicenter comparative study with chronic hepatitis B. The Hepatitis Interventional Therapy Group. Gastroenterology 1993;104: 595–603.
31. Monto A, Alonzo J, Watson JJ, Grunfeld C, Wright TL. Steatosis in chronic hepatitis C: relative contribution of obesity, diabetes mellitus and alcohol. Hepatology 2002;36:729–736.
32. Lonardo A, Trande P. Are there any sex differences in fatty liver? A study of glucose metabolism and body fat distribution. J Gastroenterol Hepatol 2000;15:775–782.
33. Charlton M, Sreekumar R, Rasmussen D, Lindor K, Nair KS. Apolipoprotein synthesis in nonalcoholic steatohepatitis. Hepatology 2002;35:898–904.
34. Lonardo A, Loria P. Apolipoprotein synthesis in nonalcoholic steatohepatitis. (Letter) Hepatology 2002;36:514–515.
35. Chitturi S, Abeygunasekera S, Farrell GC, Holmes-Walker J, Hui JM, Fung C, Karim R, Lin R, Samarasinghe D, Liddle C, Weltman M, George. NASH and insulin resistance: insulin hypersecretion and specific association with the insulin resistance syndrome. Hepatology 2002;35:373–379.
36. Paradis V, Perlemuter G, Bonvoust F, Dargere D, Parfait B, Vidaud M, Conti M, Huet S, Ba N, Buffet C, Bedossa P. High glucose and hyperinsulinemia stimulate connective tissue growth factor expression: a potential mechanism involved in progression to fibrosis in nonalcoholic steatohepatitis. Hepatology 2001;34:738–744.
37. Anania FA. Leptin, liver, and obese mice—fibrosis in the fat lane. Hepatology 2002;36:246–248.
38. Fiore G, Fera G, Napoli N, Velia F, Schiraldi O. Liver steatosis and chronic hepatitis C: a spurious association? Eur J Gastroenterol Hepatol 1996;8:125–129.
39. Czaja AJ, Carpenter HA, Santrach PJ, Moore SB. Host- and disease-specific factors affecting steatosis in chronic hepatitis C. J Hepatol 1998;29:198–206.
40. Hourigan LF, MacDonald GA, Purdie D, Whitehall VH, Shorthouse C, Clouston A, Powell EE. Fibrosis in chronic hepatitis C correlates significantly with body mass index and steatosis. Hepatology 1999;29:1215–1219.
41. Cimino L, Oriani G, D'Arienzo A, Manguso F, Loguercio C, Ascione A, Caporaso N, Del Vecchio Blanco C, Budillon G. Interactions between metabolic disorders (diabetes, gallstones, and dyslipidaemia) and the progression of chronic hepatitis C virus infection to cirrhosis and hepatocellular carcinoma. A cross-sectional multicentre survey. Dig Liver Dis 2001;33:240–246.
42. Ong JP, Younossi ZM, Speer C, Olano A, Gramlich T, Boparai N. Chronic hepatitis C and superimposed nonalcoholic fatty liver disease. Liver 2001;21:266–271.
43. Adinolfi LE, Gambardella M, Andreana A, Tripodi MF, Utili R, Ruggero G. Steatosis accelerates the progression of liver dam-

age of chronic hepatitis C patients and correlates with specific HCV genotype and visceral obesity. Hepatology 2001;33: 1358–1364.
44. Rubbia-Brandt L, Quadri R, Abid K, Giostra E, Male PJ, Mentha G, Spahr L, Zarski JP, Borisch B, Hadengue A, Negro F. Hepatocyte steatosis is a cytopathic effect of hepatitis C virus genome 3. J Hepatol 2000;33:106–115.
45. Rubbia-Brandt L, Leandro G, Spahr L, Giostra E, Quadri R, Male PJ, Negro F. Liver steatosis in chronic hepatitis C: a morphologic sign suggesting infection with HCV genotype 3. Histopathology 2001;39:119–124.
46. Fujie H, Yotsuyanagi H, Moriya K, Shintani Y, Tsutsumi T, Takayama T, Makuuchi M, Matsuura Y, Miyamura T, Kimura S, Koike K. Steatosis and intrahepatic hepatitis C virus in chronic hepatitis. J Med Virol 1999;59:141–145.
47. Kumar D, Farrell GC, Fung C, George J. Hepatitis C virus genotype 3 is cytopathic to hepatocytes. Genotype-specific reversal of hepatic steatosis after sustained response to antiviral therapy. Hepatology 2002;36:1266–1272.
48. Moriya K, Yotsuyanagi H, Shintani Y, Fujie H, Ishibashi K, Matsuura Y, Miyamura T, Koike K. Hepatitis C virus core protein induces hepatic steatosis in transgenic mice. J Gen Virol 1997; 78:1527–1531.
49. Barba G, Harper F, Harada T, Kohara M, Goulinet S, Matsuura Y, Eder G, Schaff Z, Chapman MJ, Miyamura T, Brechot C. Hepatitis C virus core protein shows a cytoplasmic localization and associates to cellular lipid storage droplets. Proc Natl Acad Sci U S A 1997;94:1200–1205.
50. Abid K, Rossi C, Latorre P, Rubbia-Brandt L, Hadengue A, Negro F. The core protein of HCV genotype 3a, 3h and 1b induces lipid accumulation in Huh7 cells. Proceedings of the 9th International Meeting on HCV and Related Viruses, San Diego, July 7–11. (Abstract P-166). 2002:143.
51. Moriya K, Fujie H, Shintani Y, Yoysuyanagi H, Tsutsumi T, Ishibashi K, Matsuura Y, Kimura S, Miyamura T, Koike K. The core protein of hepatitis C virus induces hepatocellular carcinoma in transgenic mice. Nat Med 1998;4:1065–1067.
52. Perlemuter G, Sabile A, Letteron P, Vona G, Topilco A, Chretien Y, Koike K, Pessayre D, Chapman J, Barba G, Brechot C. Hepatitis C virus core protein inhibits microsomal triglyceride transfer protein activity and very low density lipoprotein secretion: a model of viral-related steatosis. FASEB J 2002;16:185–194.
53. Serfaty L, Andreani T, Giral P, Carbonell N, Chazouilliers O, Poupon R. Hepatitis C virus induced hypobetalipoproteinemia: a possible mechanism for steatosis in chronic hepatitis C. J Hepatol 2001;34:428–434.
54. Wetterau JR, Aggerbeck LP, Bouma ME, Eisenberg C, Munck A, Hermier M, Schmitz J, Gay G, Rader DJ, Gregg RE. Absence of microsomal triglyceride transfer protein in individuals with abetalipoproteinemia. Science 1992;258:999–1001.
55. James OF, Day CP. Non-alcoholic steatohepatitis (NASH): a disease of emerging identity and importance. J Hepatol 1998; 29:495–501.
56. Cummings MH, Watts GF, Umpleby AM, Hennessy TR, Naoumova R, Slavin BM, Thompson GR, Sönksen PH. Increased hepatic secretion of very-low-density lipoprotein apolipoprotein B-100 in NIDDM. Diabetologia 1995;38:959–967.
57. Lonardo A. Fatty liver and nonalcoholic steatohepatitis: where do we stand and where are we going? Dig Dis 1999;17:80–89.
58. Wanless IR, Lentz JS. Fatty liver hepatitis (steatohepatitis) and obesity: an autopsy study with analysis of risk factors. Hepatology 1990;12:1106–1110.
59. Garcia-Monzon C, Martin-Perez E, Iacono OL, Fernandez-Bermejo M, Majano PL, Apolinario A, Larranaga E, Moreno-Otero R. Characterization of pathogenic and prognostic factors of nonalcoholic steatohepatitis associated with obesity. J Hepatol 2000;33:716–724.
60. Adinolfi LE, Utili R, Andreana A, Tripodi MF, Marracino M, Gambardella M, Giordano M, Ruggiero G. Serum HCV RNA levels correlate with histological liver damage and concur with steatosis in progression of chronic hepatitis C. Dig Dis Sci 2001;46: 1677–1683.
61. Clouston AD, Jonsson JR, Purdie DM, Macdonald GA, Pandeya N, Shorthouse C, Powell EE. Steatosis and chronic hepatitis C: analysis of fibrosis and stellate cell activation. J Hepatol 2001; 34:314–320.
62. Westin J, Nordlinder H, Lagging M, Norkrans G, Wejstal R. Steatosis accelerates fibrosis development over time in hepatitis C virus genotype 3 infected patients. J Hepatol 2002;37: 837–842.
63. Castera L, Hezode C, Roudot-Thoroval F, Bastie A, Zafrani E-S, Pawlotsky J-M, Dhumeaux D. Worsening of steatosis is an independent factor of fibrosis progression in untreated patients with chronic hepatitis C and paired liver biopsies. Gut 2003;52: 288–292.
64. Poynard T, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC groups. Lancet 1997;349: 825–832.
65. Braillon A, Capron JP, Herve MA, Degott C, Quenum C. Liver in obesity. Gut 1985;26:133–139.
66. Angulo P, Keach JC, Batts KP, Lindor KD. Independent predictors of liver fibrosis in patients with nonalcoholic steatohepatitis. Hepatology 1999;30:1356–1362.
67. Ratziu V, Giral P, Charlotte F, Bruckert E, Thibault V, Theodorou I, Khalil L, Turpin G, Opolon P, Poynard T. Liver fibrosis in overweight patients. Gastroenterology 2000;118:1117–1123.
68. Dixon JB, Bhathal PS, O'Brien PE. Nonalcoholic fatty liver disease: predictors of nonalcoholic steatohepatitis and liver fibrosis in the severely obese. Gastroenterology 2001;121:91–100.
69. Petersen KF, Befroy D, Dufour S, Dziura J, Ariyan C, Rothman DL, DiPietro, Cline GW, Shulman GI. Mitochondrial dysfunction in the elderly: possible role in Insulin resistance. Science 2003; 300:1140–1142.
70. Hickman IJ, Clouston AD, Macdonald GA, Purdie DM, Prins JB, Ash S, Jonsson JR, Powell EE. Effect of weight reduction on liver histology and biochemistry in patients with chronic hepatitis C. Gut 2002;51:89–94.
71. Kaserer K, Fiedler R, Steindl P, Muller CH, Wrba F, Ferenci P. Liver biopsy is a useful predictor of response to interferon therapy in chronic hepatitis C. Histopathology 1998;32:454–461.
72. Perry IJ, Wannamethee SG, Shaper AG. Prospective study of serum gamma-glutamyl transferase and risk of NIDDM. Diabetes Care 1998;21:732–737.
73. Hwang SJ, Luo JC, Chu CW, Lai CR, Lu CL, Tsay SH, Wu JC, Chang FY, Lee SD. Hepatic steatosis in chronic hepatitis C virus infection: prevalence and clinical correlation. J Gastroenterol Hepatol 2001;16:190–195.
74. Ebeling F, Lappalainen M, Vuoristo M, Nuutinen H, Leino R, Karvonen AL, Lehtola J, Julkunen R, Pohjanpelto P, Farkkila M. Factors predicting interferon treatment response in patients with chronic hepatitis C: late viral clearance does not preclude a sustained response. Am J Gastroenterol 2001;96:1237–1242.
75. Mihm S, Monazahian M, Grethe S, Fechner C, Ramadori G, Thomssen R. Ratio of serum gamma-GT/ALT rather than ISDR variability is predictive for initial virological response to IFN-α in chronic HCV infection. J Med Virol 1999;58:227–234.
76. Pawltosky J-M, Roudot-Thoraval F, Bastie A, Darthuy F, Remire J, Metreau JM, Zafrani E-S, Duval J, Dhumeaux D. Factors affecting treatment responses to interferon-α in chronic hepatitis C. J Infect Dis 1996;174:1–7.
77. Giannini E, Ceppa P, Testa R. Steatosis in chronic hepatitis C:

can weight reduction improve therapeutic efficacy? J Hepatol 2001;35:432–433.
78. Day CP, James OF. Steatohepatitis: a tale of two "hits"? Gastroenterology 1998;114:842–845.
79. Paradis V, Mathurin P, Kollinger M, Imbert-Bismut F, Charlotte F, Piton A, Opolon P, Holstege A, Poynard T, Bedossa P. In situ detection of lipid peroxidation in chronic hepatitis C: correlation with pathological features. J Clin Pathol 1997;50:401–406.
80. Houglum K, Venkataramani A, Lyche K, Chojkier M. A pilot study of the effects of d-α-tocopherol on hepatic stellate cell activation in chronic hepatitis C. Gastroenterology 1997;113:1069–1073.
81. Sanyal AJ, Campbell-Sargent C, Mirshahi F, Rizzo WB, Contos MJ, Sterling RK, Luketic VA, Shiffman ML, Clore JN. Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities. Gastroenterology 2001;120:1183–1192.
82. Woodcroft KJ, Hafner MS, Novak RF. Insulin signaling in the transcriptional and posttranscriptional regulation of CYP2E1 expression. Hepatology 2002;35:263–273.
83. Day CP. Pathogenesis of steatohepatitis. Balliere's Best Pract Res Clin Gastroenterol 2002;16:663–678.
84. Yang SQ, Lin HZ, Lane MD, Clemens M, Diehl AM. Obesity increases sensitivity to endotoxin liver injury: implications for the pathogenesis of steatohepatitis. Proc Natl Acad Sci U S A 1997;94:2557–2562.
85. Okuda M, Li K, Beard MR, Showalter LA, Scholle F, Lemon SM, Weinman SA. Mitochondrial injury, oxidative stress, and antioxidant gene expression are induced by hepatitis C virus core protein. Gastroenterology 2002;122:366–375.
86. Moriya K, Nakagawa K, Santa T, Shitani Y, Fujie H, Miyoshi H, Tsutsumi T, Miyazawa T, Ishibashi K, Horie T, Imai K, Todoroki T, Kimura S, Koike K. Oxidative stress in the absence of inflammation in a mouse model for hepatitis C virus-associated hepatocarcinogenesis. Cancer Res 2001;61:4365–4370.
87. James OFW, Day CP. Non-alcoholic steatohepatitis (NASH): a disease of emerging identity and importance. J Hepatol 1998; 29:495–501.
88. Marchesini G, Brizi M, Bianchi G, Tomassetti S, Bugianesi E, Lenzi M, McCullough AJ, Natale S, Forlani G, Melchionda N. Nonalcoholic fatty liver disease: a feature of the metabolic syndrome. Diabetes 2001;50:1844–1850.
89. Pagano G, Pacini G, Musso G, Gambino R, Mecca F, Depetris N, Cassader M, David E, Cavallo-Perin P, Rizzetto M. Nonalcoholic steatohepatitis, insulin resistance, and metabolic syndrome: further evidence for an etiologic association. Hepatology 2002; 35:367–372.
90. Neuschwander-Tetri BA. A resistance movement in NASH. Am J Gastroenterol 2001;96:2813–2814.
91. Kim JK, Fillmore JJ, Chen Y, Yu C, Moore IK, Pypaert M, Lutz EP, Kako Y, Velez-Carrasco W, Goldberg IJ, Breslow JL, Shulman GI. Tissue-specific overexpression of lipoprotein lipase causes tissue-specific insulin resistance. Proc Natl Acad Sci U S A 2001; 98:7522–7527.
92. Lee Y, Wang MY, Kakuma T, Wang ZW, Babcock E, McCorkle K, Higa M, Zhou YT, Unger RH. Liporegulation in diet-induced obesity: the antisteatotic role of hyperleptinemia. J Biol Chem 2001;276:5629–5635.
93. Song S. The role of increased liver triglyceride content: a culprit of diabetic hyperglycaemia? Diabetes Metab Res Rev 2002;18: 5–12.
94. Powell EE, Searle J, Mortimer R. Steatohepatitis associated with limb lipodystrophy. Gastroenterology 1989;97:1022–1024.
95. Garg A, Misra A. Editorial: hepatic steatosis, insulin resistance, and adipose tissue disorders. J Clin Endocrinol Metab 2002; 87:3019–3022.
96. Seppala-Lindroos A, Vehkavaara S, Hakkinen AM, Goto T, Westerbacka J, Sovijarvi A, Halavaara J, Yki-Jarvinen H. Fat accumulation in the liver is associated with defects in insulin suppression of glucose production and serum free fatty acids independent of obesity in normal men. J Clin Endocrinol Metab 2002;87:3023–3028.
97. Clarke SD. Nonalcoholic steatosis and steatohepatitis. I. Molecular mechanism for polyunsaturated fatty acid regulation of gene transcription. Am J Physiol Gastrointest Liver Physiol 2001;281:G865–G869.
98. Kahn BB, Flier JS. Obesity and insulin resistance. J Clin Invest 2000;106:473–481.
99. Hadzyiannis S. Karamanos B. Diabetes mellitus and chronic hepatitis C virus infection. Hepatology 1999;29:604–605.
100. Mason AL, Lau JY, Hoang N, Qian K, Alexander GJ, Xu L, Guo L, Jacob S, Regenstein FG, Zimmerman R, Everhart JE, Wasserfall C, Maclaren NK, Perrillo RP. Association of diabetes mellitus and chronic hepatitis C virus infection. Hepatology 1999;29: 328–333.
101. Labrapoulou-Karatza C, Goritsas C, Fragopanagou H, Repandi M, Matsouka P, Alexandrides T. High prevalence of diabetes mellitus among adult β-thalassemic patients with chronic hepatitis C. Eur J Gastroenterol Hepatol 1999;11:1033–1036.
102. Knobler H, Schihmanter R, Zifroni A, Fenakel G, Schattner A. Increased risk of type 2 diabetes in noncirrhotic patients with chronic hepatitis C virus infection. Mayo Clin Proc 2000;75: 355–359.
103. Mehta SH, Brancati FL, Sulkowski MS, Strathdee SA, Szklo M, Thomas DL. Prevalence of type 2 diabetes mellitus among persons with hepatitis C virus infection in the United States. Ann Intern Med 2000;133:592–599.
104. Alexander GJ. An association between hepatitis C virus infection and type 2 diabetes mellitus: what is the connection? Ann Intern Med 2000;133:650–652.
105. Mangia A, Schiavone G, Lezzi G, Marmo R, Bruno F, Villani MR, Cascavilla I, Fantasia L, Andriulli A. HCV and diabetes mellitus: evidence for a negative association. Am J Gastroenterol 1998; 93:2363–2367.
106. Adinolfi LE, Utili R, Attanasio V, Zampino R, Ragone E, Tripodi MF, Ruggiero G. Epidemiology, clinical spectrum and prognostic value of mixed cryoglobulinaemia in hepatitis C virus patients: a prospective study. Ital J Gastroenterol 1996;28:1–9.
107. Zignego AL, Ferri C, Giannini C, Monti M, La Civita L, Caroccia G, Longobardo G, Lombardini F, Bombardieri S, Gentilini P. Hepatitis C virus genotype analysis in patients with type II mixed cryoglobulinemia. Ann Int Med 1996;124:31–34.
108. Andreone P, Gramenzi A, Cursaro C, Bernardi M, Zignego AL. Monoclonal gammopathy in patients with chronic hepatitis C virus infection. Blood 1996;88:1122.
109. Hadzyiannis SJ. The spectrum of extrahepatic manifestations in hepatitis C virus infection. J Viral Hepatol 1997;4:9–28.
110. Petit JM, Bour JB, Galland-Jos C, Minello A, Verges B, Guiguet M, Brun JM, Hillon P. Risk factors for diabetes mellitus and early insulin resistance in chronic hepatitis C. J Hepatol 2001;35: 279–283.
111. Mihm S, Fayazi A, Hartmann H, Ramadori G. Analysis of histopathological manifestations of chronic hepatitis C virus infection with respect to virus genotype. Hepatology 1997;25:735–739.
112. Adinolfi LE, Utili R, Ruggiero G. Body composition and hepatic steatosis as precursors of fibrosis in chronic hepatitis C patients. Hepatology 1999;30:1530–1531.
113. Adinolfi LE, Utili R, Andreana A, Tripodi MF, Rosario P, Mormone G, Ragone E, Pasquale G, Ruggiero G. Relationship between genotypes of hepatitis C virus and histopathologic manifestations in chronic hepatitis C patients. Eur J Gastroenterol Hepatol 2000;12:299–304.
114. kai E, Honda R, Yamada Y. Serum γ-glutamyl transpeptidase level and blood pressure in nondrinkers: a possible pathoge-

netic role of fatty liver in obesity-related hypertension. J Hum Hypertens 1994;8:95–100.
115. Akahoshi M, Amasaki Y, Soda M, Tominaga T, Ichimaru S, Nakashima E, Seto S, Yano K. Correlation between fatty liver and coronary risk factors: a population study of elderly men and women in Nagasaki, Japan. Hypertens Res 2001;24:337–343.
116. Ishizaka N, Ishizaka Y, Takahashi E, Tooda E, Hashimoto H, Nagai R, Yamakado M. Association between hepatitis C virus sieropositivity, carotid-artery plaque, and intima-media thickening. Lancet 2002;359:133–135.
117. Reid EA. Nonalcoholic steatohepatitis. Gastroenterology 2001; 121:710–723.
118. Angulo P, Lindor KD. Treatment of nonalcoholic fatty liver: present and emerging therapies. Semin Liver Dis 2001;21:81–88.
119. Eriksson S, Eriksson K, Bondesson L. Nonalcoholic steatohepatitis in obese: a reversible condition. Acta Med Scand 1986; 220:83–88.
120. Keeffe E, Adesman P, Stenzel P, Palmer R. Steatosis and cirrhosis in an obese diabetic. Resolution of fatty liver by fasting. Dig Dis Sci 1987;32:441–445.
121. Vajro P, Fontanella A, Perna C, Orso G, Tedesco M, De Vincenzo A. Persistent hyperaminotransferasemia resolving after weight reduction in obese children. J Pediatr 1994;125:239–241.
122. Ueno T, Sugawara H, Sujaku K, Hashimoto O, Tsuji R, Tamaki S, Torimura T, Inuzuka S, Sata M, Tanikawa K. Therapeutic effects of restricted diet and exercise in obese patients with fatty liver. J Hepatol 1997;27:103–107.
123. Knobler H, Schattener A, Zhornicki T, Malnick S, Keter D, Sokolovskaya N, Lurie Y, Bass DD. Fatty liver. An additional and treatable feature of the insulin resistance syndrome. Q J Med 1999;92:87–96.
124. Fargion S, Mattioli M, Fracanzani AL, Sampietro M, Tavazzi D, Fociani P, Taioli E, Valenti L, Fiorelli G. Hyperferritinemia, iron overload, and multiple metabolic alterations identify patients at risk for nonalcoholic steatohepatitis. Am J Gastroenterol 2001; 96:2448–2455.
125. Loguercio C, De Girolamo V, de Sio I, Tuccillo C, Ascione A, Baldi F, Budillon G, Cimino L, Di Carlo A, Di Marino MP, Morisco F, Picciotto F, Terracciano L, Secchione R, Verde V, Del Vecchio Blanco C. Non-alcoholic fatty liver disease in an area of southern Italy: main clinical, histological, and pathophysiological aspects. J Hepatol 2001;35:568–574.
126. Laurin J, Lindor K, Crippin J, Gossard A, Gores G, Ludwing J, Rakela J, McGill DB. Ursodeoxycholic acid or clofibrate in the treatment of non-alcohol-induced steatohepatitis: a pilot study. Hepatology 1996;23:1464–1467.
127. Lavine J. Vitamin E treatment of nonalcoholic steatohepatitis in children: a pilot study. J Pediatr 2000;136:734–738.
128. Abdelmalek MF, Angulo P, Jorgensen RA, Sylvestre PB, Lindor KD. Betaine, a promising new agent for patients with nonalcoholic steatohepatitis: results of a pilot study. Am J Gastroenterol 2001;96:2711–2717.
129. Lin H, Yang S, Chuckaree C, Kuhajda F, Ronnet G, Diehl A. Metformin reverses fatty liver disease in obese, leptin-deficient mice. Nat Med 2000;6:998–1003.
130. Caldwell SH, Hespenheide EE, Redick JA, Iezzoni JC, Battle EH, Sheppard BL. A pilot study of thiazolidinedione, troglitazone, in nonalcoholic steatohepatitis. Am J Gastroenterol 2001; 96:519–525.
131. Marchesini G, Brizi M, Bianchi G, Tomassetti S, Zoli M, Melchionda N. Metformin in non-alcoholic steatohepatitis. Lancet 2001;358:893–894.
132. Neuschwander-Tetri BA, Brunt EM, Wehmeier KR, Sponseller CA, Hampton K, Bacon BR. Interim results of a pilot study demonstrating the early effects of the PPAR-γ ligand roiglitazone on insulin sensitivity, aminotransferases, hepatic steatosis and body weight in patients with non-alcoholic steatohepatitis. J Hepatol 2003;8:434–440.
133. Facchini FS, Hua NW, Stoohs RA. Effect of iron depletion in carbohydrate-intolerant patients with clinical evidence of nonalcoholic fatty liver disease. Gastroenterology 2002;122:931–939.
134. Niederau C, Berger M, Stremmel W, Starke A, Strohmeyer G, Ebert R, Siegel E, Creutzfeldt W. Hyperinsulinaemia in noncirrhotic haemochromatosis: impaired hepatic insulin degradation? Diabetologia 1984;26:441–444.
135. Mendler MH, Turlin B, Moirand R, Jouanolle AM, Sapey T, Guyader D, Le Gall JY, Brissot P, David V, Deugnier Y. Insulin resistance-associated hepatic iron overload. Gastroenterology 1999;117:1155–1163.
136. Poynard T, Marcellin P, Lee SS, Niederau C, Minuk GS, Ideo G, Bain V, Heathcote J, Zeuzem S, Trepo C, Albrecht J. Randomised trial of interferon α2b plus ribavirin for 48 weeks or for 24 weeks versus interferon α2b plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. International Hepatitis Interventional Therapy Group (IHIT). Lancet 1998;352:1426–1432.
137. McHutchison JG, Gordon SC, Schiff ER, Shiffman ML, Lee WM, Rustgi VK, Goodman ZD, Ling MH, Cort S, Albrecht JK. Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. Hepatitis Interventional Therapy Group. N Engl J Med 1998;339:1485–1492.
138. Andreone P, Cursaro C, Gramenzi A, Fiorino S, Di Giammarino L, Miniero R, D'Errico A, Grigioni WF, Gasbarrini G, Bernardi M. Interferon α plus ketoprofen or interferon α plus ribavirin in chronic hepatitis C non-responder to interferon α alone: results of a pilot study. Ital J Gastroenterol Hepatol 1999;31:688–694.
139. Yuan M, Konstantopoulos N, Lee J, Hansen L, Li Z-W, Karin M, Shoelson SE. Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of IKKβ. Science 2001;293:1673–1677.
140. Hundal SR, Petersen KF, Mayerson AB, Randhawa PS, Inzucchi S, Shoelson SE, Shulman GI. Mechanism by which high-dose aspirin improves glucose metabolism in type 2 diabetes. J Clin Invest 2002;109:1321–1326.
141. Tanaka K, Kondo M, Sakaguchi T, Saito S, Arata S, Ikeda M, Kitamura T, Morimoto M, Sekihara H. Efficacy of ursodoxycholic acid in combination with interferon-α in treating chronic hepatitis C: results of a long-term follow-up trial. J Gastroenterol Hepatol 1996;11:1155–1160.
142. Lirussi F, Beccarello A, Bortolato L, Morselli-Labate AM, Crovatto M, Ceselli S, Santini G, Crepaldi G. Long-term treatment of chronic hepatitis C with ursodeoxycholic acid: influence of HCV genotypes and severity of liver disease. Liver 1999;19:381–388.
143. Look MP, Gerard A, Rao GS, Sudhop T, Fischer HP, Sauerbruch T, Spengler U. Interferon/antioxidant combination therapy for chronic hepatitis C: a controlled pilot trial. Antiviral Res 1999; 43:113–122.
144. Sartori M, Andorno S, Rigamonti C, Grossini E, Nicosia G, Boldorini R. Chronic hepatitis C is mild in menstruating women. J Gastroenterol Hepatol 2000;15:1411–1417.
145. Fong TL, Han SH, Tsai NC, Morgan TR, Mizokami M, Qian D, Phan C, Goad K, Redeker AG. A pilot randomized, controlled trial of the effect of iron depletion on long-term response to α-interferon in patients with chronic hepatitis C. J Hepatol 1998;28: 369–374.
146. Cagnoni C, Corsini F, Pancotti D, Carrara G. Effect of iron depletion on long-term response to interferon in patients with chronic hepatitis C with increased plasma iron without accumulation of liver iron. Ann Ital Med Int 2000;15:132–138.
147. Di Bisceglie AM, Bonkowski HL, Chopra S, Flamm S, Reddy RK,

Grace N, Killinberg P, Hunt C, Tamburro C, Tavil AS, Ferguson R, Krawitt E, Banner B, Bacon BR. Iron reduction as an adjuvant to interferon therapy in patients with chronic hepatitis C who have previously not responded to interferon: a multicenter, prospective, randomized, controlled trial. Hepatology 2000;32:135–138.
148. Yano M, Hayashi H, Wakusawa S, Sanae F, Takikawa T, Shiono Y, Arao M, Ukai K, Ito H, Watanabe K, Yoshioka K. Long-term effects of phlebotomy on biochemical and histological parameters of chronic hepatitis C. Am J Gastroenterol 2002;97:133–137.
149. Fargion S, Fracanzani AL, Rossini A, Borzio M, Riggio O, Belloni G, Bissoli F, Ceriani R, Ballare M, Massari M, Trischitta C, Fiore P, Orlandi A, Morini L, Mattioli M, Oldani S, Cesana B, Fiorelli G. Iron reduction and sustained response to interferon-α therapy in patients with chronic hepatitis C: results of an Italian multicenter randomized study. Am J Gastroenterol 2002;97:1204–1210.
150. Griffiths A, Olynk JK. Iron and the response to treatment of hepatitis C. Am J Gastroenterol 2002;97:788–790.
151. Garrido Serrano A, Guerrero Igea FJ, Lepe Jiménez JA, Palomo Gill S, Grilo Reina A. Hepatitis C virus infection, increased serum ferritin and hyperinsulinemia. Revista Española de Enfermedades Digestivas 2001;93:644–647.
152. Kakizaki S, Takagi H, Horiguchi N, Toyoda M, Takayama H, Nagamine T, Mori M, Kato N. Iron enhances hepatitis C virus replication in cultured human hepatocytes. Liver 2000;20:125–128.
153. Bonkowsky HL. Iron as a comorbid factor in chronic viral hepatitis. Am J Gastroenterol 2002;97:1–4.
154. Erhardt A, Maschner-Olberg A, Mellenthin C, Kappert G, Adams O, Donner A, Willers R, Niederau C, Haussinger D. HFE mutations and chronic hepatitis C: H63D and C282Y heterozygosity are independent risk factors for liver fibrosis and cirrhosis. J Hepatol 2003;38:335–342.
155. Tung BY, Emond MJ, Bronner MP, Raaka SD, Cotler SJ, Kowdley KV. Hepatitis C, iron status, and disease severity: relationship with HFE mutations. Gastroenterology 2003;124:318–326.
156. Younossi ZM, Gramlich T, Bacon BR, Matteoni CA, Boparai N, O'Neill R, McCullough AJ. Hepatic iron and nonalcoholic fatty liver disease. Hepatology 1999;30:847–850.
157. Chitturi S, Weltman M, Farrell GC, McDonald D, Liddle C, Samarasinghe D, Lin R, Abeygunasekera S, George J. HFE mutations, hepatic iron, and fibrosis: ethnic-specific association of NASH with C282Y but not with fibrotic severity. Hepatology 2002;36:142–149.
158. Piatti PM, Monti LD, Valsecchi G, Magni F, Setola E, Marchesi F, Galli-Kienle M. Long-term oral L-arginine administration improves peripheral and hepatic insulin sensitivity in type 2 diabetic patients. Diabetes Care 2001;24:875–880.
159. Hui JM, Kench J, Farrell GC, Lin R, Samarasinghe D, Liddle C, Byth K, George J. Genotype-specific mechanisms for hepatic steatosis in chronic hepatitis C infection. J Gastroenterol Hepatol 2002;17:873–881.
160. Sabile A, Perlemuter G, Bono F, Kohara K, Demaugre F, Kohara M, Matsuura Y, Miyamura T, Barba G. Hepatitis C virus core protein binds to apolipoprotein AII and its secretion is modulated by fibrates. Hepatology 1999;1064–1076.
161. Shi ST, Polyak SJ, Tu H, Taylor DR, Gretch DR, Lai MMC. Hepatitis C virus NS5A colocalizes with core proteins on lipid droplets and interacts with apolipoproteins. Virology 2002;292:198–210.
162. Med Thomssen R, Bonk S, Propfe C, Heermann KH, Kochel HG, Uy A. Association of hepatitis C virus in human sera with β-lipoprotein. Microbiol Immunol 1992;181:293–300.
163. Monazahian M, Bohme I, Bonk S, Koch A, Scholz C, Grethe S, Thomssen R. Low density lipoprotein receptor as a candidate receptor for hepatitis C virus. J Med Virol 1999;57:223–229.
164. Andre P, Komurian Pradel F, Deforges S, Perret M, Berland JL, Sodoyer M, Pol S, Brechot C, Parnhos-Baccala G, Lotteau V. Characterization of low- and very-low density hepatitis C virus RNA-containing particles. J Virol 2002;76:6919–6928.
165. Brown MS, Goldstein JL. A receptor-mediated pathway for cholesterol homeostasis. Science 1986;232:34–47.
166. Vu-Dac N, Schoonjans K, Kosykh V, Dallongeville J, Fruchart JC, Staels B, Auwerx J. Fibrates increase human apolipoprotein A-II expression through activation of the peroxisome proliferator-activated receptor. J Clin Invest 1995;96:741–750.
167. Bernard S, Touzet S, Personne I, Lapras V, Bondon PJ, Berthezene F, Moulin P. Association between microsomal triglyceride transfer protein gene polymorphism and the biological features of liver steatosis in patients wih type II diabetes. Diabetologia 2000;43:995–999.
168. Day CP, Saksena S, Leathart JB, Burt AD, James OFW, Daly AK. Genetic evidence supporting the two-hit model of NASH pathogenesis. Hepatology 2002;36:383A.
169. Raynard B, Balian A, Fallick D, Capron F, Bedoss P, Chaput J-C, Naveau S. Risk factors of fibrosis in alcohol-induced liver disease. Hepatology 2002;35:635–638.

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