

## Non-alcoholic Steatohepatitis—An Endocrine Disorder

a report by

**Bogdan Balas, MD, Rafeeq Ali, MD and Kenneth Cusi, MD**

*Division of Diabetes, University of Texas Health Science Center at San Antonio*

DOI: 10.17925/USE.2007.00.2.79

### Non-alcoholic Steatohepatitis

Non-alcoholic fatty liver disease (NAFLD) is a chronic liver condition frequently associated with type 2 diabetes and characterized by insulin resistance and hepatic fat accumulation. Liver fat may range from simple steatosis to severe steatohepatitis with necroinflammation and variable degrees of fibrosis (non-alcoholic steatohepatitis (NASH)). About 40% of patients with NAFLD develop NASH, with an early study reporting progression to fibrosis and/or cirrhosis in 15–20% of NASH.<sup>1</sup> More recent series have shown that this figure may be much higher, ranging from 32 to 41%.<sup>2</sup> Moreover, very recent evidence suggests that the lifespan of patients with NAFLD is not only significantly shortened by liver-related morbidity, but also by a higher incidence of cardiovascular disease.<sup>3,4</sup> Thus, substantial benefit may be expected from improving current screening strategies, early detection, and finding potential treatments of NAFLD.

### Prevalence

The true prevalence of NAFLD and its different stages is unknown. It has been recently estimated that fatty liver disease affects approximately one-third of the adult population or ~80 million Americans and as many as approximately two-thirds of obese subjects in the US.<sup>5,6</sup> Obese diabetic patients have the highest risk of progression to severe forms of the disease.

NAFLD continues to be overlooked by most physicians, as the majority of patients with NAFLD (70–80%) have normal liver enzymes<sup>5,7</sup> and the sensitivity of ultrasound (U/S) or computed tomography (CT) is poor overall when the degree of steatosis is less than 30%.<sup>8,9</sup> From our experience, using the more sensitive magnetic resonance spectroscopy (MRS) technique,<sup>10,11</sup> many diabetics go under the radar of conventional imaging studies such as U/S and CT scans. When patients with type 2 diabetes are systematically screened for NAFLD by MRS, about 80% of patients have a fatty liver.<sup>11</sup>

The overall prevalence of NASH, the more severe form of fatty liver disease, is even less certain and there are no exact numbers about the magnitude of the problem in the US. In obese subjects undergoing bariatric surgery, steatosis was found in about 90% of them. The prevalence of NASH in this patient population was 42%.<sup>12</sup> In these patients there was a correlation between liver enzyme elevations and the presence of steatosis and/or steatohepatitis.

NASH is estimated to affect between 3 and 23% of adults,<sup>13</sup> with suspected higher prevalence rates in patients with the metabolic syndrome. NASH is now recognized as a plausible explanation for many cases of

cryptogenic cirrhosis.<sup>1</sup> Hepatocellular carcinoma is also a documented complication of fatty liver disease.<sup>14</sup> Although initially it was thought to be a rather benign condition, fatty liver has gradually been recognized as a disease with potentially serious and life-threatening consequences. Risk factors associated with cryptogenic cirrhosis shed more light on its strong metabolic similarities with NASH. In a group of patients with cryptogenic cirrhosis, type 2 diabetes or obesity were significantly more prevalent in this patient population, representing a risk factor in about 73% of patients.<sup>15</sup> In the same study, 70% of NASH patients did have type 2 diabetes or obesity, a similarity suggesting that many cases of cryptogenic cirrhosis could represent an advanced stage of NASH.

### Pathogenesis

The current accepted hypothesis recognizes insulin resistance as the primordial factor that promotes the accumulation of fat in the liver. The disease can remain in a benign state or progress to steatohepatitis in the presence of (presumably) oxidative stress as a result of either overall inflammation or increased local lipid peroxidation (see *Figure 1*). It is not clear which is the exact trigger that promotes inflammation because not all liver steatosis progresses to NASH. It is possible that genetic factors



Bogdan Balas, MD, is a Post-doctoral Fellow in the Division of Diabetes at the University of Texas Health Science Center at San Antonio. His research focuses on delineating the pathogenesis of type 2 diabetes mellitus and the mechanism of action of drugs that improve insulin sensitivity and insulin secretion in humans and animals. Dr Balas obtained his medical degree from the University of Bucharest in 2000.

E: [bbalas74@yahoo.com](mailto:bbalas74@yahoo.com)



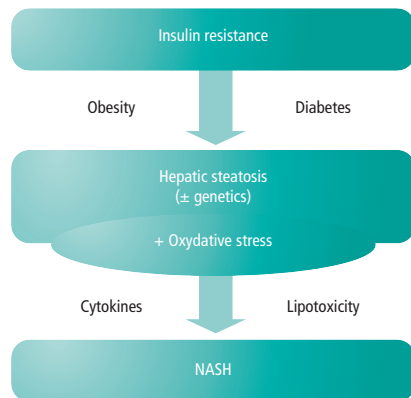
Rafeeq Ali, MD, is a Clinical Fellow in the Division of Endocrinology, Diabetes, and Metabolism at the University of Texas Health Science Center at San Antonio. He has clinical and research interests in non-alcoholic fatty liver disease. Dr Ali received his medical degree from King Abdulaziz University in Saudi Arabia in 1999.



Kenneth Cusi, MD, is an Associate Professor in the Division of Diabetes at the University of Texas Health Science Center at San Antonio. His research has focused on the mechanisms leading to pancreatic  $\beta$ -cell failure and to insulin resistance at the level of muscle and liver. He was the principal investigator for the first randomized controlled trial of an effective pharmacological treatment (pioglitazone) in non-alcoholic steatohepatitis.

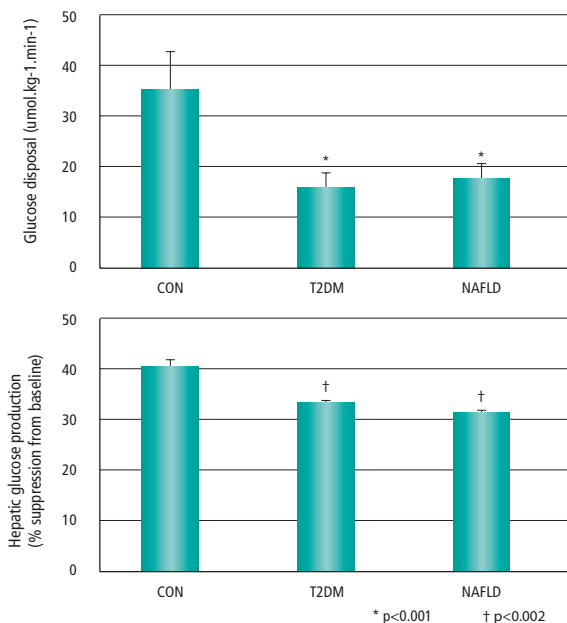
# Non-alcoholic Steatohepatitis

**Figure 1: The Pathophysiology of Non-alcoholic Fatty Liver Disease**



Non-alcoholic fatty liver disease (NAFLD) recognizes insulin resistance as the main cause of hepatic steatosis. Once developed, steatosis may remain benign or progress to non-alcoholic steatohepatitis (NASH) via multiple mechanisms.

**Figure 2: Hyperinsulinemic Euglycemic Clamps Show Insulin Resistance in Both the Skeletal Muscle and Liver of Patients with Non-alcoholic Fatty Liver**



Source: Marchesini et al., 2001.<sup>25</sup>

may play a role in the progression of the disease and, by coupling with the specific environmental and local factors, determine the evolution of steatosis to NASH.<sup>16</sup>

## Insulin Resistance

Insulin resistance syndrome is defined by a deficiency of various target tissues to respond to the action of insulin.<sup>17</sup> In response, the beta cell of the pancreas will secrete larger amounts of insulin to compensate the diminished response of tissues to insulin action.<sup>18</sup> Liver, skeletal muscle, and adipose tissues are the main sites of insulin resistance.<sup>19</sup> Accumulation of fat in the liver has been almost always associated with insulin resistance.<sup>20-24</sup> Patients with NAFLD show insulin resistance in both

muscle and liver (see Figure 2).<sup>25</sup> Increased plasma levels of insulin promote liver steatosis and, eventually, fibrosis through different mechanisms.<sup>26</sup> It has been shown that patients subjected to peritoneal dialysis develop hepatic steatosis when insulin is added to the dialysate.<sup>27</sup> This effect may be mediated through upregulation of a lipogenic protein, sterol regulatory elemental binding protein (SREBP).<sup>28</sup> Insulin also appears to have a direct effect in promoting fibrosis as it may stimulate connective tissue growth.<sup>29</sup>

## Lipotoxicity

Insulin-resistant states are also characterized by increased circulating free fatty acids (FFAs) levels as a result of uninhibited lipolysis caused by resistance to the action of insulin in the adipocyte.<sup>30</sup> This leads to an increased metabolic flux of FFAs in the hepatocyte that results in the generation of metabolites, in particular peroxides of lipids that have been shown to be increased in NAFLD.<sup>31,32</sup> NASH patients have even higher levels of oxidative stress compared with patients with steatosis alone.<sup>31</sup>

FFAs are the likely source of oxidative stress within the liver in these patients. Elevated FFAs within the liver<sup>33</sup> act as ligands for the transcription factor peroxisome proliferator-activated receptor (PPAR)- $\alpha$ , which upregulates the oxidation of FFAs in mitochondria.<sup>34</sup> It is believed that the inability of mitochondria to adapt to chronic high levels of FFA supply/flux leads to its ultimate demise, followed by cell injury and death.

## Oxidative Stress and Mitochondrial Dysfunction

There is an increasing consensus that mitochondrial dysfunction is essential to the development of NASH in insulin-resistant states such as obesity and type 2 diabetes.<sup>35-37</sup> When mitochondria are unable to adapt to an overload of fat, the release of reactive oxygen species (ROS) will be triggered with activation of inflammatory pathways (i.e. c-Jun N-terminal kinase (JNK), NF- $\kappa$ B), steatohepatitis, and progressive liver damage.<sup>36,38</sup> A number of mechanisms have been described by which steatosis induces an inflammatory response from the local macrophages (Kupfer cells), with the release of a number of cytokines that will induce hepatocyte necrosis, inflammation, and eventual apoptosis.<sup>39</sup> In this setting, activation of stellate cells by local inflammation promotes fibrogenesis. Unfortunately, almost all of the information available about NAFLD/NASH arises from animal models of the disease (largely rodents) with very limited information from human tissue. This shortcoming in our knowledge has been recently made evident when examining the discrepant effects of fibrates and of rosiglitazone in mice compared with human studies. For example, fenofibrate markedly reduces hepatic steatosis and improves hepatic/muscle insulin sensitivity in mice models of obesity.<sup>40</sup> In contrast, no such effect of fenofibrate on liver enzymes or hepatic/muscle insulin sensitivity (assessed by the gold-standard insulin clamp technique) was observed in studies performed in obese subjects with type 2 diabetes.<sup>41</sup> In a similar way, while rosiglitazone has been reported to improve elevated liver function tests (LFTs) and steatosis in humans,<sup>42</sup> in mice rosiglitazone increases hepatic transaminases and worsens necroinflammation and steatosis.<sup>43</sup>

## Tumor Necrosis Factor-alpha and Systemic Inflammation

Insulin resistance is *per se* a pro-inflammatory state. Many authors consider that liver fat alone might not be detrimental in itself and that host factors (genetic predisposition) are required in order to promote

damage through inflammation and necrosis.<sup>44</sup> Adipose tissue in obesity is associated with dysfunctional adipocytes that promote systemic inflammation and appear to contribute to the induction of local inflammation. The intimate mechanisms are not yet elucidated. One major culprit may be tumor necrosis factor (TNF)- $\alpha$ , a powerful inflammatory signaling molecule secreted by the distressed adipocyte.<sup>45</sup> It is believed that the message from adipose tissue to the hepatocyte may be transmitted by this small molecule and this leads to attraction of macrophages<sup>46,47</sup> in the sinusoids and the promotion of local inflammation that will eventually lead to fibrosis and scarring.

### Adiponectin

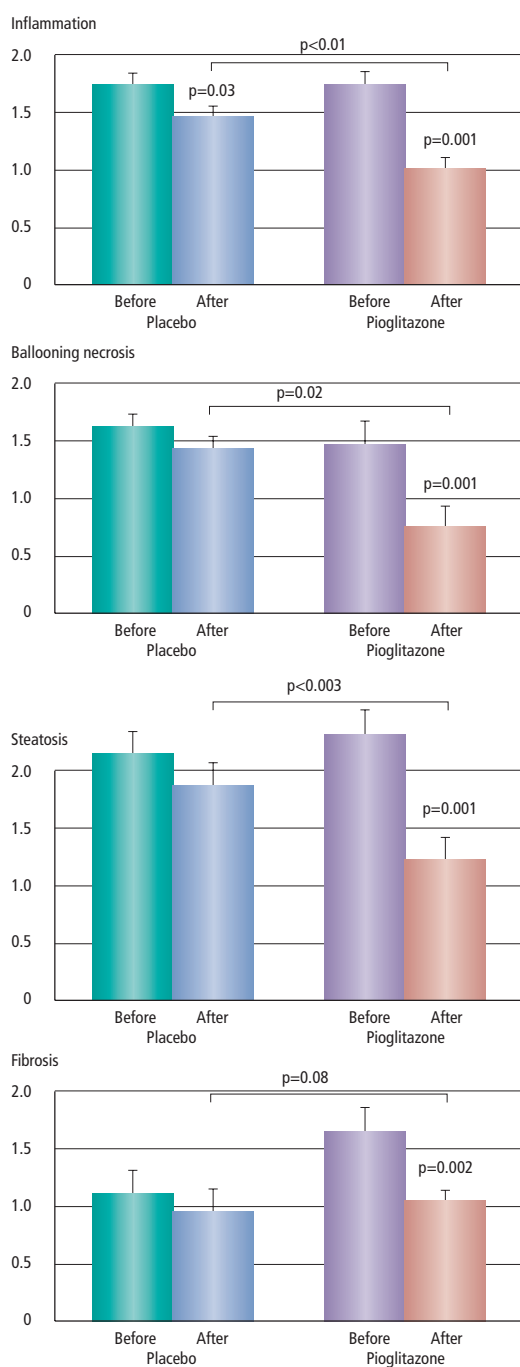
Adiponectin is an insulin-sensitizing hormone secreted by the adipocyte. Plasma adiponectin levels have been shown to closely correlate with the level of insulin sensitivity.<sup>48,49</sup> Not surprisingly, patients with NASH show decreased levels of circulating adiponectin.<sup>50</sup> It is possible that an anti-inflammatory effect mediated by adiponectin may influence the regression of local hepatic inflammation and necrosis. It has been shown that successful interventions in NASH have the effect of increasing plasma adiponectin levels.<sup>10</sup>

### Diagnosis and Natural History

One major characteristic and pre-requisite for the diagnosis of NASH is the absence of significant alcohol intake. How much alcohol intake can induce liver steatosis is still a matter of debate, but it is generally recognized that the maximum accepted alcohol consumption is two standard drinks per day for a man (20g) and one standard drink for a woman (10g). The symptoms and signs of NASH are extremely non-specific and frequently overlooked. They include general malaise and vague right upper abdominal pain. Findings on physical examination are sparse and the most common abnormality may be an enlarged liver. In the late stages of cirrhosis, findings of chronic liver disease dominate the picture. Other associated features of obesity and signs of insulin resistance (i.e. acanthosis nigricans) can be detected.<sup>51</sup>

The disease is usually detected by mild elevations of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), typically with ALT>AST in the early stages of the disease, but may reverse in advanced stages, when fibrosis develops. This is actually the most common laboratory abnormality in this condition, and the levels of those liver enzymes can fluctuate over the course of the disease and may be normal in end-stage cirrhosis.<sup>52</sup> Angulo et al.<sup>53</sup> found that diabetes mellitus, obesity, advanced age, and AST/ALT ratio greater than 1 are significant predictors of more severe liver fibrosis. The firm diagnosis of NAFLD/NASH depends on confirmed steatosis (+ inflammation  $\pm$  ballooning-necrosis and fibrosis for NASH) on a liver biopsy in the absence of significant alcohol consumption. Other culprits of liver steatosis (e.g. medications: glucocorticoids, anti-estrogens), viral hepatitis B and C, and other conditions that may increase liver transaminases (i.e. autoimmune disorders, Weber-Christian disease, HIV infection) should be excluded before labeling a patient with NAFLD. The biopsy is also necessary to grade the liver abnormalities, including the presence and stage of fibrosis. It is important to understand, though, that a liver biopsy carries its own limitation given the nature of the disease. Fat deposition and inflammation/fibrosis are not always uniform throughout the liver parenchyma, and sampling error can be a limiting factor of the liver

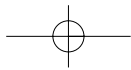
**Figure 3: Effect of Six Months of Pioglitazone 45mg/day Treatment on Liver Histology in Patients with Impaired Glucose Tolerance, Type 2 Diabetes, and Biopsy-proven Non-alcoholic Steatohepatitis**



Source: Belfort et al., 2006.<sup>10</sup>

biopsy. A non-invasive test to accurately diagnose liver steatosis and quantify liver fat content would therefore make a very attractive and useful aid in the evaluation of patients with NAFLD.

U/S and CT scans are most commonly used for this in clinical practice. In relatively small studies using U/S or CT scans, the prevalence of steatosis



## Non-alcoholic Steatohepatitis

in type 2 diabetes patients has been reported to be higher than in matched non-diabetic subjects, ranging between 50 and 80%.<sup>8,54,55</sup> This variability is related, at least in part, to the suboptimal sensitivity to detect steatosis that these imaging techniques have. Ultrasonography, the most frequently used tool in clinical practice, is very operator-dependent, affected considerably by body mass (obesity) and has a sensitivity of only ~65–80% to assess liver fat.<sup>8,54,55</sup> It may also lead to an incorrect diagnosis of NAFLD in 10–30% of cases.<sup>8</sup> The sensitivity of ultrasound improves considerably to ~80% when liver fat exceeds 30%, but drops to  $\leq$ 50% in morbid obesity or when liver fat content is <20%.<sup>56,57</sup> Thus, ultrasonography leaves many patients undiagnosed, as steatosis of  $\leq$ 20% is common in many diabetics with NAFLD.<sup>5,10,11,58</sup>

Recently, MRS has allowed a fast and highly reproducible measure of liver fat with steatosis being defined as liver fat content in excess of 5% and validated in the multiethnic population-based Dallas Heart Study in 2,287 subjects.<sup>5</sup> This 5% cut-off is consistent with our own experience over the past 10 years. By MRS we have found that NAFLD is present in >80% of unselected diabetic patients<sup>10,11,58</sup> (also in unpublished observations). In NASH patients the correlation we have found between MRS and liver biopsy fat measurements has been excellent ( $r=0.84$ ,  $p<0.0001$ ; unpublished observations).<sup>10,11,58</sup> If these results are confirmed in a larger cohort of patients, it will increase awareness about the seriousness of NAFLD as a major public health problem in type 2 diabetes.

Therefore, it would be reasonable to predict that in the future a diagnostic algorithm for NASH may include an MRS screening in patients having a relevant history (symptoms and signs in the absence of alcohol consumption) and elevated liver function tests. This may be particularly true as some pharmacological treatments appear to offer promise in NASH.<sup>10</sup> Should the screening be positive (>5% liver fat on MRS), subjects could be offered to undergo a U/S-guided liver biopsy. In patients with type 2 diabetes and NAFLD by MRS but normal liver enzymes, there are no studies on the natural history of the disease or clear guidelines as to the best way to manage them. Therefore, a liver biopsy may be considered only if liver fat is clearly elevated (i.e. two-fold above the upper limit of normal for MRS or >10% liver fat). This approach is likely to ensure that only type 2 diabetes patients with normal liver enzymes but the worst prognosis receive a diagnostic liver biopsy. Many studies have suggested that age >50 years, obesity, metabolic syndrome, insulin resistance, and type 2 diabetes are all powerful predictors of disease progression.<sup>59</sup> A recent large study has confirmed the validity of such an approach as disease progression was common in patients with the above risk factors.<sup>60</sup>

### Treatment

Weight loss remains the standard of care because no other therapy has conclusively proven to be effective in the long term. However, weight loss is rarely achieved or maintained over time, with the disease progressing relentlessly in a substantial number of subjects.<sup>2</sup>

Weight loss alone was observed to lead to resolution of liver steatosis.<sup>61</sup> The initial consideration was to induce significant weight loss by markedly reducing caloric intake to the point of starvation. It was noticed, though, that inflammation is exacerbated in patients with severe fatty infiltration and more rapid weight loss.<sup>62</sup> Weight loss was shown to reduce fatty liver infiltration also in hepatitis C patients with hepatic steatosis.<sup>63</sup>

Bariatric surgery has contributed significantly to our understanding of the effect of weight loss on liver steatosis. This technique, however, selected severely obese patients who probably do not reflect the majority of patients suffering from NAFLD. In this subset of patients, NAFLD and its more aggressive form, NASH, are more prevalent and show histological improvement with weight loss after surgery.<sup>64</sup> Steatosis improves more remarkably than inflammation or fibrosis. This could reflect the decrease in FFA supply to the liver, which acts as the substrate to promote liver steatosis.<sup>65</sup> Insulin resistance in these patients plays a more influential role than their weight and its persistence predicts steatosis even after weight loss.<sup>66</sup> It is well accepted that diet and exercise reduce insulin resistance even at lesser degrees of weight loss. It also improves hyperlipidemia and systemic inflammation. Using a hypocaloric diet associated with moderate exercise in patients with biopsy-proven NAFLD, Ueno et al.<sup>67</sup> observed reduction in liver fat over a three-month period. This occurred with modest reduction in body mass index (BMI) when compared with a control group of patients. Levels of ALT and aspartate aminotransferase also normalized in the treatment group. However, there was no significant reduction of liver inflammation or fibrosis on repeat biopsy.

The main caveat of weight loss programs is that they are not sustainable over longer periods of time. More than usual, there is a rebound that annihilates the initial benefits and this is normally associated with a relapse in insulin resistance, inflammation, and liver steatosis.<sup>68</sup> There is an increased need for alternative interventions that would promote weight loss, but only small, short-term, uncontrolled studies in NASH are available (discussed below).

### Drug Therapy

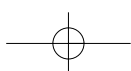
While many drugs have shown promising results in animal models of the disease, the reality is that many of these therapies have had modest benefits when administered to humans. Pharmacological therapies with some effects have included pentoxifylline, orlistat, vitamin E, cytoprotective agents, ursodeoxycholic acid, and lipid-lowering agents.<sup>69</sup> In contrast, insulin-sensitizers, such as metformin (70) and thiazolidinediones, have yielded more provocative results in NASH and improved LFTs/insulin resistance.

### Pentoxifylline

The anticipated anti-TNF- $\alpha$  properties of this compound led to its use in NASH.<sup>71,72</sup> Small open label trials evaluated this drug over a period of six to 12 months. In these trials, only surrogate markers of liver injury were used to assess the response to therapy. Liver enzymes and TNF- $\alpha$  levels were reduced significantly by the end of the treatment period. No histological evaluation was performed and therefore nothing can be said on the effect of the drug to alter the natural course of the disease.

### Orlistat

Orlistat is an oral medication that has recently been approved by the US Food and Drug Administration (FDA) as an adjuvant therapy to diet in weight loss programs. It inhibits gastric and pancreatic lipases and thus impairs fat absorption. To be effective it must go hand in hand with a fat-restricted diet. Weight reduction by this drug was observed to improve hepatic steatosis in some patients with NASH.<sup>73</sup> However, the efficacy of this therapy added no significant benefits compared with dietary management alone in a double-blind, randomized, placebo-controlled trial.<sup>7</sup>



### Antioxidants

Vitamins E and C gained interest as antioxidants and as potential therapies in NASH in which oxidative stress could play a role in liver cell injury and death. In a recent study,<sup>74</sup> daily supplementation with vitamins E and C was compared with placebo in a randomized double-blind trial. Unfortunately, the results of the study were controversial: although vitamin therapy improved fibrosis, there was no beneficial effect on necroinflammation or ALT levels.

### Other Therapies

The need for an effective therapy for NASH is reflected by the many interventions that have been assessed over time in search of a cure. Ursodeoxycholic acid<sup>75,76</sup> is a popular medication for hepatologists due to its safety profile and its possible cytoprotective effect on liver cells. However, a large randomized trial of two years duration did not demonstrate any significant advantage of this medication over placebo in NASH.<sup>77</sup>

The renin–angiotensin system plays an important role in modulating insulin resistance, with a reported effect of angiotensin receptor blockers (ARBs) in improving insulin sensitivity<sup>78</sup> and decreasing the number of hepatic stellate cells (involved in the development of fibrosis).<sup>79</sup> Although hepatic steatosis did not change in a small study (n=7) of losartan in patients with NASH, inflammation and fibrosis improved in five and four patients, respectively.

Lipid lowering by probucol (not marketed in the US) in NASH subjects was evaluated as it may have some antioxidative effects and may inhibit tissue deposition of low-density lipoprotein cholesterol (LDL-C). It had modest effects in lowering ALT levels compared with placebo, but unfortunately there was no histological proof of efficacy.<sup>80</sup>

### Insulin Sensitizers

Given the strong association between insulin resistance and NASH, it made sense to test insulin sensitizers in an attempt to treat the liver condition. Metformin and thiazolidinediones (TZDs) are both drugs that improve insulin sensitivity in muscle, liver, and adipose tissue (TZDs).

Metformin is a biguanide drug that exerts its glucose-lowering effects partly because it activates AMPK,<sup>81</sup> which in turn promotes a decrease in gluconeogenesis.<sup>82</sup> Its first use in humans for NAFLD was in a small group of non-diabetic patients.<sup>83</sup> Over a period of four months, it progressively reduced serum ALT levels in treated patients while no changes were noticed in the untreated group. Liver enzymes and liver volume as assessed by U/S were used as surrogate markers.

A similar effect of liver enzyme improvement was noted in an open-label randomized trial.<sup>84</sup> Metformin improves insulin resistance and liver enzymes to a greater extent than dietary measures, but no significant improvement in hepatic inflammation was found on liver biopsy.

The largest study with metformin compared this agent with vitamin E treatment or dietary measures.<sup>70</sup> In an open-label trial, 110 patients were randomized to receive metformin 2g/day, vitamin E 800IU/day, or a prescriptive weight-reducing diet. The study lasted for one year and demonstrated the superiority of metformin in reducing ALT levels

compared with prescriptive diet or vitamin E administration. The effect of metformin on liver histology should be interpreted with caution. Of the 17 subjects who had a repeated biopsy, inflammation or fibrosis improved in 10, worsened in one, and did not change in six.

Thiazolidinediones (TZDs) have gained significant attention due to their numerous beneficial metabolic effects associated with glucose-lowering abilities in diabetic patients. They decrease peripheral insulin resistance and therefore inhibit lipolysis and FFA influx to the liver. TZDs also promote hepatic fatty acid oxidation and increase adiponectin levels. Troglitazone (later withdrawn due to its idiosyncratic hepatotoxic effect) was the first agent to be tested in humans. In a pilot study of 10 patients with NASH,<sup>85</sup> ALT normalized in seven subjects by the end of the study (six months). Necroinflammation essentially was not changed.

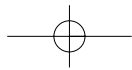
Neuschwander-Tetri et al.<sup>42</sup> studied the effects of another TZD (rosiglitazone) in patients with NASH. The study lacked a control group. It involved 30 subjects with biopsy-proven NASH treated with 4mg of rosiglitazone for 48 weeks. All patients were overweight and half of them had impaired glucose tolerance. At the end of the treatment there was a significant reduction in liver enzymes and an improvement in insulin sensitivity assessed by HOMA and QUICKI indexes. In 22 patients who had a repeat biopsy there was a significant reduction in steatosis, ballooning, and overall inflammation score. Fibrosis score was unaffected.

In a preliminary report of a controlled trial with rosiglitazone, there was also a significant improvement of liver steatosis and biochemical markers,<sup>86</sup> in spite of increased weight gain, but not in necroinflammation or fibrosis. The reasons for the differences in response among studies remain unclear.

Another TZD, pioglitazone, has been evaluated in several studies. Non-diabetic patients with biopsy-proven NASH (n=18) were enrolled in a pilot study of pioglitazone (30mg daily) for 48 weeks.<sup>87</sup> Liver enzymes improved in all patients and normalized in 72% of the patients and FFA concentration was reduced by 16%. All patients underwent a second biopsy at 48 weeks and all had at least one histological marker of NASH improved. A “significant histological response” was observed in 67% of subjects.

Sanyal et al.<sup>88</sup> compared pioglitazone with daily vitamin E supplementation in another pilot study of 20 non-diabetic subjects. Both treatment groups had reduction in liver steatosis, but reduction in inflammation was significant only in the pioglitazone + vitamin E group. No significant effects were seen on hepatic fibrosis.

We recently demonstrated in a randomized, double-blind, placebo-controlled trial in patients with impaired glucose tolerance or type 2 diabetes and NASH that pioglitazone treatment for six months significantly improved glycemic control, glucose tolerance, insulin sensitivity, and systemic inflammation.<sup>10</sup> This was associated with a ~50% decrease in steatohepatitis (p<0.001) and a ~40% reduction of fibrosis within the pioglitazone-treated group (p<0.002), although this fell short of statistical significance when compared with placebo (p=0.08) (see Figure 3). Our results provided ‘proof-of-concept’ that pioglitazone may



## Non-alcoholic Steatohepatitis

be the first agent capable of altering the natural history of the disease. However, definitive proof requires establishing its safety and efficacy in a large number of subjects treated for a longer period of time. Pioglitazone proved to be safe and effective in our patient population of patients with NASH, although the associated weight gain is an undesirable side effect of therapy that can be mitigated with proper lifestyle changes.

Of note, weight gain in our patients was from an expansion in adipose tissue (which becomes more insulin-sensitive<sup>89</sup>) and not from water retention.<sup>90</sup> Since pioglitazone use in NASH may entail long-term administration, it is reassuring that a recent meta-analysis found that its use was associated with a reduction in cardiovascular disease, in contrast to rosiglitazone, whose long-term safety has been questioned.<sup>91</sup>

### Conclusion

The true magnitude and social impact of NASH is probably underestimated by current statistics. These factors are also difficult to assess because obesity is increasing dramatically in Western populations. Non-alcoholic fatty liver is a disease with potentially severe outcomes expected to become a rising silent epidemic faced with under-recognition, underdiagnosis, and undertreatment. Recently, TZDs were shown to be effective in reversing the metabolic and histological abnormalities of fatty liver disease, but larger-scale trials need to be undertaken to establish whether these drugs may have an effect on altering the natural course of the disease. Meanwhile, the practitioner should address this problem by continuously fighting obesity and the lack of physical exercise that seem to characterize modern society. ■

- Powell EE, Cooksley WG, Hanson R, Searle J, *Hepatology*, 1990;11:74–80.
- Angulo P, *Aliment Pharmacol Ther*, 2007;25:883–9.
- Targher G, Bertolini L, Padovani et al., *Diabetes Care*, 2007;30:1212–18.
- Hamaguchi M, Kojima T, Takeda N, et al., *World J Gastroenterol*, 2007;13:1579–84.
- Browning JD, Szczepaniak LS, Dobbins R, et al., *Hepatology*, 2004;40:1387–95.
- Wieckowska A, McCullough AJ, Feldstein A, *Hepatology*, 2007;46:582–9.
- Zelber-Sagi S, Kessler A, et al., *Clin Gastroenterol Hepatol*, 2006;4:639–44.
- Hamaguchi M, Kojima T, Takeda N, et al., *Ann Intern Med*, 2005;143:722–8.
- Saadeh S, Younossi ZM, Remer EM, et al., *Gastroenterology*, 2002;123:745–50.
- Belfort R, Harrison SA, Brown K, et al., *Engl J Med*, 2006;355:2297–2307.
- Gastaldelli A, Cusi K, Pettiti M, et al., *Gastroenterology*, 2007;133:496–506.
- Frantzides CT, Carlson MA, Moore RE, et al., *J Gastrointest Surg*, 2004;8:849–55.
- Clark JM, Brancati FL, Diehl AM, *Gastroenterology*, 2002;122:1649–57.
- Bugianesi E, Leone N, Vanni E, et al., *Gastroenterology*, 2002;123:134–40.
- Caldwell SH, Oelsner DH, Lezzone JC, et al., *Hepatology*, 1999;29:664–9.
- Alisi A, Nobili V, *J Hepatol*, 2007
- DeFronzo RA, *Med Clin North Am*, 2004;88:787–835, ix.
- Kashyap SR, DeFronzo RA, *Diab Vasc Dis Res*, 2007;4:13–19.
- Saltiel AR, Kahn CR, *Nature*, 2001;414:799–806.
- Chitturi S, Abeygunasekera S, Farrell GC, et al., *Hepatology*, 2002;35:373–9.
- Chalasanani N, Deeg MA, Persohn S, Crabb DW, *Am J Gastroenterol*, 2003;98:1849–55.
- Musso G, Gambino R, De Michieli F, et al., *Hepatology*, 2003;37:909–16.
- Cassader M, Gambino R, Musso G, et al., *Lipids*, 2001;36:1117–24.
- Sanyal AJ, Campbell-Sargent C, Mirshahi F, et al., *Gastroenterology*, 2001;120:1183–92.
- Marchesini G, Brizi M, Bianchi G, et al., *Diabetes*, 2001;50:1844–50.
- Goldstein BJ, Mahadev K, Wu X, *Diabetes*, 2005;54:311–21.
- Wanless IR, Bargman JM, Oreopoulos DG, Vas SI, *Mod Pathol*, 1989;2:69–74.
- Li XL, Man K, Ng KT, et al., *Liver Transpl*, 2004;10:1173–82.
- Paradis V, Perlemuter G, Bonvoust F, et al., *Hepatology*, 2001;34:738–44.
- DeFronzo RA, *Int J Clin Pract*. 2004;(Suppl. 143):9–21.
- Videla LA, Rodrigo R, Orellana M, et al., *Clin Sci (Lond)*, 2004;106:261–8.
- Koruk M, Taysi S, Savas MC, *Ann Clin Lab Sci*, 2004;34:57–62.
- Mavrelis PG, Ammon HV, Gleysteen JJ, et al., *Hepatology*, 1982;3:226–31.
- Clarke SD, *Am J Physiol Gastrointest Liver Physiol*, 2001;281:G865–9.
- Caldwell SH, Chang CY, Nakamoto RK, Krugner-Higby L, *Clin Liver Dis*, 2004;8:595–617, x.
- Diehl AM, Li ZP, Lin HZ, Yang SQ, *Gut*, 2005;54:303–6.
- Pessayre D, Fromenty B, *J Hepatol*, 2005;42:928–40.
- Elsharkawy AM, Mann DA, *Hepatology*, 2007;46:590–97.
- Choi S, Diehl AM, *Curr Opin Gastroenterol*, 2005;21:702–7.
- Chakravarthy MV, Pan Z, Zhu Y, et al., *Cell Metab*, 2005;1:309–22.
- Bajaj M, Suraamornkul S, Hardies LJ, et al., *Diabetologia*, 2007;50:1723–31.
- Neuschwander-Tetri BA, Brunt EM, Wehmeier KR, et al., *Hepatology*, 2003;38:1008–17.
- Garcia-Ruiz I, Rodriguez-Juan C, Diaz-Sanjuan T, et al., *Hepatology*, 2007;46:414–23.
- Sanyal AJ, *Nat Clin Pract Gastroenterol Hepatol*, 2005;2:46–53.
- Ryden M, Arner P, *J Intern Med*. 262:431–438, 2007
- Chitturi S, Farrell GC, *J Gastroenterol Hepatol*, 2007;22:613–14.
- Manco M, Marcellini M, Giannone G, Nobili V, *Am J Clin Pathol*, 2007;127:954–60.
- Hanley AJ, Bowden D, Wagenknecht LE, et al., *J Clin Endocrinol Metab*, 2007;92:2665–71.
- Higashiura K, Ura N, Ohata J, et al., *Clin Endocrinol (Oxf)*, 2004;61:753–9.
- Pagano C, Soardo G, Esposito W, et al., *Eur J Endocrinol*, 2005;152:113–18.
- Angulo P, *N Engl J Med*, 2002;346:1221–31.
- Ipekci SH, Basaranoglu M, Sonsuz A, *J Clin Gastroenterol*, 2003;36:371.
- Angulo P, Keach JC, Batts KP, Lindor KD, *Hepatology*, 1999;30:1356–62.
- Gupte P, Amarapurkar D, Agal S, et al., *J Gastroenterol Hepatol*, 2004;19:854–8.
- Younossi ZM, Gramlich T, Matteoni CA, et al., *Clin Gastroenterol Hepatol*, 2004;2:262–5.
- Palmentieri B, de Sio I, La Mura V, et al., *Dig Liver Dis*, 2006;38:485–9.
- Adams LA, Angulo P, *Clin Liver Dis*, 2007;11:25–35, viii.
- Bajaj M, Suraamornkul S, Piper P, et al., *J Clin Endocrinol Metab*, 2004;89:200–206.
- Clark JM, *J Clin Gastroenterol*, 2006;40:55–10.
- Fracanzani AL, Valenti L, Bugianesi E, et al., *Hepatology*, 2007;46:741A.
- Keeffe EB, Adesman PW, Stenzel P, Palmer RM, *Dig Dis Sci*, 1987;32:441–5.
- Andersen T, Gluud C, Franzmann MB, Christoffersen P, *J Hepatol*, 1991;12:224–9.
- Hickman IJ, Jonsson JR, Prins JB, et al., *Gut*, 2004;53:413–19.
- Dixon JB, Bhathal PS, Hughes NR, O'Brien PE, *Hepatology*, 2004;39:1647–54.
- Klein S, Mittendorfer B, Eagon JC, et al., *Gastroenterology*, 2006;130:1564–72.
- Mathurin P, Gonzalez F, Kerdraon O, et al., *Gastroenterology*, 2006;130:1617–24.
- Ueno T, Sugawara H, Sujaku K, et al., *J Hepatol*, 1997;27:103–7.
- Duvnjak M, Lerotic I, Barsic N, et al., *World J Gastroenterol*, 2007;13:4539–50.
- Bugianesi E, Marzocchi R, Villanova N, Marchesini G, *Best Pract Res Clin Gastroenterol*, 2004;18:1105–16.
- Bugianesi E, Gentilecore E, Manini R, et al., *Am J Gastroenterol*, 2005;100:1082–90.
- Crespo J, Cayon A, Fernandez-Gil P, et al., *Hepatology*, 2001;34:1158–63.
- Adams LA, Zein CO, Angulo P, Lindor KD, *Am J Gastroenterol*, 2004;99:2365–8.
- Harrison SA, Ramrakhiani S, Brunt EM, et al., *Am J Gastroenterol*, 2003;98:926–30.
- Harrison SA, Torgerson S, Hayashi P, et al., *Am J Gastroenterol*, 2003;98:2485–90.
- Laurin J, Lindor KD, Crippin JS, et al., *Hepatology*, 1996;23:1464–7.
- Dufour JF, Oneta CM, Gonvers JJ, et al., *Clin Gastroenterol Hepatol*, 2006;4:1537–43.
- Lindor KD, Kowdley KV, Heathcote EJ, et al., *Hepatology*, 2004;39:770–78.
- Zandbergen AA, Lamberts SW, Janssen JA, Bootsma AH, *Eur J Endocrinol*, 2006;155:293–6.
- Yokohama S, Tokusashi Y, Nakamura K, et al., *World J Gastroenterol*, 2006;12:322–6.
- Merat S, Malekzadeh R, Sohrabi MR, et al., *J Hepatol*, 2003;38:414–18.
- Zhou G, Myers R, Li Y, et al., *J Clin Invest*, 2001;108:1167–74.
- Musi N, Hirschman MF, Nygren J, et al., *Diabetes*, 2002;51:2074–81.
- Marchesini G, Brizi M, Bianchi G, et al., *Lancet*, 2001;358:893–94.
- Uygun A, Kadayifci A, Isik AT, et al., *Aliment Pharmacol Ther*, 2004;19:537–44.
- Caldwell SH, Hespdenheide EE, Redick JA, et al., *Am J Gastroenterol*, 2001;96:519–25.
- Ratziu V, Giral P, Jacqueminet S, et al., Group TLS: A one-year randomized, placebo-controlled, double-blind trial of rosiglitazone in non alcoholic steatohepatitis: Results of the flirt pilot trial. *Diabetes Annual Meeting*, 2006/
- Promrat K, Lutchman G, Uwaifo GI, et al., *Hepatology*, 2004;39:188–96.
- Sanyal AJ, Mofrad PS, Contos MJ, et al., *Clin Gastroenterol Hepatol*, 2004;2:1107–15.
- Balas B, Belfort R, Gastaldelli A, et al., abstract at 42st EASD Annual Meeting Copenhagen, Denmark, 2006.
- Balas B, Belfort R, Harrison SA, et al., *J Hepatol*, 2007;47:565–70.
- Lincoff AM, Wolski K, Nicholls SJ, Nissen SE, *JAMA*, 2007;298:1180–88.

