Treatment of nonalcoholic fatty liver disease

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INTRODUCTION

Insulin resistance and obesity represent the most important risk factors for development of NAFLD. Nonalcoholic fatty liver disease (NAFLD) is the most common cause for elevated liver enzymes in the developed nations. Beyond prevention programs which are of particular interest because of the increasing number of overweight children, treatment should be focussed on the most important risk factors, obesity and insulin resistance. As a consequence of elucidating the pathomechanisms of NAFLD, the number of potential therapeutic options increased. However, many studies investigating the therapeutic effect show shortcomings in at least one of the following points: lack of a serial liver biopsy, short term of treatment and limited number of included patients. The second generation insulin sensitizer pioglitazone and rosiglitazone show the most promising improvements in NAFLD, but weight gain and potential hepatotoxicity calls for attention. In conclusion, a general recommendation for the application of specific drugs cannot be given. Besides controlled clinical trials, weight reduction and physical activity to improve insulin sensitivity in obese patients should be the priority objective.

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REDUCTION OF BODY WEIGHT

Although insulin resistance occurs in patients with normal BMI and anthropometric measurements, the majority of these patients are adipose with increased visceral fat. So, in overweight or obese patients, weight loss is usually recommended as the first line management. The American Gastroenterological Association recommends a target of 10% of baseline weight as an initial goal of weight loss if BMI exceeds 25 kg/m². Weight loss should proceed at a rate of 1-2 lb/wk. Rapid weight loss due to a very low energy diet (<500 kcal daily) or jejunoileal bypass has been associated with exacerbation of steatohepatitis in obese patients. Here we discuss the role of a drug-free management in improvement of insulin resistance and NASH and give a critical summary of recent data on medical treatment. The potential concepts of treatment are summarized in Figure 1.
risk of complications associated with obesity[19]. However, it is not clear whether patients with NAFLD would benefit from merely increasing physical activity. The goal of weight management is to induce a negative calorie balance. A calorie deficit of 500-1000 calories/d for those who are overweight or obese appears to be rational, although there are no specific studies on this subject. Dietary recommendations should include reduction of dietary carbohydrates, because lipid profile of overweight patients improves[20, 21]. However, weight loss is rarely achieved or maintained over a long period. Most studies to date have been short term with a small number of patients included. Children with NASH may benefit from weight loss, because serum liver enzymes normalized and sonographic abnormalities disappeared[22]. Another study investigated 48 patients with elevated liver enzymes and clinical, histological, and sonographic characteristics of fatty liver disease[23]. Eighty-one percent of these patients were obese, 73% were glucose-intolerant or diabetic, and 85% had dyslipidemia. Dietary intervention as well as lipid-lowering medication and oral antidiabetics as needed were included into the treatment protocol. These dietary interventions resulted not only in a moderate weight loss, but also in a reduction of serum liver enzymes in 96% of patients. However, it remains unclear, if improved liver enzymes were accompanied by an improvement of liver histology, because serial liver biopsies were not performed. In another study, 15 obese patients followed a restricted diet (25 calories per kilogram ideal body weight) and exercise regimen over a 3 mo period[24]. Liver enzymes decreased in all patients and steatosis determined by biopsy was reduced compared with patients from control group.

A recently published study analyzed the effect of short term weight loss on liver histology[25]. Twenty-three obese patients (BMI>25kg/m²) with biopsy-proven NASH received standardized nutritional counseling to reduce insulin resistance and body weight. Each subject received individualized nutritional counseling in order to achieve dietary goals. The dietary adjustments included increased intake of fiber and a decreased intake of calories. The daily calory intake consists of 40%-45% carbohydrates with an emphasis on complex carbohydrates with fiber, 35%-40% fat with emphasis on mono- and polyunsaturated fats, and 15%-20% protein. In addition, all participants were encouraged to increase their physical activity to achieve a heart rate of 70% of the calculated target heart rate. Food frequency questionnaires were performed to assess dietary intake and the Paffenberger Activity Questionnaire was used to evaluate the level of physical activity. Sixteen patients successfully completed 12 mo of intense dietary intervention. Mean weight decreased from 98.3 kg to 95.4 kg. There was also a reduction of mean waist circumference, visceral fat, fasting glucose, insulin resistance, triglycerides, serum levels of liver enzymes and histological score, but the differences were not statistically significant. Fifteen patients underwent repeat liver biopsy. Nine of these 15 patients had a histological response, 6 patients had a stable score and none had worsened.

Pharmacological treatment of obesity may be applied to patients with a BMI>27 kg/m² and obesity-associated comorbidities. Sibutramine is a serotonin reuptake antagonist which should not be used in patients with coronary heart disease and moderate or severe hypertension[26]. The intake of orlistat results in fat malabsorption. Sabuncu and coworkers analyzed the effects of orlistat and sibutramine in obese patients with clinically presumed NASH. Both orlistat and sibutramine improved liver enzymes and decreased the sonographic hallmarks of fatty liver. However, liver biopsies were not performed and the level of alkaline phosphatase increased[27].

Those patients with a BMI>35 kg/m² and obesity-associated comorbidities may be considered for more aggressive weight management, including bariatric surgery. Because liver failure occurs after jejuno-ileal bypass, the latter has been replaced by the proximal gastric bypass operation. Two studies demonstrated improvement of liver histology after weight reduction and stabilization of weight for long term[28, 29]. However, occasional cases of worsening liver function can also occur during period of rapid weight loss following this procedure. The results of studies investigating the safety of such surgery in patients with severe NASH have to be awaited. Patients considered for this procedure should be monitored carefully and the pros and cons should be discussed with the patient in detail.

**ANTIOXIDANTS**

Oxidative stress is proposed to act as the “second hit” in...
the pathogenesis from steatosis to NASH and fibrosis. Therefore, using antioxidant substances seems to be rational in the treatment of steatohepatitis. Several in vitro and animal in vitro studies revealed that application of vitamin E decreased levels of profibrogenic TGF beta, improved liver histology and inhibited hepatic stellate cell activation\[^{30-32}\]. Two open-label pilot trials examined the effect of vitamin E in patients with NAFLD\[^{33, 34}\]. Eleven pediatric patients with presumed NASH were prescribed 400-1200 IU of oral vitamin E. Diagnosis was based on the presence of chronically elevated levels of AST and ALT, and fatty liver on ultrasound. Other causes for hepatitis were excluded. Two patients had biopsy-proven NASH. Treatment resulted in normalization of liver function test. However, serial biopsies were not performed, liver remained increased echogenic during treatment and improvement of enzymatic values was not sustained after discontinuation of vitamin E\[^{33}\]. In another study\[^{34}\], 10 patients with the clinical diagnosis of NAFLD and 12 patients with biopsy proven NASH were treated with vitamin E (300 mg/d) for 1 year. Treatment resulted in a significant improvement of liver enzymes. In the nine patients with steatohepatitis who had a posttreatment liver biopsy, the degree of steatosis, inflammation, or fibrosis also improved or remained unchanged. The plasma levels of TGF-β decreased significantly with vitamin E. However, these promising results were not confirmed in a subsequent randomized, double-blind, placebo-controlled trial\[^{35}\]. In this study, vitamin E (1000 IU/d) in combination with vitamin C (1000 mg/d) to potentially enhance the regeneration of oxidized vitamin E was given to 23 patients with NASH, while 22 patients were randomized to placebo. The duration of treatment was 6 mo. In addition, a low fat, low calorie diet in combination with increased physical activity was recommended. The degree of adherence to these recommendations remained unclear. The results showed a significant improvement of ALT levels in the placebo group but not in the treatment group. The fibrosis stage of 11 (48%) patients of the vitamin group and 9 (41%) of the placebo group improved by at least one stage. The authors concluded from this within-group comparison that vitamin C and vitamin E are effective in improving liver fibrosis, although only two more patients in the vitamin group showed a regression of fibrosis. Adams and Angulo\[^{36}\] criticized that the effect of placebo treatment was ignored, because no comparison between groups was performed. A between group analysis revealed that 6 mo of therapy with the combination of vitamin E and C is not better than placebo for patients with NASH.

**URSODEOXYCHOLIC ACID**

This hydrophilic bile acid is approved for the treatment of primary biliary cirrhosis. Ursodeoxycholic acid (UDCA) has been shown to reduce the portion of hydrophobic bile acids which contribute to oxidative stress. This is of particular importance, because fatty hepatocytes reveal an increased sensitivity to hydrophobic bile acids\[^{37}\]. A pilot study published in 1996 analyzing the effect of UDCA on serum liver enzymes and histology in patients with NAFLD showed promising results\[^{38}\]. The hepatic steatosis decreased on repeat liver biopsy in 12 of 19 patients and there was also a statistically significant improvement in liver enzymes, but there were no changes in the histological grade of inflammation or fibrosis. In a subsequent controlled trial 166 patients were randomized with liver-biopsy proven NASH to receive 13 and 15 mg/kg of UDCA daily\[^{39}\]. One hundred and twenty-six patients completed 2 years of therapy and serial liver biopsies were available in 107 patients. Analysis of serum liver chemistry, changes in the degree of steatosis, necro-inflammation or fibrosis revealed no significant difference between the verum and placebo-treated groups. However, the results from this study showed a high rate of spontaneous improvement in hepatic steatosis in the placebo arm probably explaining in part why the data were negative. In addition, the dose of 13 and 15mg/kg per day was possibly insufficient to improve NAFLD, so effect of higher doses needs to be evaluated in further studies.

**INSULINSENSITIZER**

The association of insulin resistance and hyperinsulinemia with NAFLD suggests a possibility of therapeutical intervention. The first evidence came from leptin-deficient, obese ob/ob mice. Metformin, a biguanide that reduces hyperinsulinemia and improves hepatic insulin resistance, reduced hepatomegaly and hepatic steatosis in ob/ob mice, whereas caloric restriction did not result in a substantial improvement\[^{40}\]. The authors postulated that metformin improve hepatic insulin resistance by decreasing hepatic expression of TNF-α, a cytokine that promotes insulin resistance. A pilot study evaluated the effect of metformin in 20 patients with histological proven NASH\[^{41}\]. When compared with the six patients not complying with treatment, intake of metformin for 4 mo significantly reduced levels of transaminases. They normalized in 50% of treated individuals. Also, insulin sensitivity improved significantly and liver volume decreased by 20%. Metformin was well tolerated and there was no case of lactic acidosis. However, the authors did not provide a serial liver biopsy to evaluate the effect of metformin on liver histology. These promising results were only in part supported by another open label trial\[^{42}\]. Fifteen patients with biopsy proven NAFLD completed 12 mo of treatment with metformin (20 mg/kg). During the initial 3 mo, liver enzymes improved significantly. There was also an improvement in insulin sensitivity detectable. However, after 3 mo, insulin sensitivity did not further improve and levels of AST and ALT gradually increased back to pretreatment levels. Among the 10 patients with posttreatment biopsy, three showed improvement in steatosis, two showed improvement in inflammation score and one in fibrosis.

Another trial evaluating the effect of metformin was performed by a Turkish group from Ankara\[^{43}\]. Uygun and coworkers randomized 36 patients with NASH into two groups. The first group was given lipid and calorie-restricted diet alone, while the second group was treated with metformin in addition to the diet for a period of 6 mo. The comparison between both groups showed no...
significant differences in inflammatory activity or fibrosis, although more patients in the treatment group showed an improved liver histology. The improvements of liver enzymes, insulin, insulin resistance index and c-peptide levels in the metformin group were significantly greater than those detected in the group with dietary treatment alone. The most recently published controlled trial by Bugianesi and colleagues demonstrated a better effect of metformin on improvement of liver enzymes compared to a prescriptive diet or the administration of vitamin E\(^{[44]}\). Unfortunately, the histological data were limited to support an association between improvement of liver chemistry and histological findings.

Another class of agents presumably improving insulin sensitivity is the thiazolidinediones. These compounds are ligands for the peroxisome proliferator-activated receptor gamma (PPAR gamma), which is expressed at high levels in adipocytes. Troglitazone, a first generation thiglitazone, and metformin were shown to inhibit the expression of sterol regulatory element binding protein-1 (SREBP-1), a key regulator of lipogenic enzymes\(^{[40, 45]}\). Troglitazone was investigated in a pilot study including 6 patients with biopsy-proven NASH\(^{[46]}\). Patients received 200 mg of troglitazone twice daily, which was well tolerated. Levels of ALT normalized in 4 of 6 patients on therapy and they persisted in normal range 3 mo after discontinuation of troglitazone. However, the Food and Drug Administration removed troglitazone from the market in March 2000 because of serious hepatotoxicity\(^{[47]}\). The second-generation thiglitazones rosiglitazone and pioglitazone appear to be safer, although their use is currently contraindicated in the presence of active liver disease or of ALT more than 2.5 times normal. An open label trial including 26 biopsy-proven NASH patients analyzed the effect of rosiglitazone, 4 mg twice daily for 48 wk\(^{[48]}\). All patients were overweight, and 23% had a BMI >35 kg/m\(^2\). Twenty-six patients had posttreatment biopsy. The mean necro-inflammatory score significantly improved with treatment and biopsies of 10 patients did not fulfill published criteria for NASH anymore after treatment. Twenty-five patients completed 48 wk of treatment and showed a significant improvement in liver enzymes and insulin resistance. However, 3 patients had to be withdrawn because of adverse events. One of these individuals discontinued because of increased ALT levels. In addition, weight gain occurred in more than two-thirds of participants and liver enzyme levels increased to near pretreatment level 6 mo after discontinuation of study medication. Another pilot study demonstrated similar results in 18 patients with biopsy proven NASH, who were treated with pioglitazone\(^{[50]}\). By 48 wk, levels of ALT normalized in 72% of patients. Hepatic fat content and size decreased which was determined by magnetic resonance imaging. There was also a significant improvement in liver histology regarding features of steatosis, inflammation and fibrosis. Histological improvement occurred in two-thirds of patients. The main side effect in this study was also weight gain and increase in total body adiposity.

In a recently published pilot study by Sanyal and coworkers 20 nondiabetic patients with biopsy-confirmed NASH were randomized to take the combination of pioglitazone (30 mg daily) and vitamin E or vitamin E (400 IU daily) alone for a period of 6 mo\(^{[51]}\). ALT levels normalized in all patients within 6 mo. Compared to baseline, treatment with vitamin E alone resulted in a significant decrease in steatosis, whereas the combination therapy produced a significant improvement in steatosis, cytologic ballooning, Mallory’s hyaline and pericellular fibrosis. Although vitamin E did not have any significant effects on metabolic endpoints, combination therapy improved insulin sensitivity, lowered fasting free fatty acid (FFA) levels and decreased metabolites of FFA oxidation. However, like in the previous trial by Neuschwander-Tetri and coworkers\(^{[52]}\), one of 10 patients receiving pioglitazone plus vitamin E had a significant increase in ALT level and was withdrawn from the study.

## LIPID LOWERING DRUGS

Because NAFLD frequently occurs with a disordered lipid homeostasis, lipid-lowering drugs are considered as possible treatment for NAFLD. Hypertriglyceridemia and reduced HDL-cholesterol level are typical dyslipidemias associated with NAFLD. Gemfibrozil reduces very low-density lipoprotein triglyceride production. In a small controlled study of 46 patients with NASH, levels of AST were significantly decreased in 74% of the gemfibrozil group compared with 30% in the control group after 4 wk of treatment\(^{[53]}\). There was no correlation with pretreatment serum triglyceride levels. Posttreatment liver biopsies were not performed and the duration of biochemical response was not evaluated.

In NASH patients with hyperlipidemia statins are another potential treatment option. However, existing data are predominantly uncontrolled with a small number of patients. One study analyzed 28 hyperlipidemic patients with biopsy-proven NASH. Patients were given atorvastatin 20 mg daily for 24 wk. Both significant reduction of LDL-cholesterol and liver enzymes were detectable after treatment\(^{[54]}\). Statin-induced hepatotoxicity did not occur and the risk seems to be not increased in patients with presumed NAFLD\(^{[55]}\). However, controlled trials with a bigger number of patients are required to demonstrate the benefit and elucidate potential risks of administrating statins.

## BLOCKADE OF TNF-α

Adipose tissue produces several cytokines and biologically active proteins, denoted as adipokines, regulating hepatic and peripheral glucose and lipid metabolism. These adipokines include leptin, resistin, adiponectin and TNF-α. Expression of resistin is not increased in patients with insulin resistance, although resistin inhibits insulin action in animal models\(^{[56]}\). NASH patients show increased serum leptin levels, suggesting the attempt to overcome hepatic leptin resistance to stimulate hepatic lipid turnover\(^{[57]}\). In several studies investigating the pathomechanisms of fatty liver disease increased TNF-α levels have also been demonstrated\(^{[58, 59]}\). TNF-α contributes to insulin resistance and thereby increases hepatic steatosis and plays a
potentially proinflammatory role\(^{[29]}\). This was supported by studies in leptin deficient ob/ob mice. Treatment of anti-TNF-\(\alpha\) antibody improved liver histology, reduced hepatic total fatty acid content, and decreased ALT levels\(^{[13]}\). However, studies of ob/ob mice lacking type I and II TNF receptors have suggested that TNF-\(\alpha\) is not involved in the liver disease\(^{[60]}\). Further evidence of the involvement of TNF-\(\alpha\) came from studies of pentoxifylline which acts as an inhibitor of TNF-\(\alpha\)\(^{[61, 62]}\). In these two studies 20 patients and 18 patients, respectively, with biopsy confirmed NASH were enrolled. Pentoxifylline was given for 6 or 12 mo. Both studies demonstrated a significant improvement of AST and ALT levels after application of pentoxifylline in patients with NASH, although histological evidence of its benefit remains unknown.

Adiponectin is exclusively secreted from adipose tissue in inverse proportion to BMI\(^{[63, 64]}\). Although the three dimensional structure of adiponectin closely resembles that of TNF-\(\alpha\)\(^{[65]}\), these two proteins have completely opposite effects. Adiponectin and TNF-\(\alpha\) suppress each other’s production and antagonize their biological effects\(^{[66]}\). Adiponectin acts to reduce body fat\(^{[67]}\), improve hepatic and peripheral insulin sensitivity\(^{[68]}\) and decrease fatty acid levels\(^{[69]}\). Adiponectin has also antiinflammatory effects which could prevent liver disease. Xu and coworkers replenished recombinant adiponectin in mice fed with a high fat ethanol containing diet and in obese ob/ob mice with NASH. In both mice, administration of adiponectin ameliorated hepatomegaly, steatosis, and elevated ALT levels\(^{[70]}\). Both hepatic TNF-\(\alpha\) expression and serum levels of TNF-\(\alpha\) significantly decreased, which is further evidence for a harmful role of TNF-\(\alpha\).

This concept was further supported by a study of over 100 patients with NAFLD\(^{[71]}\). Multivariate analysis revealed that decreased serum adiponectin levels and increased TNF-\(\alpha\) and soluble TNFR2 levels correlated with the presence of NASH independent of the presence of insulin resistance. NASH patients showed lower adiponectin levels than patients with simple steatosis. Levels of adiponectin correlated with the degree of hepatic necroinflammation. These data provide evidence for the involvement of TNF-\(\alpha\) and adiponectin in human NAFLD. Therefore, studies evaluating the effect of adiponectin in treatment of NASH are required.

**LIVER TRANSPLANTATION**

NASH is considered the most common cause of cryptogenic cirrhosis\(^{[72]}\). Patients with pure steatosis have a benign prognosis, whereas the risk for developing cirrhosis and hepatocellular carcinoma in NASH patients is increased\(^{[73, 74]}\). Complications of cirrhosis or hepatocellular carcinoma may require liver transplantation. In one study by Laurin and coworkers six of eight patients who underwent transplantation for NASH developed recurrent NASH. In three of these six patients, perivenular fibrosis recurred\(^{[75]}\). The patients with recurrences revealed post-transplant hyperlipidemia and increases in body weight. In two subsequent studies the recurrence rate of steatosis was between 60 to 100% of transplant recipients\(^{[76, 77]}\). In one third of these patients progression to steatohepatitis occurred.

**CONCLUSIONS**

Because of increasing incidence of obesity and insulin resistance NAFLD has become increasingly the focus of basic and clinical research. Whereas fatty liver shows a benign prognosis, patients with NASH should be treated. Progress in understanding the pathomechanisms which contribute to aggravation of fatty liver pathology offers potential treatment options. However, a standard therapy has not been established. The most promising results came from trials with second generation insulin sensitizer in obese patients with insulin resistance. Blockade of TNF-\(\alpha\) by adiponectin showed impressive improvement of NASH in an animal model. Clinical trials investigating therapeutic effect of inhibiting TNF-\(\alpha\) in NAFLD have to be awaited. So, beyond clinical studies, the first step in treatment should be improvement of insulin sensitivity by weight loss and physical activity.

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