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Non-alcoholic steatohepatitis: An update in pathophysiology, diagnosis and therapy

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ARTICLE INFO	ABSTRACT
Article Type: Review Article *Article history:* Received: 23 May 2010 Revised: 14 Jul 2010 Accept: 27 Aug 2010 *Keywords:* Non - alcoholic steatohepatitis Diagnosis Therapy	Non-alcoholic steatohepatitis (NASH), first described by Ludwig et al, in 1980, is a stage in the wide spectrum of non-alcoholic fatty liver diseases (NAFLDs) and one of the leading causes of chronic liver disease. Several scientists have tried to more distinctly discover and describe different aspects of NASH. In contrast with its counterpart in the NAFLDs—the NAFL—NASH consists of inflammation as well as necrosis in the liver tissue resulting in a poor outcome. NASH is also a known etiology for cryptogenic liver cirrhosis. Evidence suggests that cirrhosis developing due to NASH have a relatively worse outcome compared to that of hepatitis C-related cirrhosis. In this review article, we try to review and present all relevant articles about NASH.

▸ *Implication for health policy/practice/research/medical education:*
The incidence of NASH is gradually increasing due to increasing in the risk factors. This review article endeavors to collect some important pathophysiological aspects of the disorder for attention of nutritionists, general practitioners and internists.

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Background

Non-alcoholic fatty liver diseases (NAFLDs) is a health dilemma for the recent three decades provoking quite less concerns in the past (1, 2). However, nowadays, its prevalence has grew to 30% in the United States general population and like other gastrointestinal disorders it also grew in developing countries (3). Non-alcoholic steatohepatitis (NASH), first described by Ludwig et al. in 1980, is a stage in the wide spectrum of NAFLDs and one of the leading causes of chronic liver disease (4). Powell and colleagues (5) recommended the following diagnostic criteria for NASH: a liver biopsy specimen that shows moderate to gross macrovesicular fatty degeneration with inflammation (lobular or portal) with or without Mallory hyaline bodies, fibrosis, or cirrhosis, convincing evidence of negligible alcohol consumption (<40 g of ethanol per week) that includes a detailed history taken by three physicians independently and interrogation of family members and local medical practitioners. Results of random blood assays for the estimation of ethanol levels or of desialylated transferrin, a marker of alcohol consumption, should be negative, absence of serologic evidence of previous infection with hepatitis B virus or a clinical course that hepatitis C. In contrary to its counterpart in the NAFLDs—the NAFL—NASH consists of inflammation and necrosis in the liver tissue and has a poor outcome (6). NASH is also a known etiology for cryptogenic liver cirrhosis. After development of cirrhosis, steatosis disappears in the liver tissues leading to an underdetermined diagnosis of the underlying disease in cirrhotic patients (7, 8). According to a study by Ratziu, et al (9), cirrhosis developed due to NASH has a relatively worse outcome than that due to hepatitis C in one study. It can lead to hepatocellular carcinoma (7, 9-11). In this article, we try to review and present all relevant articles about NASH.

Methods

Search for articles were performed through Medline and Google Scholar using the keywords "non-alcoholic steato-

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hepatitis," "steatohepatitis," "NASH," "non-alcoholic fatty liver." Only articles published in English language journals to 2010 were reviewed. We also used the bibliographies of relevant articles as well as their citations to enhance our search.

Epidemiology

It is estimated that about 8% of general population has elevated serum aminotransferase levels (12) and majority of them are NAFLD. The most important risk factors for NAFLD are male sex, age, obesity, insulin resistance and cardio-metabolic alterations that define the metabolic syndrome. The prevalence of NAFLD is 80%–90% in obese adults and up to 90% in patients with hyperlipidemia; the prevalence of NAFLD among children is 3%–10%, rising up to 40%–70% among obese children (13). Twenty percent of NAFLD patients are supposed to have necro-inflammation and degrees of peri-cellular fibrosis in their liver biopsy specimens and "NASH" is the term used to address this condition. Since definite diagnosis of NASH needs liver biopsy and histopathological studies, the exact prevalence of the disease has not yet been defined in the general population. But, studies on patients undergone liver biopsies for routine indications have demonstrated a prevalence of 1.2% in Japan (14) to approximately 7%–9% in western countries (4).

Demography

NASH generally occurs within the 5th and 6th decades of life (4, 15-18), although cases in earlier decades of life have also been reported (11, 12). NASH is also believed to occur more frequently in women (4, 5, 16, 19-22), although Bacon, *et al*, (17) reported that a higher proportion of NASH patients were male (58%). There is nothing about the reason behind this gender disparity in NASH patients, although hormonal effects and a higher prevalence of obesity in women, may make NASH more prevalent in them (23). Female gender and younger age tend to have a relatively lower prevalence of hepatocellular carcinoma (HCC) due to NASH (11). Obesity is the condition most often associated with NASH: The first evidence showing an association between obesity and liver histopathological abnormalities including steatosis, inflammation, and fibrosis was achieved in 1958 (24). Different studies have reported that a high proportion of NASH patients are obese with a body weight >10% above the normal ranges. Obesity was also strongly associated with NASH (5, 16, 17, 25-27), although studies recommended obesity as a confounding variable which is more likely to exist concomitant with diabetes mellitus (28); in accordance with this conclusion, Bacon and colleagues (17) reported a low prevalence of obesity of 39%. Although weight reduction is one of the most proposed treatment strategies in obese people with NASH (29), surgical weight reduction has also been reported as an etiological factor associated with liver dysfunction (16). While, it is proposed that gradual weight reduction of about 0.5 to 1.6 kg per week to work as a curative method for NASH management, accelerated weight loss may worsen the clinical picture (30-32).

Diabetes mellitus

Non-insulin-dependent diabetes mellitus and elevated blood glucose values are noted in 21%–75% of patients with

NASH (4, 5, 20, 33, 34) and is one of the major risk factors of the disease (19, 35-40); although Baldridge and colleagues (41) found normal fasting blood sugar levels in all their pediatric patients with NASH. In an autopsy study, Wanless and Lentz (26) found a higher prevalence of NASH in patients with type II diabetes mellitus. Hyperlipidemia has been reported in 20%–92% of patients with NASH (15-17, 19, 27, 42). Elevated serum triglycerides and/or low serum high-density lipoprotein (HDL) cholesterol are found in up to 82% of patients with NASH and is also reported to be significantly associated with the disease (27, 43-45). Serum low-density lipoprotein (LDL) cholesterol and total cholesterol are less commonly reported to be associated with NASH. A recent study on mice (46), showed a prominent role of cholesterol during the development, inhibition and reversal of hepatic inflammation in NASH. Other conditions that are less commonly associated with NASH include total parenteral nutrition (47), jejunal diverticulosis with bacterial overgrowth (48), biliopancreatic diversion (49), Weber-Christian disease (50), partial lipodystrophy (51), abetalipoproteinemia (52), and therapy with certain drugs (53-56).

Pathogenesis and pathophysiology

It seems that NASH is multifactorial and its pathogenesis is not well defined. Since now, several factors have been proposed to cause or contribute to development of NASH *(Table 1)*. Accumulation of triglyceride-filled vacuoles within the hepatocyte cytoplasm which leads to fatty liver is generally considered as a benign condition; however, NASH develops inevitably subsequent to fatty liver. This shows that fatty liver can result in consequences that lead to NASH.Pathogenesis of NASH is proposed to be based on a two-hit model (57-59). The first hit is hepatic steatosis that makes the liver susceptible to some hepatic insults leading to inflammation, steato-necrosis and fibrosis (60). Hepatic steatosis has been demonstrated to be associated with insulin resistance (27, 61-63). However, insulin resistance is shown to be a mechanism protecting tissues from fat accumulation which provokes through an increase in plasma free fatty acid and cytokine levels (64, 65). Free fatty acids activate an enzymatic cascade that impairs normal tyrosine phosphorylation of insulin receptor substrates, resulting in failure to transportation of glucose into the cell (22, 60, 65, 66).There are a number of factors that have been reported to contribute to interruptions in physiologic pathways in NASH patients: Diets consisting of excessive saturated fatty acids and cholesterol (63, 67, 68), a reduced ability of insulin to inhibit lipolysis (61), and decreased levels of proteins involving in VLDL metabolism (61, 69, 70).The second hit includes excessive amounts of oxidative stress and pro-inflammatory cytokines but reduction in hepatic ATP production. Accumulations of fat in the liver and augmented levels of free fatty acids are major players in the pathogenesis of NASH. High levels of fat within the liver can occur due to an increased free fatty acids absorption by the liver, decreased oxidation of them, excessive synthesis of them in the liver, and decreased production of VLDL cholesterol by the liver (71, 72). Metabolism of free fatty acids in the liver provokes reactive oxygen species (ROS) that produces free radicals. These highly reactive molecules induce a destructive metabolism for free fatty acids known as lipid peroxidation that results in cellular membrane disruption and cellular dysfunction (73). Intraperitoneal injec-

tion of insulin during long-term peritoneal dialysis results in steatohepatitis in the liver (74). This fact may suggest that insulin plays a role in NASH associated with obesity. Insulin reduces metabolism of free fatty acids, so as a result, levels of toxic free fatty acids in the liver raise up (75); moreover, obese patients are also resistant to insulin and have higher plasma levels of free fatty acids (76). Insulin levels in obese patients are sufficient to inhibit free fatty acids delivery by the liver, thus, elevated insulin levels leads to accumulation of free fatty acids in the liver. The pathogenesis of NASH following jejunal (including jejunocolic and jejunoileal) bypass surgery for weight reduction is complex and multifactorial. Mechanisms suggested to explain the pathogenesis include bacterial products absorbed from the blind loop as well as the bile acids (77-80), protein-energy malnutrition

(81), vitamins deficiency (78), and pathologic mobilization of free fatty acids during weight loss (82). For prevention of hepatic failure after jejunal bypass, a dietary regimen with low fat and carbohydrates but high in protein has been recommended (83). Intravenous carbohydrates have been shown to induce fatty liver in the critically ill patients (26). Fatty acids are synthesized within the liver when there is a need for, and enhances by high-carbohydrate and low-fat regimens. Hyperinsulinemia occurring due to carbohydrate overuse, insulin resistance and critical illness, is responsible for the elevated de novo fatty acid synthesis (84, 85). Another mechanism suggested for the pathogenesis of NASH is that lipolysis can mobilize free fatty acids from adipose tissue to liver (86). This mechanism of action is proposed in patients with decreased insulin action (as seen in hypoinsulinemia and

Table 1: Potential etiologies of NASH

1. Drugs or Toxins	
Metals	
Antimony	Carbon disulfide
Barium salts	Thallium compounds
Antibiotics	
Bleomycin	Tetracycline
Other drugs	
Amiodarone	Tetracycline
Coumadin	Valproic acid
Synthetic estrogens	Methotrexate
Glucocorticosteroids	Cocaine
Perhexilene maleate	Didanosine
Aspirin	Fialuridine
Calcium-channel blockers	Zidovudine
Tamoxifen	
2. Metabolic disorders	
Abetalipoproteinemia	Glycogen storage disease
Lipodystrophy	Weber-Christian syndrome
Dysbetalipoproteinemia	Wilson's disease
Hyperlipidemia	Wolman's disease
Galactosemia	
3. Nutritional	
Obesity	Total parenteral nutrition
Diabetes and hyperglycemia	Rapid weight loss
Intravenous glucose therapy in the week before death	Kwashiorkor
4. Surgical	
Jejuno colic bypass	Gastroplasty
Jejunoileal bypass	Extensive small bowel resection
Biliopancreatic diversion	
5. Other disorders	
Inflammatory bowel disease	Hepatotoxins: phosphorus,
Jejunal diverticulosis with bacterial overgrowth	Petrochemicals, organic solvents
Small bowel bacterial overgrowth	Acute starvation
Human immunodeficiency virus infection	

insulin resistance), rapid weight reduction or acute starvation, or exposed to high level of corticosteroids, leptin, and tumor necrosis factor alpha (TNF-α) (64, 87, 88). When obese people fast for a weight reduction, insulin levels decrease; however, insulin levels in obese patients who have had gastrointestinal bypass surgery for weight reduction augments since they continue to receive carbohydrates stimulating insulin secretion. Increased insulin levels in these patients result in the accumulation of free fatty acids in the liver, as mentioned above. Other proposed pathophysiological mechanisms for the development of NASH include attenuated mitochondrial beta oxidation causing increased forma-

tion of reactive oxygen species and ATP depletion (89). Three main mechanisms have been proposed for triggering steatohepatitis and fibrosis by mitochondria which are generally considered as the main sources of reactive oxygen species: lipid peroxidation, cytokine induction and induction of Fas ligand. Reactive oxygen species (ROS) cause lipid peroxidation, releases malon-dialdehyde (MDA) and μ-hydroxynoneal (HNE) and induces formation of cytokines TNF-α, TGF-beta and IL-8. Mitochondrial ROS cause expression of Fas ligand on hepatocytes, which may interact with Fas ligand of other hepatocytes causing death (90). MDA and HNE may further facilitate cell death and collagen synthesis. Moreover, con-

centration of free fatty acids in the hepatocytes may result in mitochondrial swelling, increased fragility, and membrane permeability that may lead to an increased ratio of mitochondrial AST to total AST in NASH patients.

Clinical Features

About 48%–100% of NASH patients are asymptomatic (5, 17, 91). Nonspecific constitutional symptoms including feeling of fullness, discomfort, or vague pain in the upper right abdominal quadrant (in about 25% of patients), profound prolonged lethargy (17, 92), weakness, fatigue and malaise (17) exist in a third of NASH patients (17). Pediatric NASH patients more frequently present with clinical symptoms other than those of adults (41, 92). Most patients have abnormal liver function tests incidentally found when performing laboratory tests for checkup or other conditions (such as hypertension, gallstones, coronary artery disease, congestive heart failure, cancer, peripheral vascular disease, hypothyroidism, and gynecologic or psychiatric conditions) (23). Although significant alcohol consumption is an exclusion criterion for the diagnosis of NASH, most patients report an alcohol intake of less than 20–40 g/day. However determining the amount of alcohol consumed by patients who gave history of alcohol consumption is difficult to verify, and even many patients who consume excessive amounts of alcohol underestimate or deny alcohol ingestion (93), due to memory mistakes, cultural issues as well as legal anxieties in certain countries like Iran. Pinto and colleagues (33) suggest that it is difficult to differentiate patients with NASH from alcoholic hepatitis based on clinical and biochemical evaluations alone. Baker, *et al* (94), suggested that alcohol may contribute to the development of NAFLD in human subjects.NASH has no direct physical signs that can directly lead the physician to diagnose the disease. The most common finding at the initial presentation is obesity which is present in up to 100% of NASH patients (95). Asymptomatic hepatomegaly, without evidence of chronic liver disease is the second most common physical sign of NASH that has been reported in up to 75% of patients when measured by palpation and percussion (16, 17); the rate is 90% by ultrasonographic evaluations (5). Splenomegaly is the third most commonly recognized sign of NASH reported in 6% to 25% (4) of patients. A recent study on 84 NAFLD patients (62 had NASH) (96), demonstrated that spleen body index (SBI) measured by computed tomography might be a non-invasive and simple method of differentiating NASH and simple steatosis particularly in the early stages of the disease. In this study, the SBI was significantly correlated with the discrimination of simple steatosis and NASH with mild fibrosis on multiple logistic regression analysis.

Laboratory evaluations

NASH patients typically present with mild to moderate elevations of serum aminotransferase levels, reported 1.5–4 times the upper limit of normal (22, 34, 42, 97, 98). Although serum aminotransferase levels are the most commonly used method for screening of NASH, these lack sufficient sensitivity to detect patients with NASH; these test cannot predict hepatic injury as well (99). Less than one-third of NASH patients present with elevated alkaline phosphatase. It has been demonstrated that alanine transaminase (ALT)

has positive correlations both with dyslipidemia and family history of liver disease (98). In up to 95% of patients with alcoholic liver disease, serum ALT level is higher than AST (17, 100-102). However, this is not a general rule and conflicting results have been reported (20). On the other hand, in those with severe NASH, the serum level of AST is higher than ALT. There was no significant difference between fibrotic and non-fibrotic disease in one study (102), though in another study, abnormal AST was associated with fibrotic NAFLD (103). AST can better differentiate simple steatosis and NASH than ALT in morbidly obese patients (103). A recent study by Puljiz, *et al* (104), on patients with elevated ALT showed that 30% had NASH and that high triglyceride, low HDL and high serum ferritin levels correspond with NASH. Although it is generally believed that, liver function indices such as prothrombin time (PT), albumin, total protein, and bilirubin, are almost always within the normal range (15, 17, 23, 40, 97, 105), there are exceptions possibly due to hepatic injury and malnutrition in which laboratory abnormalities including hypoalbuminemia, prolonged PT, and hyperbilirubinemia, may be found in patients with end-stage liver disease (57). Prothrombin activity level may also be able to predict fibrosis progression in NASH patients; it is reported that patients with progressive fibrosis have lower prothrombin activity than those with non-progressive fibrosis NASH (40). Hyperinsulinemia as well as an impaired glucose tolerance is a common finding in NASH patients. It is found in more than half of the patients; C-peptide levels are increased in 96% of patients, reflecting insulin hypersecretion (27). This is also suggested as a major factor in the pathogenesis of pediatric NASH (106). It is suggested that insulin resistance is the main predictor of NASH. Moreover, insulin level is higher in those with higher histological classes of NASH (20). On the other hand, hyperinsulinemia is frequently observed in the conditions with which NASH is highly associated, including visceral obesity, type 2 diabetes mellitus, high serum ferritin levels, and parenteral nutrition in critically ill patients (25, 26, 107-110). Hyperinsulinemia is also shown to be associated with the stage of fibrosis in hepatitis C virus infection (111). Although, as it is mentioned earlier, hyperinsulinemia elevates de novo fatty acid synthesis (82, 83)—a condition which is the main pathogenesis of NASH, we have no strict evidence showing whether hyperinsulinemia is an independent factor associated with NASH or it is only seen concurrent to these conditions.Dyslipidemias are also common abnormalities observed in NASH and have been reported in up to 81% of patients (4, 5, 15-17, 33, 112-114). Although Bacon, *et al,* (17) found no statistically significant difference between NASH patients and controls in terms of prevalence of dyslipidemia. It is suggested that hypertriglyceridemia is more likely to increase the risk of NAFLD than hypercholesterolemia (115). Evidence suggests that among different types of cholesterol, abnormality of HDL is the most frequent lipid profile abnormality in NASH patients, while LDL as well as total cholesterol are more likely to be within normal ranges (61, 103). However, data also exist reporting adverse results (114, 116). In one study (102), no significant difference was observed among different classes of the disease in terms of triglyceride and cholesterol levels. Those with moderate NASH had 1.5 and 2 times higher serum levels of serum triglyceride and cholesterol than those in patients with severe NASH, respectively. Imaging modalities as well as other noninvasive

methods are not accurate for the diagnosis of NASH. The presence of fat in the liver can be diagnosed by ultrasonography, computed tomography (CT), and magnetic resonance imaging (MRI). However, these techniques lack enough power to differentiate between fatty liver and NASH. At the moment, ultrasonography is the imaging technique of choice for evaluation of steatosis because it is relatively cheap, riskless, available, and yielding the best results (22). When patients present with elevated liver enzymes, ultrasound of the liver is frequently performed to detect steatosis. It manifests itself as a hyperechoic texture or a bright liver compared to kidney echogenicity (117), dark shadows posterior to the liver with vague intrahepatic vascular pattern; fibrosis can enhance echogenicity producing a coarse echo pattern showing well-defined portal veins (97, 118). Ultrasonography also has more sensitivity in detecting abnormalities in the total amount of hepatic fatty content, compared to CT (119). Despite all advantages of ultrasonography in detecting hepatic steatosis, it is not a specific method and cannot be used to diagnose NASH. When changes in fatty content are focal, CT and MRI are superior to ultrasonography. Several new techniques in MRI like fast gradient echo techniques (modified Dixon method) have resulted in substantial advances in the ability to diagnose a fatty liver by MRI (120).

Treatment

Since the pathogenesis as well as distinct molecular pathophysiological course of NASH have not yet been elucidated and for lack of properly designed large trials on NASH patients, there is no general consensus on the treatment strategy for NASH. However, based on prognosis of patients with NASH, need for effective therapies to prevent its progression seems evident.

Weight Management

Lifestyle modifications should include nutritional interventions as well as exercise implementation (121). Protein-energy imbalance such as excessive energy intake has been shown to be consistently associated with higher prevalence of hepatic steatosis (122). In obese patients, moderate weight loss of about 6% via caloric restriction has been shown to result in improvements in intrahepatic lipid content (123). Another study showed that weight loss through caloric restrictions results in improvement in steatosis in serial liver histological evaluations (124). It also has been shown that weight reduction can normalize serum aminoteransferase activities in overweight people who present with aminoteransferase abnormalities both in children and adults (125, 126). Clinical trials have also demonstrated a substantial reduction in aminoteransferase levels in patients with NAFLD when they are put on calorie-restricted diets whether or not on an exercise program (2, 125-131). On the other hand, it has been shown that the degree of improvement in insulin sensitivity is related to the exercise intensity possibly due to its role in increasing the oxidative capacity and utilization of fatty acids by muscles (132). Moreover, it is known that type of diet and rates of weight loss are important components in the efficacy of the treatment. For example, insulin resistance can be improved by a fiber-rich diet but not by diets consisting of high levels of saturated fats (133). Furthermore, a very rapid weight loss may increase portal

inflammation and fibrosis; it is recommended that weight reduction should not exceed 1.6 kg per week (32). A reasonable diet in adults also has been suggested to contain 45–100 g high quality animal protein, less than 100 g carbohydrates (avoiding high fructose value regimens) (134) and less than 10 g fat (with decreased intake of saturated and trans lipids) (135) per day comprising around 600 to 800 kcal of energy (28). Through this approach, it is highly recommended that gradual weight loss should be performed through the combination of dietary restrictions along with regular aerobic exercise regimen of at least 30–45 minutes three to seven times a week. A recent article by Centis, *et al* (136), suggest that cognitive-behavior treatment would also be a good approach and should be considered for patients with NAFLD. This treatment can be coupled with prevention strategies at the population level.

Pharmacologic intervention

A number of studies have investigated the usefulness of medications for weight reduction. Since now, a number of drugs are in phase III trials to get approval for treatment of NASH. The drugs include orlistat, metformin, vitamin E, rosiglitazone, and pioglitazone (63, 137). Orlistat, a reversible inhibitor of gastric and pancreatic lipase, has been proven in weight reduction; however, its value in the treatment of NASH needs more evaluations. A clinical trial showed that orlistat can achieve a body weight reduction of over 10 kg as well as significant reductions in serum transaminase levels in obese patients with NASH (138, 139); but side effects of this drug including diarrhea and bloating has made it less attractive. On the other hand, in a more recent study, Harrison, *et al*, showed that orlistat has no superiority in terms of weight loss or histological improvement to calorie restriction alone (140). Rimonabant, a selective antagonist for endocannabinoid receptor 1, is approved for weight reduction. In addition, it is proposed that this agent may have some favorable anti-steatotic and anti-fibrotic values (141-144). Ursodeoxycholic acid (UDCA), a cytoprotective agent, has been demonstrated to be useful and safe in patients with chronic liver diseases such as primary biliary cirrhosis and primary sclerosing cholangitis (145). In NASH, it caused significant decreases in the levels of liver enzymes and normalization in 77%–87% of patients (146-149). After one year after drug use, it has also improved steatosis at ultrasonogram (149) and histological grade (146) but it had no effect on fibrosis (149). On the other hand, a randomized trial on NAFLD patients showed no advantage by using UDCA over placebo when added to a hypocaloric diet (150). A review of four randomized controlled trials showed no significant difference regarding mortality or improvement in liver function tests after treatment with UDCA (151). In an open-label pilot study, Laurin, *et al*, (146) tested the effect of UDCA against clofibrate on NAFLD patients. They found that significant improvements have been achieved in serum hepatic enzymes (alkaline phosphatase, aminotransferases, gamma-GT) as well as grade of steatosis at liver biopsy. Kiyici, *et al*, (149) also confirmed results of the said study against atorvastatin. However, two other studies found no advantages for UDCA on hepatic enzyme abnormalities and NASH (152, 153). Lindor, *et al*, also suggest that there is a possibility for a spontaneous improvement in liver enzymes in patients with NAFLD associated with improvements in the degree of steatosis and fibrosis. In another

study by Obinata, *et al*, (154) taurine, an agent in the same drug category, was found to improve aminotransferase levels regardless of weight changes in children with NASH.

Metformin

Several studies have consistently showed association between insulin resistance and elevated aminotransferase levels in NASH (155, 156). This forms a basis for the use of insulin sensitizers with the presumption that it could improve NAFLDs. Metformin, a biguanide, reduces hyperinsulinemia and has therapeutic advantages as an anti-diabetic agent and insulin sensitizer. Its anti-diabetic action is through diminishing glucose absorption in the intestinal tract, down-regulating hepatic gluconeogenesis, and facilitating glucose uptake and utilization by diverting fatty acids from lipid production to mitochondrial oxidation (157, 158). It also has stimulatory effects on AMP-activated protein kinase and modulation of hepatic TNF-α expression (159, 160). In an open label controlled study, Marchesini, *et al* (105), found that metformin significantly reduces liver volume, moderately improves insulin sensitivity, and normalizes aminotransferase levels in 50% of cases; medication withdrawal led to a return of aminotransferase levels to pre-treatment values. It also has been shown that its beneficial effect on serum aminotransferase normalization is greater than that can be achieved through dietary modification alone (161). It has already been shown that treatment with metformin improved inflammation and hepatic steatosis in steatotic animal models (160). However, there are few small controlled studies in which effect of metformin on hepatic histology in patients with NASH is examined. For example, a randomized study on 36 patients with NASH treated with dietary counseling with or without metformin confirmed the preliminary knowledge of improved serum aminotransferases and insulin sensitivity by metformin but failed to show a significant improvement in necro-inflammation and fibrosis on repeated liver biopsy (162). The same results were achieved when metformin was examined against rosiglitazone (163); on the other hand, in an open-label randomized study, repeated liver biopsies in 17 patients with NASH who received metformin demonstrated significant improvement in steatosis, necroinflammation, and fibrosis (164). We therefore, recommend use of metformin in patients with NASH, since most patients with NASH including non-diabetics have glucose intolerance and the least advantage of using metformin is a decrease in the risk of developing diabetes in these patients (165). Loomba, *et al*, also confirmed beneficial effects of metformin in the treatment of NASH(166). Schwimmer, *et al*, also found the same results in a pediatric group with NASH(167).

Thiazolidinediones (Glitazones) improve insulin sensitivity in adipose tissue by activating the nuclear transcription factor on peroxisome proliferators-activated receptor gamma (PPAR-gamma) (168). The first evidence on a potential beneficial effect of thiazolidinediones use in NASH patients was achieved by Caldwell, *et al* (169, 170), in which 7 out of 10 patients receiving troglitazone normalized aminotransferase levels—although no significant histological response was attained. This agent has been withdrawn from the market due to its hepatotoxicity (171). In a study by Promrat, *et al*, (172) 72% of patients who received pioglitazone for almost a year experienced normalization of serum ALT, steatosis, necro-inflammation, and fibrosis on liver histology. Radio-

logical evaluations by MRI showed significant decreases in liver volume and fat content. In another study, pioglitazone at a dose of 15 mg/day for three months normalized ALT levels in all the 12 patients with NASH (173). A more recent randomized clinical trial by Belfort, *et al*, showed biochemical and histological improvements associated with the use of pioglitazone (174). Similar results have been achieved through other studies on pioglitazone (175-177). Oruc, *et al*, demonstrated that rosiglitazone 4 mg/day for three months improves aminotransferases and insulin sensitivity in fatty liver diseases (178). Another large study employing rosiglitazone revealed the same results plus histological improvements in patients with NASH (179). The only adverse effects associated with thiazolidindiones are mild weight gain and rare hepatotoxicity (180, 181). Moreover, rosiglitazone is suggested to rarely cause silent cardiac failure which is more likely to occur in conditions most prevalently existing in patients with NASH (hypertension and metabolic syndrome) (182). On the other hand, discontinuation of pioglitazone is shown associated with a reversal of its beneficial effects on both biochemical markers as well as histological findings (183).

Lipid-lowering agents

Since dyslipidemia is a strong factor associated with NAFLD, attempts have been made to ameliorate lipid homeostasis and hypertriglyceridemia as a possible treatment for NASH. Although clofibrate is considered one of the main medications used for hypertriglyceridemia and reduces hepatic triglyceride in experimental models of hepatic steatosis, in human models, Laurin, *et al* (146), found no significant improvements in either enzyme levels or liver histology when the agent was used at a dose of 2 g/day for one year. However, in a controlled study, Basaranoglu, *et al* (184), found that gemfibrozil, another drug in the same drug category as clofibrate, was significantly effective in reducing aminotransferase levels in 74% of patients after four weeks of administration. In a report of two cases with NASH under treatment with tamoxifen (185), bezafibrate prevented the histological damage progression of NASH. To our knowledge, these are the only published data existing in the literature on potential effects of clofibrate, bezafibrate, and gemfibrozil in NASH patients. Therefore, future studies with larger NASH population as well as more perfect methodologies (double blind randomized controlled trials) seems necessary.Statins, inhibitors of 3-hydroxy-3-methyglutaryl-coenzyme A reductase, have also suggested as safe and having healing effects in patients with underlying liver diseases and serious hepatotoxicity is quite rare. The effect is almost universally reversible upon prompt discontinuation of the drug (186-189). Kiyici, *et al*, reported significant improvements in cholesterol and liver enzyme levels with atorvastatin when administered at a dosage of 10 mg/day for six months in patients with NASH and hyperlipidemia(149). However, fibrosis and necroinflammation were not improved compared to patients receiving UDCA. Another study on humans comparing the effects of atorvastatin with omega-3 fatty acids and orlistatin in patients with NAFLD (190), showed that orlistatin causes a greater improvement in histological indices while omega-3 fatty acids are more effective in reducing AST and triglyceride levels. Atorvastatin was also effective in reducing serum transaminase levels as well as ultrasonographic manifestations of NAFLD in patients presenting with hyper-

cholesterolemia. In another study, atorvastatin use was associated with improved serum aminotransferase levels and lipid levels in patients with NAFLD (191). As well, pravastatin at 20 mg/day for six months improved both liver enzymes and histological findings in patients with NASH (192). A recent study on 11 patients showed that long-term consumption of olive oil enriched with n-3 PUFA in patients with NAFLD is able to decrease circulating liver enzymes and triglycerides, with a significant improvement of adiponectin levels (193). Georgescu, *et al*, found that atorvastatin and losartan are efficient in the treatment of dyslipidemia- and hypertension-associated NASH, by improving both biochemical parameters and steatosis(194). They found similar efficacy for pentoxifylline in non-hypertensive/non-dyslipidemic patients, while UDCA did not improve the histological score, although it improved the biochemical parameters.

Antioxidants

At this time, there is a shortage of data supporting the use of vitamin E, as a potent therapeutic approach in NASH patients. Antioxidants first became candidate as potential therapeutic agents for NASH because oxidative stress is a well-known relevant factor in the pathogenesis of NASH (195). Moreover, serum levels of antioxidants such as vitamin E, have been shown to be low in obese children (196, 197). Lavine, demonstrated that supplementation with vitamin E results in normalization of ALT in children aged less than 16 years(198). Another one-year study on patients with NASH receiving vitamin E as well as dietary advice showed substantial improvement in histological findings of liver biopsy specimen (199). In a double-blind randomized study, 45 patient received vitamin E, vitamin C or placebo together with dietary counseling. Histological evaluations showed significant improvements in fibrosis score, although there were no changes in serum aminotransferase levels or inflammation (200). Kugelmas, *et al* (201), found no beneficial effect of vitamin E on serum cytokine levels. Silybin, a formulation of vitamin E showed biochemical improvement in patients with NASH but histological follow-up was not addressed in this study (202). On the other hand, Bugianesi, *et al*, in their open label randomized study, found that vitamin E has similar efficacy to prescriptive diet but inferior to metformin when looking at improvement in serum aminotransferase levels(164). Although, histological improvement was achieved by metformin, for lack of follow-up for vitamin E group, no data was achieved. Another randomized study showed that pioglitazone and vitamin E were more potent in improving steatosis and histological features of NASH than vitamin E alone (203). Vitamin E at a dosage of 300 mg/day for one year resulted in a significant reduction in AST, ALT, and g-GT and improvement in steatosis, inflammation, and fibrosis in liver biopsy of 12 patients with NASH (198). In another pilot study, vitamin E was administered for six months. After this time, liver enzymes, cholesterol, ultrasonographic and histological grade of steatosis, and inflammation were substantially improved (198). N-acetylcystein, a well-known antioxidant, was administered to 11 patients with NASH at a dosage of 1 g/day which resulted in significant decreases in serum aminotransferase levels after three months of therapy (204). Similar study employing N-acetylcysteine for one month demonstrated biochemical improvement in patients with NASH (205). Betaine is a component of the metabolic cycle of methionine, capable of increasing S-adenosylmethionine levels and, as a result, decreasing the rate of hepatic steatosis. A 12-month treatment was associated with a significant improvement in ALT, AST, and histological grade of steatosis, inflammation, and fibrosis (206). When administered concomitantly with diethanolamine glucuronate and nicotinamide ascorbate in NASH patients for eight weeks, betaine caused a 10% improvement in aminotransferase levels as well as a 25% improvement in hepatic steatosis in a randomized controlled trial (207, 208). A review of six trials (209) showed that treatment with antioxidant supplements causes a statistical significant though not clinically important, amelioration of AST levels. However, ALT levels had no change. Moreover, gamma-glutamyl-transpeptidase was decreased, albeit not significantly in the treatment arm.

Other drugs

A recent study by Yoneda, *et al* (210), demonstrated improvements in the clinical parameters as well as histological observations by treatment with ezetimibe in 10 patients with NASH. Probiotics (211), anti-tunor necrosisi factor alpha antibodies (212), pentoxifylline (213), and probucol (214). Treatment with silymarin has also been shown to have significant beneficial effects on biochemical factors in patients with NAFLD (215, 216).

Phlebotomy

Since association between NASH and elevated serum ferritin, transferrin and iron concentration or transferrin saturation is a proven fact (217), and that increased hepatic iron and excessive fat accumulation may interact to generate hepatic inflammation causing liver injury (164), one may consider phlebotomy as a potential procedure likely to reduce the inflammatory process. Facchini, *et al*, showed that severe iron depletion by phlebotomy, causes reduced glucose tolerance as well as a significant improvement in aminotransferase and glucose levels(218).

Advice regarding bariatric surgery

Since bariatric surgery is a known procedure that is associated with substantial improvement in metabolic syndrome (219), scientists considered this treatment as a potential therapy for overweight patients with NASH. Dixon, *et al*, demonstrated positive effects of gastric banding on steatosis, necro-inflammatory activity and fibrosis in 22 obese patients with NASH undergoing liver biopsies after a mean losing weight of 34 kg about two years after bariatric surgery(220). These beneficial effects were more pronounced in patients with metabolic syndrome. The studied population experienced a high remission rate of 82%. Similar results have not been observed in those who had undergone gastric bypass. Another study by Luyckx, *et al*, on patients undergoing gastric bypass also showed elevated inflammatory activities in liver biopsies of patients who achieved a faster weight loss than in those who lost weight by gastric banding (221). On the other hand, Klein, *et al*, showed significant improvement in metabolic parameters and hepatic expression of factors involved in liver inflammation in seven patients one year after Roux-en-Y gastric bypass(222). A larger study by Bakker, *et al*, showed significant improvements in steatosis, lobular

inflammation, and lobular and portal fibrosis in 19 morbidly obese patients with NASH who lost a mean of 52 kg of body weight, and repeated liver biopsies after almost two years post-Roux-en-Y gastric bypass(223). Dixon also reported beneficial effects for Roux-en-Y gastric bypass for patients with NASH(224). Furuva, *et al*, evaluated liver biopsies two years after gastric bypass in 18 patients with NAFLD and showed substantial improvements in all histopathologic findings of steatohepatitis(225). Jejunoileal bypass has also been proposed as a beneficial procedure for patients with NASH (82, 226). A study on 104 patients underwent second liver biopsy also showed significant improvement in fibrosis and inflammation after biliopancreatic diversion (158). A recent study on 116 patients who underwent laparoscopic bariatric surgery, including Roux-en-Y gastric bypass, adjustable gastric banding and biliopancreatic diversion with duodenal switch (227), revealed complete regression of NAFLD in 83% of patients; most of the remaining patients presented with mild NAFLD. Complete regression of necroinflammatory activity was observed in 93% of patients. Among the 12 patients presenting with fibrosis in the first biopsy, complete remission was observed in 10 and improvement in two. Two patients continued to show the same degree of fibrosis without evidence of disease activity. None of the patients had worsening of steatosis, necroinflammatory activity or fibrosis; none progressed to cirrhosis.A recent review of 25 cohort studies evaluating effects of bariatric surgery in patients with NASH (228) showed that in 21 studies, patients experienced improvement in steatosis or inflammation scores; four studies described some deterioration in the degree of fibrosis.

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