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## Modern approach to the clinical management of non-alcoholic fatty liver disease

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### Abstract

Non-alcoholic fatty liver disease (NAFLD) is the most common and emerging form of chronic liver disease worldwide. It includes a wide spectrum of liver diseases ranging from simple fatty liver to steatohepatitis, which may progress to cirrhosis, liver cancer, and liver mortality. Common metabolic diseases, which are well established cardiovascular risk factors, have been associated to NAFLD and cardiovascular disease is the single most important cause of morbidity and mortality in this patient population. The pathogenesis of NAFLD appears multifactorial and many mechanisms have been proposed as possible causes of fatty liver infiltration. Management of fatty liver has become a major challenge to healthcare systems as the consequence of the increasing rates of obesity worldwide. First-line management focuses on lifestyle modifications. Moderate weight reduction either by dietary restriction or by increased habitual physical activity is safe and highly recommended. Several therapeutic interventions have been proposed. These include insulin sensitizer agents,

lipid lowering drugs, antioxidants such as vitamin E and supplementation of vitamin D<sub>3</sub>. However, therapeutic strategies have been largely empirical so far, and experimental trials have mostly been carried out in uncontrolled settings with small sample sizes. Metabolic conditions such as diabetes mellitus, obesity, hypertension and hyperlipidemia, should be strongly considered and a multidisciplinary approach should be personalized for individual patients. Treatment of co-morbidities should be regarded as of paramount importance in the management of these patients. The purpose of this review is to examine different approaches for the clinical management of non-alcoholic fatty liver disease.

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**Key words:** Non-alcoholic fatty liver disease; Non-alcoholic steatohepatitis; Insulin resistance; Oxidative stress; Cardiovascular risk; Statins; Vitamin D; Vitamin E; Pioglitazone

**Core tip:** Management of fatty liver has become a major challenge to healthcare systems as the consequence of the increasing rates of obesity worldwide. Several therapeutic interventions have been proposed, which include moderate weight loss, insulin sensitizer agents, lipid lowering drugs, antioxidants such as vitamin E and treatment of vitamin D<sub>3</sub> deficiency. However, therapeutic approaches have been largely empirical and experimental trials have mostly been carried out in uncontrolled settings with small sample sizes so far. Treatment of coexisting metabolic conditions should be regarded as of paramount importance in the management of these patients.

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## INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) represents the most common and emerging form of chronic liver disease worldwide. It is increasingly detected by routine abdominal ultrasound examination. Indeed, NAFLD reached epidemic proportions and the general prevalence of this condition is reported ranging between 20%-30% and 70%-90% respectively in patients with severe obesity or with type 2 diabetes mellitus<sup>[1-4]</sup>.

NAFLD includes a wide spectrum of liver diseases ranging from simple fatty liver to non-alcoholic steatohepatitis (NASH), which may progress to fibrosis and even cirrhosis and hepatocellular carcinoma. Simple steatosis generally represents a benign condition following a non-progressive clinical course. By contrast, a subset of patients with NASH, in particular those with a more severe fibrosis, are at higher risk for progressing to liver disease complications<sup>[5]</sup>. In fact, about 25% of patients affected by NASH can develop cirrhosis with the subsequent risk of hepatocellular carcinoma (HCC)<sup>[6]</sup>. Moreover, the rising number of HCCs developing into non cirrhotic NASH liver is becoming a cause for concern<sup>[7,8]</sup>.

The pathogenesis of non-alcoholic and virus-negative liver steatosis and NASH appears multifactorial and many mechanisms have been proposed as possible causes of fatty liver infiltration<sup>[9]</sup>. The association of steatosis with a number of different clinical conditions has been suggested. Common metabolic diseases, such as hyperlipidaemia, type 2 diabetes and central obesity, are well established cardiovascular risk factors and have been associated to both benign liver steatosis and progressive NASH<sup>[10]</sup>. Moreover, it has recently been proposed that the metabolic syndrome may play a causal role in the pathogenesis of NASH<sup>[11-14]</sup>.

Management of NAFLD and NASH has become a major challenge to healthcare systems as the consequence of the increasing rates of obesity worldwide. Since NAFLD is usually associated to a number of insulin resistance related conditions, treatment of comorbidity should be regarded as of paramount importance in the management of these patients. Several therapeutic interventions have been proposed, which include weight reduction, insulin sensitizer agents, lipid lowering drugs, antioxidants such as vitamin E and treatment of vitamin D<sub>3</sub> deficiency (Table 1). However, therapeutic strategies have been largely empirical so far and experimental trials have mostly been carried out in uncontrolled settings with small sample sizes.

## NAFLD AND OBESITY: A ROLE FOR LOW-CALORIE DIET, EXERCISE AND BARIATRIC SURGERY

Diet is an important contributor to the pathogenesis of

**Table 1 Major proposed interventions for nonalcoholic fatty liver disease**

Lifestyle intervention
Calorie restriction
Mediterranean diet
Exercise
Medication
Weight loss drugs
Orlistat
Sibutramine
Antidiabetics
Metformin
Pioglitazone
DPP IV inhibitors
GLP-1 receptors agonists
Antioxidants
Vitamin E
Statins
Pravastatin
Pitavastatin
Atorvastatin
Rosuvastatin
PUFA
Vitamin D
UDCA
Probiotics
Berberine
Silymarin
Silybin
Bariatric surgery

NAFLD and a healthy low-fat diet may have benefits independent of weight loss.

Some studies demonstrated favourable effects of the Mediterranean diet on NAFLD. Recently, Ryan *et al*<sup>[15]</sup> demonstrated in an insulin-resistant population with NAFLD a reduction of liver steatosis and an improvement of insulin sensitivity after 6 wk of the Mediterranean diet, compared to current dietary advice. Moreover, Kontogianni *et al*<sup>[6]</sup> reported that higher adherence to the Mediterranean diet was not associated with a less likelihood of having NAFLD, but it was associated with a lower degree of insulin resistance and less severe liver disease among patients with NAFLD. Finally, in a post hoc analysis of a controlled trial conducted in Israel, a modified Mediterranean diet was associated with the greatest reduction in alanine aminotransferase levels (ALT) in obese type 2 diabetes patients<sup>[17]</sup>.

There is evidence that saturated fat and fructose are more likely to stimulate hepatic lipid accumulation and progression in NASH, whereas unsaturated fat, choline, antioxidants and high-protein diets rich in isoflavones seem to have a more preventive effect<sup>[18-22]</sup>. Among polyunsaturated fatty acids, in particular, the n-3 fatty acids seem to reduce the accumulation of triglycerides and ameliorate hepatic steatosis<sup>[23]</sup>.

Since the majority of patients with NAFLD are overweight or obese, weight reduction either by dietary restriction or by increased habitual physical activity or by both, is highly recommended. Weight loss is safe in patients with nonalcoholic fatty liver disease, and it improves liver histology<sup>[24]</sup>. Moreover, diet is also effective

for the treatment of metabolic syndrome and is able to control its clinical and metabolic features; in fact, central obesity, insulin resistance, blood pressure and atherogenic dyslipidemia are improved after a modest weight loss of 5%-10%<sup>[25]</sup>. In addition, lifestyle modifications in the form of moderate calorie restriction and weight loss have also been consistently associated with reduced oxidative stress<sup>[25]</sup>.

The only two registered drugs for pharmacological weight reduction, Orlistat and Sibutramine, gave some positive results on serum liver enzymes, but not on liver histology in patients with NAFLD<sup>[26]</sup>.

Bariatric surgery is considered a quite safe<sup>[27]</sup> and effective<sup>[28]</sup> option for obese patients in reducing weight. Despite its known efficacy on decreasing obesity related disease<sup>[29]</sup>, at present there is a lack of randomized controlled trials evaluating the effects of bariatric surgery in patients with NAFLD and NASH. The available studies in fact are retrospective or prospective cohort studies. Based on these data, a recent Cochrane review concluded that because of a high risk of bias, a reliable summary of their data is not possible<sup>[30]</sup>. Thus, well-designed randomised trials to assess benefits and harms of bariatric surgery as a therapeutic approach for patients with NASH are required.

Short-term aerobic exercise can improve fatty liver composition in patients with NAFLD and recent studies reported reduction in hepatic lipid content also after exercise intervention programs which did not induce weight loss<sup>[31-33]</sup>. However, the independent role of regular physical activity for NAFLD has been assessed only in few studies and the optimal physical activity regimen still remains to be determined.

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## INSULIN RESISTANCE AND THE METABOLIC SYNDROME: A POSSIBLE ROLE FOR INSULIN SENSITIZER AGENTS AND INCRETINS

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Insulin resistance is a frequent metabolic abnormality affecting approximately 20% of the non-diabetic population and occurring in association with many cardiovascular and metabolic abnormalities. It has been suggested that the presence of insulin resistance is an essential requirement for the accumulation of hepatocellular fat. In fact, according to current opinion, the pathogenesis of NAFLD is based on a “two hits hypothesis”<sup>[5,34]</sup>. The “first hit” involves hepatic triglyceride accumulation (*i.e.*, simple steatosis) and is mainly due to insulin resistance thus increasing vulnerability to further damage. In fact, insulin resistance is responsible for causing abnormalities of lipid storage and lipolysis in insulin sensitive tissues, which may induce an increased fatty acid flux from adipose tissue to the liver and cause steatosis.

Insulin resistance may also cause lipid peroxidation which in turn may activate inflammatory cytokines and promote the progression of “innocent” steatosis to non-

alcoholic steato-hepatitis and liver fibrosis.

In a previous study by our group, when non-diabetic subjects were simultaneously categorized by insulin resistance and insulin secretion status, those with a predominant insulin resistance had a higher prevalence of severe liver steatosis at ultrasound, thus confirming the pathophysiological role of hyperinsulinemia in the events leading to the development of fatty liver<sup>[11]</sup>. The same subjects had significantly higher body mass index and waist circumference and similar blood pressure and serum lipids as compared to those with a prevalent insulin secretory defect. Moreover, insulin resistance was associated with severe steatosis, independently from potentially confounding factors such as age, BMI, high fasting glucose and high serum triglycerides<sup>[9]</sup>.

Insulin resistance has been demonstrated to unify NAFLD to the metabolic syndrome. In fact, it has been suggested that fatty liver can be considered as the hepatic consequence of the insulin resistance metabolic syndrome, emerging as a distinct high cardiovascular risk entity which has been recently indicated as the secondary target of therapy for the reduction of coronary risk after serum low-density-lipoprotein cholesterol<sup>[35]</sup>. Metabolic syndrome is characterized by the clustering of glucose intolerance and/or diabetes, hyperinsulinemia, increased levels of triglycerides and decreased HDL cholesterol, hypertension and central and overall obesity<sup>[36]</sup>. In a previous study, we demonstrated a strong positive association between the prevalence of some common metabolic diseases and the severity of liver steatosis. Obesity, impaired glucose tolerance and type 2 diabetes mellitus were 5.3-fold, 3.6-fold and 4.0-fold, more common among subjects with more severe steatosis, as compared to controls without steatosis. In the same group the prevalence of hypertriglyceridemia was seven times more frequent than in the control group and about three times higher than in the group with mild steatosis. Among subjects with severe steatosis obesity, impaired glucose metabolism and hyperlipidemia were present in over 50% of patients. These findings were paralleled by the presence of a statistically significant increase in mean BMI, plasma glucose and triglyceride levels from the group of subjects without steatosis to the three groups with increasing severity of fatty liver<sup>[10]</sup>.

Based on the above considerations, the use of drugs improving insulin resistance has been proposed for patients with fatty liver. Metformin was the first insulin sensitizer used; however, only marginal benefits on transaminases and no improvement in steatosis or inflammation have been reported so far, and metformin is not recommended for patients with NAFLD<sup>[37]</sup>. Recently, it has been suggested that metformin may prevent liver tumorigenesis *via* suppression of liver fat accumulation in the early stage, before the onset of NAFLD. However, the suggested potential role of metformin as antitumor agent should be further confirmed by large-scale clinical trials<sup>[38,39]</sup>. Concerning thiazolidinediones, treatment with pioglitazone was associated with improvements of liver

steatosis and inflammation in recent controlled clinical trials<sup>[40-42]</sup>. Therefore, pioglitazone may be recommended for the treatment of adults with biopsy proven NASH<sup>[43]</sup>.

Recently, some studies investigated the possible effects of analogues of glucagon-like peptide-1 (GLP-1), an incretin that increases insulin sensitivity and aids glucose metabolism, on NAFLD. *In vivo* and *in vitro* studies demonstrated that GLP-1 analogues reduce fatty acid accumulation and endoplasmic reticulum stress-related apoptosis in human hepatocytes treated with fatty acids as well as in an animal model of NAFLD<sup>[35]</sup>. This fact could improve hepatic steatosis by modulation of lipid metabolism and hepatic insulin signaling in NAFLD animal models<sup>[45,46]</sup>. These effects could be explained by possible direct stimulation of hepatic GLP-1 receptor.

Cuthbertson *et al*<sup>[46]</sup> recently conducted a prospective study in 25 obese, T2DM patients with hepatic steatosis, treated with metformin and sulphonylureas/DPP-IV inhibitors. After a 6 mo treatment with GLP-1 receptor agonists (exenatide or liraglutide), reduction of mean HbA1c, body weight and intrahepatic lipid was demonstrated<sup>[46]</sup>. The relative reduction in intrahepatic lipid correlated with that in HbA1c but was not significantly correlated with that in total body weight. Adequately powered, randomised, placebo-controlled intervention studies using GLP-1 analogues in patients with NAFLD, with or without T2DM, are needed to demonstrate the potential role of incretins in ameliorating NAFLD and to investigate the inter-relationship between the biochemical, metabolic and histological responses.

## OXIDATIVE STRESS AND REDUCED ANTIOXIDANT STATUS: A POSSIBLE ROLE FOR VITAMIN E

Several lines of evidence suggest that chronic oxidative stress is one of the key mechanisms responsible for liver damage and disease progression in NAFLD. In particular, according to the “two-hit” theory, oxidative stress is a major player triggering the progression of steatosis to NASH as the result of an imbalance between pro-oxidant and anti-oxidant chemicals that lead to liver cell damage<sup>[5,34]</sup>. Indeed, the increased production of reactive oxygen species (ROS) is known to cause lipid peroxidation, followed by inflammation, and activation of stellate cells leading to fibrogenesis.

Consistent with the above theory, subjects with NASH had significantly increased levels of lipid peroxidation products<sup>[47-52]</sup>. Moreover, in a small study performed in 21 subjects with NASH and 19 controls, subjects with NASH had significantly higher levels of oxidized LDL and of thiobarbituric acid-reacting substances (TBARS) suggesting an increased cardiovascular risk<sup>[51]</sup>. Similar results were reported in India, where TBARS levels were significantly elevated and GSH/GSSG ratio was significantly decreased in NAFLD subjects without and with type 2 diabetes<sup>[49]</sup>. Increased systemic levels of malondialdehyde were ob-

served in 58 male patients with histologically proven NAFLD compared to healthy age matched males<sup>[48]</sup>. In a further study, NAFLD children with immune responses against MDA derived antigens showed more severe lobular inflammation and had a 13-fold higher prevalence of overt NASH suggesting the presence of oxidative stress in a high proportion of NAFLD children<sup>[50]</sup>. In two more studies the percentage of hepatocytes, positive for 8-OHdG expression, and serum 8-OHdG levels were significantly higher in patients with NASH than simple fatty liver, while the oxidative stress marker GGT was increased in both conditions<sup>[51,53]</sup>. Finally, in a recent cross-sectional study, oxidative stress, detected as the ratio of plasma total antioxidant status to total oxidant status, was associated with insulin resistance in obese adolescents with NAFLD<sup>[52]</sup>.

In a previous study<sup>[54]</sup>, we demonstrated an increased systemic oxidative stress in subjects with fatty liver, as compared to those without. This increase was associated to severity of liver steatosis evaluated either by liver ultrasonography, or by fatty liver index or by serum cytokeratin-18 levels, a marker of apoptosis reflecting liver disease severity.

In fact, we found increased systemic oxidative stress assessed by increased levels of urinary 8-iso-PGF2 $\alpha$ , currently regarded as the best measure of oxidative stress *in vivo* and of serum sNOX2-dp, a marker of NOX2 activation by blood cells, which plays an important role in ROS production. The same findings were also observed after the exclusion of obese subjects, or subjects with diabetes or with metabolic syndrome and in those not taking statin medication. In our study, a correlation was also found between HOMA-IR, urinary 8-iso PGF2 $\alpha$  and sNOX2-dp, confirming the interdependency of insulin resistance and oxidative stress in the pathogenesis of NAFLD. Moreover, both oxidative stress markers were also highly correlated with serum cytokeratin-18, thus suggesting a possible role in the progression from simple fatty liver to NASH.

Therefore, although the mechanisms underlying disease progression remain poorly understood, a therapeutic strategy targeting oxidative stress reduction has been proposed and supplementation with vitamin E has been suggested by recent guidelines for the treatment of NASH in non-diabetic subjects<sup>[37]</sup>.

In pioglitazone vs vitamin E vs placebo for the Treatment of nondiabetic patients with nonalcoholic steatohepatitis (PIVENS) trial, vitamin E therapy demonstrated a significant improvement in steatosis, inflammation, ballooning, and resolution of steatohepatitis in adult patients with aggressive NASH, who did not have diabetes or cirrhosis<sup>[42]</sup>. Conversely, in the TONIC trial, where 173 children were randomized to receive vitamin E (400 IU twice daily), metformin (500 mg twice daily), or placebo for 96 wk, neither agent was superior to placebo in attaining the primary outcome, a reduction in ALT level 50% or less of the baseline<sup>[55]</sup>. Finally, it should be taken into consideration that vitamin E supplementation has



not been associated to benefits in cardiovascular death or cerebrovascular events<sup>[56]</sup>. Moreover, the results of two recent reviews showed no evidence of benefits by vitamin E supplementation for the prevention of cardiovascular events and there was no evidence for a possible association of vitamin E with higher all-cause mortality<sup>[57,58]</sup>.

## NAFLD AND CARDIOVASCULAR RISK: A POSSIBLE ROLE FOR STATINS

NAFLD has been traditionally interpreted as a condition, which may progress into liver related complications such as cirrhosis, liver cancer, and liver mortality. In particular, liver fibrosis predicts disease progression and the risk for hepatocellular carcinoma. However, most people with NAFLD in the absence of significant hepatic fibrosis do not develop serious liver problems. Conversely, people with NAFLD have an increased chance of developing cardiovascular diseases, such as myocardial infarction and stroke, which represent the major causes of death in this setting<sup>[25]</sup>. Indeed, cardiovascular disease is the single most important cause of morbidity and mortality in this patient population<sup>[59]</sup>.

Therefore, it appears that the increased mortality of patients is primarily a result of cardiovascular diseases and, to a lesser extent, of liver related diseases. Indeed, many epidemiological, clinical, and pathophysiological observations support a strong association between NAFLD and increased cardiovascular risk, which in some studies was found to be independent of traditional risk factors and aspects of the metabolic syndrome.

Moreover, patients with NASH seem to be at higher risk for atherosclerosis than patients with simple steatosis as a consequence of chronic inflammation and oxidative stress. Finally, an increased risk of developing type 2 diabetes mellitus has also been demonstrated among NAFLD patients<sup>[60]</sup>. Therefore, based on the above considerations, we may perhaps consider patients with NAFLD at increased global cardiometabolic risk, although the precise mechanisms by which NAFLD contributes to cardiovascular disease (CVD) and diabetes are still the subject of ongoing research. In a recent review, we discussed the epidemiological, clinical, and pathophysiological evidence of an association between NAFLD and cardiovascular diseases<sup>[61]</sup>.

Recent genetic studies<sup>[62,63]</sup>, such as those showing a strong correlation between presence of the *I148M* polymorphism in the *PNPLA3* gene and elevated serum levels of ALT and AST and liver inflammation, will probably be helpful in improving the assessment of cardiovascular risk in NAFLD patients with and without metabolic syndrome.

NASH is also linked to accelerated atherogenesis through the presence of abnormal production of triglyceride- and cholesterol-rich remnant particles, leading to accelerated atherosclerosis, together with other features of the metabolic syndrome. Dyslipidemia is characterized by increased serum triglycerides, low high-density

lipoprotein (HDL) cholesterol and increased small, dense, low-density lipoprotein (LDL) particles, *i.e.*, the so-called “atherogenic lipid triad”<sup>[64,65]</sup>. The presence of small dense LDL particles is associated with increased cardiovascular risk. Therefore, aggressive treatment of dyslipidemia and the other features of the metabolic syndrome should be a primary target in patients with NAFLD.

Statins are first line drugs for the treatment of LDL cholesterol. According to ATPIII Guidelines, the current recommendation that liver biochemistries be checked before and periodically after starting statin therapy is not evidence-based and is controversial<sup>[36]</sup>.

On the other end, measuring aminotransferases at baseline cannot adequately identify those who have underlying liver disease and there is no sound rationale why statins should not be used in patients with chronic liver disease who otherwise need statin therapy. In fact, in the GRACE Study, atorvastatin treatment was safe in patients with mild to moderately abnormal liver tests that were potentially attributable to NAFLD<sup>[66]</sup>. Moreover, in small uncontrolled studies, pravastatin, atorvastatin, rosuvastatin and pitavastatin were safe and effective in NASH patients with dyslipidemia<sup>[67,68]</sup>. In all these studies a decrease of serum aminotransferase was also observed and liver histology improved in some patients.

Therefore, the AASLD Guidelines recommend that statins can be used to treat dyslipidemia in patients with NAFLD and NASH (Strenght 1; Quality B)<sup>[37]</sup>.

## VITAMIN D DEFICIENCY: A POSSIBLE ROLE FOR VITAMIN D<sub>3</sub>

Vitamin D deficiency is a highly prevalent condition worldwide. Low serum 25(OH)D<sub>3</sub> concentrations have been reported among adults and children affected by NASH<sup>[69,70]</sup>.

Furthermore, we have recently demonstrated the existence of a strong and independent association between NAFLD and low 25(OH)D<sub>3</sub> levels in a large adult population with normal serum liver enzymes<sup>[71]</sup>. Vitamin D is present in the diet and dietary supplements, but its primary source is the photo-mediated conversion of 7-dehydrocholesterol in the skin<sup>[72]</sup>. To become biologically active, vitamin D requires 25-hydroxylation in the liver and subsequent 1-hydroxylation in the kidney<sup>[73]</sup>. The 25-hydroxylation occurs exclusively in the hepatocyte and is mediated by CYP27A1 and CYP2R1, two liver-expressed cytochromes characterized by different intracellular locations, specificity and affinity for vitamin D<sub>3</sub>. The biological effects of vitamin D<sub>3</sub> are mediated by the vitamin D receptor (VDR)<sup>[74]</sup>.

In a recent study<sup>[75]</sup>, we suggest that the vitamin D-vitamin D receptor system may play an important role in the response of the liver to chronic damage induced by different pathogenic stimuli. In fact, low hepatic VDR expression which was closely associated with more severe liver histology in this study, could represent the primary event leading to progression of hepatitis.

Based on the above considerations, treatment of vitamin D<sub>3</sub> deficiency to prevent or treat NAFLD to NASH progression has been proposed. However, large placebo-controlled randomized trials have not been performed so far. Thus, it is premature to recommend vitamin D<sub>3</sub> for the specific treatment of NAFLD/NASH.

## OTHER TREATMENTS

Many other agents have been investigated as potential therapeutic options in NAFLD and NASH in small, proof-of-concept studies.

Omega-3 fatty acids are currently approved to treat severe hypertriglyceridemia and have also proven beneficial for cardiovascular disease. Recently, they have been suggested as a treatment for NAFLD and some studies support the use of omega-3 fatty acids in this setting<sup>[24]</sup>. Their potential mechanisms of action may be mediated by regulation of hepatic gene expression, improvement of insulin sensitivity, reduction of inflammation and oxidative stress.

In a cross-sectional study by Zelber-Sagi *et al.*<sup>[76]</sup> dietary habits of NAFLD patients have been investigated, finding that the NAFLD group consumed less fish rich in omega-3 and almost double the quantity of soft drinks and also consumed more meat compared with the general population. Moreover, these dietary differences were associated with an increased risk of NAFLD independent of traditional risk factors. Evidence emerging from animal studies show a reduction in hepatic steatosis, inflammation and oxidative stress and an improvement in insulin sensitivity.

Moreover, some preliminary clinical studies in humans reported a reduction in hepatic steatosis on imaging and an improvement of insulin sensitivity and serum liver function tests. In any case, these studies have small sample size and methodological flaws. Results from ongoing large multicenter studies with histological outcomes are needed<sup>[77]</sup>. Thus, AASLD Guidelines currently concluded that “it is premature to recommend omega-3 fatty acids for the specific treatment of NAFLD or NASH, but they may be considered as first line agents to treat hypertriglyceridemia in patients with NAFLD” (Strength-1; Quality-B)<sup>[37]</sup>.

Furthermore, ursodeoxycholic acid (UDCA) has been proposed as a treatment for NAFLD and NASH and several studies have been conducted concerning this treatment<sup>[78,79]</sup>. All but one study are with small sample size and/or surrogate end-points. The only available large RCT with histological endpoints showed lack of any histological benefit of UDCA in patients with NASH<sup>[79]</sup>.

During the last years, there has been renewed interest in the potential efficacy of silymarin and silybin, two natural products derived from the milk thistle plant *Silybum marianum*, traditionally used in classical Greece to treat hepatic and biliary diseases. A higher bioavailability of silybin has been shown and experimental studies have demonstrated its antifibrotic, antioxidant and metabolic

effects<sup>[80,81]</sup>. Treatment with silymarin was associated with improvement in liver enzymes in a small placebo-controlled study in patients with NAFLD<sup>[82]</sup>. Silybin, combined with phosphatidylcholine and vitamin E, induced improvements of insulin resistance and liver histology in a large multicenter RCT<sup>[83]</sup>.

A growing body of evidence supports a relation between overgrowth of gut microbiota with NAFLD and NASH. In fact, it has been suggested that liver injury could be partly caused by exposure to bacterial and fermentation agents produced by microflora which are metabolized in the liver. Therefore, the use of probiotics to better balance the gut flora has been proposed. So far, various experimental studies and clinical trials revealed promising effects<sup>[84]</sup>. However, larger trials with longer-term follow-up are needed.

Recently, treatment with berberine has been suggested for patients with NAFLD, although its clinical application should be better defined. In fact, few small experimental and clinical studies have demonstrated favorable effects on insulin resistance, hepatic lipoprotein secretion and gut microenvironment modifications<sup>[85]</sup>.

Finally, mitochondrial dysfunction may induce overproduction of free radicals that, in turn, trigger lipid peroxidation and cell death. As a result, impaired mitochondrial function is thought to contribute to NAFLD and insulin resistance<sup>[86,87]</sup>. However, so far, improvement of mitochondrial dysfunction has only been associated to increased physical exercise and few experimental studies have assessed the potential role of antioxidant molecules targeted to mitochondria<sup>[88,89]</sup>.

## CONCLUSION

NAFLD is a multifactorial disease and the exact mechanism is unknown. Therefore, it is important to carefully evaluate for competing etiologies and clinically important co-morbidities. Metabolic conditions, such as diabetes mellitus, obesity, hypertension, and hyperlipidemia, should be treated aggressively and a multidisciplinary approach should be personalized for individual patients. The main targets of therapy are reported in Table 2.

Since NAFLD is largely a manifestation of obesity and metabolic syndrome, first-line management of NAFLD focuses on lifestyle modifications. Based on the available evidence, Mediterranean diet seems to be the best dietary regimen for patients with NAFLD. Weight loss generally reduces hepatic steatosis, and is achieved either by low-calorie diet alone or in conjunction with increased physical activity. Many patients with NAFLD have severe obesity. The role of bariatric surgery for these patients is still under evaluation.

As insulin resistance is nearly universal in patients with NASH, therapeutic measures to reduce insulin resistance have been suggested. Metformin was the first insulin sensitizer used; however, only marginal benefits on transaminases and no significant effect on liver steatosis or inflammation have been reported so far. Treatment

**Table 2 Main targets of intervention in nonalcoholic fatty liver disease**

Overweight and obesity
Sedentary lifestyles
Insulin resistance
Oxidative stress
Atherogenic dyslipidemia
Vitamin D deficiency
Hepatocyte protection
Cardiovascular risk
Treatment of comorbidities
Mitochondrial dysfunction

with pioglitazone is associated with improvements of liver histology and therefore may be recommended for the treatment of patients with biopsy-proven NASH.

Proposed pharmacologic therapies for NASH include antioxidants such as vitamin E (α-tocopherol) administered at daily dose of 800 IU/d, which could improve liver histology in non-diabetic adults with biopsy-proven NASH.

The cardiovascular morbidity and mortality is perhaps one of the most important and new aspects of NAFLD and several recent studies have shown that statins can be safely used to treat dyslipidemia in patients with NAFLD, but are not recommended to specifically treat NASH. UDCA is not recommended and it is premature to recommend omega-3 fatty acids and vitamin D supplementation.

Patients should be advised about the limited effectiveness of most drug therapies except possibly insulin sensitizers for some patients with diabetes and vitamin E for those patients without diabetes. Finally, it should be considered that there are no medications approved by the United States Food and Drug Administration. Our clinical approach to the treatment of NAFLD is based on few relatively small studies demonstrating benefits from some of the above treatments.

## REFERENCES

- Browning JD**, Szczepaniak LS, Dobbins R, Nuremberg P, Horton JD, Cohen JC, Grundy SM, Hobbs HH. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. *Hepatology* 2004; **40**: 1387-1395 [PMID: 15565570 DOI: 10.1002/hep.20466]
- Vernon G**, Baranova A, Younossi ZM. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. *Aliment Pharmacol Ther* 2011; **34**: 274-285 [PMID: 21623852 DOI: 10.1111/j.1365-2036.2011.04724.x]
- Leite NC**, Salles GF, Araujo AL, Villela-Nogueira CA, Cardoso CR. Prevalence and associated factors of non-alcoholic fatty liver disease in patients with type-2 diabetes mellitus. *Liver Int* 2009; **29**: 113-119 [PMID: 18384521 DOI: 10.1111/j.1478-3231.2008.01718.x]
- Bellentani S**, Saccoccio G, Masutti F, Crocè LS, Brandi G, Sasso F, Cristanini G, Tiribelli C. Prevalence of and risk factors for hepatic steatosis in Northern Italy. *Ann Intern Med* 2000; **132**: 112-117 [PMID: 10644271 DOI: 10.7326/0003-4819-132-2-200001180-00029]
- Angulo P**. Nonalcoholic fatty liver disease. *N Engl J Med* 2002;

- 346**: 1221-1231 [PMID: 11961152 DOI: 10.1056/NEJMra011775]
- Farrell GC**, Larter CZ. Nonalcoholic fatty liver disease: from steatosis to cirrhosis. *Hepatology* 2006; **43**: S99-S112 [PMID: 16447287 DOI: 10.1002/hep.20973]
- Torres DM**, Harrison SA. Nonalcoholic steatohepatitis and noncirrhotic hepatocellular carcinoma: fertile soil. *Semin Liver Dis* 2012; **32**: 30-38 [PMID: 22418886 DOI: 10.1055/s-0032-1306424]
- Kawada N**, Imanaka K, Kawaguchi T, Tamai C, Ishihara R, Matsunaga T, Gotoh K, Yamada T, Tomita Y. Hepatocellular carcinoma arising from non-cirrhotic nonalcoholic steatohepatitis. *J Gastroenterol* 2009; **44**: 1190-1194 [PMID: 19672551 DOI: 10.1007/s00535-009-0112-0]
- Schuppan D**, Schattenberg JM. Non-alcoholic steatohepatitis: pathogenesis and novel therapeutic approaches. *J Gastroenterol Hepatol* 2013; **28** Suppl 1: 68-76 [PMID: 23855299 DOI: 10.1111/jgh.12212]
- Clark JM**. The epidemiology of nonalcoholic fatty liver disease in adults. *J Clin Gastroenterol* 2006; **40** Suppl 1: S5-10 [PMID: 16540768 DOI: 10.1097/01.mcg.0000168638.84840.ff]
- Angelico F**, Del Ben M, Conti R, Francioso S, Feole K, Fiorello S, Cavallo MG, Zalunardo B, Lirussi F, Alessandri C, Violi F. Insulin resistance, the metabolic syndrome, and nonalcoholic fatty liver disease. *J Clin Endocrinol Metab* 2005; **90**: 1578-1582 [PMID: 15598693 DOI: 10.1210/jc.2004-1024]
- Angelico F**, Del Ben M, Conti R, Francioso S, Feole K, Maccioni D, Antonini TM, Alessandri C. Non-alcoholic fatty liver syndrome: a hepatic consequence of common metabolic diseases. *J Gastroenterol Hepatol* 2003; **18**: 588-594 [PMID: 12702052 DOI: 10.1046/j.1440-1746.2003.02958.x]
- Williams CD**, Stengel J, Asike MI, Torres DM, Shaw J, Contreras M, Landt CL, Harrison SA. Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: a prospective study. *Gastroenterology* 2011; **140**: 124-131 [PMID: 20858492 DOI: 10.1053/j.gastro.2010.09.038]
- Ota T**, Takamura T, Kurita S, Matsuzawa N, Kita Y, Uno M, Akahori H, Misu H, Sakurai M, Zen Y, Nakanuma Y, Kaneko S. Insulin resistance accelerates a dietary rat model of nonalcoholic steatohepatitis. *Gastroenterology* 2007; **132**: 282-293 [PMID: 17241878 DOI: 10.1053/j.gastro.2006.10.014]
- Ryan MC**, Itsiopoulos C, Thodis T, Ward G, Trost N, Hofferberth S, O'Dea K, Desmond PV, Johnson NA, Wilson AM. The Mediterranean diet improves hepatic steatosis and insulin sensitivity in individuals with non-alcoholic fatty liver disease. *J Hepatol* 2013; **59**: 138-143 [PMID: 23485520 DOI: 10.1016/j.jhep.2013.02.012]
- Kontogianni MD**, Tileli N, Margariti A, Georgoulis M, Deutsch M, Tiniakos D, Fragopoulou E, Zafiropoulou R, Manios Y, Papatheodoridis G. Adherence to the Mediterranean diet is associated with the severity of non-alcoholic fatty liver disease. *Clin Nutr* 2014; **33**: 678-683 [PMID: 24064253 DOI: 10.1016/j.clnu.2013.08.014]
- Fraser A**, Abel R, Lawlor DA, Fraser D, Elhayany A. A modified Mediterranean diet is associated with the greatest reduction in alanine aminotransferase levels in obese type 2 diabetes patients: results of a quasi-randomised controlled trial. *Diabetologia* 2008; **51**: 1616-1622 [PMID: 18597068 DOI: 10.1007/s00125-008-1049-1]
- Vilar L**, Oliveira CP, Faintuch J, Mello ES, Nogueira MA, Santos TE, Alves VA, Carrilho FJ. High-fat diet: a trigger of non-alcoholic steatohepatitis? Preliminary findings in obese subjects. *Nutrition* 2008; **24**: 1097-1102 [PMID: 18640006]
- Cortez-Pinto H**, Jesus L, Barros H, Lopes C, Moura MC, Camilo ME. How different is the dietary pattern in non-alcoholic steatohepatitis patients? *Clin Nutr* 2006; **25**: 816-823 [PMID: 16677739 DOI: 10.1016/j.clnu.2006.01.027]
- de Wit NJ**, Afman LA, Mensink M, Müller M. Phenotyping the effect of diet on non-alcoholic fatty liver disease. *J Hepatol* 2012; **57**: 1370-1373 [PMID: 22796155 DOI: 10.1016/



- jjhep.2012.07.003]
- 21 **Ouyang X**, Cirillo P, Sautin Y, McCall S, Bruchette JL, Diehl AM, Johnson RJ, Abdelmalek MF. Fructose consumption as a risk factor for non-alcoholic fatty liver disease. *J Hepatol* 2008; **48**: 993-999 [PMID: 18395287 DOI: 10.1016/j.jhe.2008.02.011]
  - 22 **Thuy S**, Ladurner R, Volynets V, Wagner S, Strahl S, Königsrainer A, Maier KP, Bischoff SC, Bergheim I. Nonalcoholic fatty liver disease in humans is associated with increased plasma endotoxin and plasminogen activator inhibitor 1 concentrations and with fructose intake. *J Nutr* 2008; **138**: 1452-1455 [PMID: 18641190]
  - 23 **Masteron GS**, Plevris JN, Hayes PC. Review article: omega-3 fatty acids - a promising novel therapy for non-alcoholic fatty liver disease. *Aliment Pharmacol Ther* 2010; **31**: 679-692 [PMID: 20415840 DOI: 10.1111/j.1365-2036.2010.04230.x]
  - 24 **Promrat K**, Kleiner DE, Niemeier HM, Jackvony E, Kearns M, Wands JR, Fava JL, Wing RR. Randomized controlled trial testing the effects of weight loss on nonalcoholic steatohepatitis. *Hepatology* 2010; **51**: 121-129 [PMID: 19827166 DOI: 10.1002/hep.23276]
  - 25 **Angelico F**, Loffredo L, Pignatelli P, Augelletti T, Carnevale R, Pacella A, Albanese F, Mancini I, Di Santo S, Del Ben M, Violi F. Weight loss is associated with improved endothelial dysfunction via NOX2-generated oxidative stress downregulation in patients with the metabolic syndrome. *Intern Emerg Med* 2012; **7**: 219-227 [PMID: 21512794 DOI: 10.1007/s11739-011-0591-x]
  - 26 **Zelber-Sagi S**, Kessler A, Brazowsky E, Webb M, Lurie Y, Santo M, Leshno M, Blendis L, Halpern Z, Oren R. A double-blind randomized placebo-controlled trial of orlistat for the treatment of nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol* 2006; **4**: 639-644 [PMID: 16630771 DOI: 10.1016/j.cgh.2006.02.004]
  - 27 **Brethauer SA**, Chand B, Schauer PR. Risks and benefits of bariatric surgery: current evidence. *Cleve Clin J Med* 2006; **73**: 993-1007 [PMID: 17128540 DOI: 10.3949/ccjm.73.11.993]
  - 28 **Ali MR**, Fuller WD, Choi MP, Wolfe BM. Bariatric surgical outcomes. *Surg Clin North Am* 2005; **85**: 835-52, vii [PMID: 16061089 DOI: 10.1016/j.suc.2005.03.005]
  - 29 **Nguyen NT**, Goldman C, Rosenquist CJ, Arango A, Cole CJ, Lee SJ, Wolfe BM. Laparoscopic versus open gastric bypass: a randomized study of outcomes, quality of life, and costs. *Ann Surg* 2001; **234**: 279-89; discussion 289-91 [PMID: 11524581]
  - 30 **Chavez-Tapia NC**, Tellez-Avila FI, Barrientos-Gutierrez T, Mendez-Sanchez N, Lizardi-Cervera J, Uribe M. Bariatric surgery for non-alcoholic steatohepatitis in obese patients. *Cochrane Database Syst Rev* 2010; (1): CD007340 [PMID: 20091629 DOI: 10.1002/14651858.CD007340.pub2]
  - 31 **Shojaee-Moradie F**, Baynes KC, Pentecost C, Bell JD, Thomas EL, Jackson NC, Stolinski M, Whyte M, Lovell D, Bowes SB, Gibney J, Jones RH, Umpleby AM. Exercise training reduces fatty acid availability and improves the insulin sensitivity of glucose metabolism. *Diabetologia* 2007; **50**: 404-413 [PMID: 17149589 DOI: 10.1007/s00125-006-0498-7]
  - 32 **Johnson NA**, Sachinwalla T, Walton DW, Smith K, Armstrong A, Thompson MW, George J. Aerobic exercise training reduces hepatic and visceral lipids in obese individuals without weight loss. *Hepatology* 2009; **50**: 1105-1112 [PMID: 19637289 DOI: 10.1002/hep.23129]
  - 33 **van der Heijden GJ**, Wang ZJ, Chu ZD, Sauer PJ, Haymond MW, Rodriguez LM, Sunehag AL. A 12-week aerobic exercise program reduces hepatic fat accumulation and insulin resistance in obese, Hispanic adolescents. *Obesity* (Silver Spring) 2010; **18**: 384-390 [PMID: 19696755 DOI: 10.1038/oby.2009.274]
  - 34 **Day CP**, James OF. Steatohepatitis: a tale of two "hits"? *Gastroenterology* 1998; **114**: 842-845 [PMID: 9547102]
  - 35 **Targher G**, Day CP, Bonora E. Risk of cardiovascular disease in patients with nonalcoholic fatty liver disease. *N Engl J Med* 2010; **363**: 1341-1350 [PMID: 20879883]
  - 36 **National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III)**. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. *Circulation* 2002; **106**: 3143-3421 [PMID: 12485966]
  - 37 **Chalasanani N**, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, Charlton M, Sanyal AJ. The diagnosis and management of non-alcoholic fatty liver disease: practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. *Hepatology* 2012; **55**: 2005-2023 [PMID: 22488764 DOI: 10.1002/hep.25762]
  - 38 **Leone A**, Di Gennaro E, Bruzzese F, Avallone A, Budillon A. New perspective for an old antidiabetic drug: metformin as anticancer agent. *Cancer Treat Res* 2014; **159**: 355-376 [PMID: 24114491 DOI: 10.1007/978-3-642-38007-5\_21]
  - 39 **Tajima K**, Nakamura A, Shirakawa J, Togashi Y, Orime K, Sato K, Inoue H, Kaji M, Sakamoto E, Ito Y, Aoki K, Nagashima Y, Atsumi T, Terauchi Y. Metformin prevents liver tumorigenesis induced by high-fat diet in C57Bl/6 mice. *Am J Physiol Endocrinol Metab* 2013; **305**: E987-E998 [PMID: 23964070 DOI: 10.1152/ajpendo.00133.2013]
  - 40 **Belfort R**, Harrison SA, Brown K, Darland C, Finch J, Hardies J, Balas B, Gastaldelli A, Tio F, Pulcini J, Berria R, Ma JZ, Dwivedi S, Havranek R, Fincke C, DeFronzo R, Bannayan GA, Schenker S, Cusi K. A placebo-controlled trial of pioglitazone in subjects with nonalcoholic steatohepatitis. *N Engl J Med* 2006; **355**: 2297-2307 [PMID: 17135584 DOI: 10.1056/NEJMoa060326]
  - 41 **Aithal GP**, Thomas JA, Kaye PV, Lawson A, Ryder SD, Spendlove I, Austin AS, Freeman JG, Morgan L, Webber J. Randomized, placebo-controlled trial of pioglitazone in nondiabetic subjects with nonalcoholic steatohepatitis. *Gastroenterology* 2008; **135**: 1176-1184 [PMID: 18718471 DOI: 10.1053/j.gastro.2008.06.047]
  - 42 **Sanyal AJ**, Chalasanani N, Kowdley KV, McCullough A, Diehl AM, Bass NM, Neuschwander-Tetri BA, Lavine JE, Tonascia J, Unalp A, Van Natta M, Clark J, Brunt EM, Kleiner DE, Hoofnagle JH, Robuck PR. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. *N Engl J Med* 2010; **362**: 1675-1685 [PMID: 20427778 DOI: 10.1056/NEJMoa0907929]
  - 43 **Sharma S**, Mells JE, Fu PP, Saxena NK, Anania FA. GLP-1 analogs reduce hepatocyte steatosis and improve survival by enhancing the unfolded protein response and promoting macroautophagy. *PLoS One* 2011; **6**: e25269 [PMID: 21957486 DOI: 10.1371/journal.pone.0025269]
  - 44 **Ding X**, Saxena NK, Lin S, Gupta NA, Anania FA. Exendin-4, a glucagon-like protein-1 (GLP-1) receptor agonist, reverses hepatic steatosis in ob/ob mice. *Hepatology* 2006; **43**: 173-181 [PMID: 16374859 DOI: 10.1002/hep.21006]
  - 45 **Gupta NA**, Mells J, Dunham RM, Grakoui A, Handy J, Saxena NK, Anania FA. Glucagon-like peptide-1 receptor is present on human hepatocytes and has a direct role in decreasing hepatic steatosis in vitro by modulating elements of the insulin signaling pathway. *Hepatology* 2010; **51**: 1584-1592 [PMID: 20225248 DOI: 10.1002/hep.23569]
  - 46 **Cuthbertson DJ**, Irwin A, Gardner CJ, Daousi C, Purewal T, Furlong N, Goenka N, Thomas EL, Adams VL, Pushpakom SP, Pirmohamed M, Kemp GJ. Improved glycaemia correlates with liver fat reduction in obese, type 2 diabetes, patients given glucagon-like peptide-1 (GLP-1) receptor agonists. *PLoS One* 2012; **7**: e50117 [PMID: 23236362 DOI: 10.1371/journal.pone.0050117]
  - 47 **Chalasanani N**, Deeg MA, Crabb DW. Systemic levels of lipid peroxidation and its metabolic and dietary correlates



- in patients with nonalcoholic steatohepatitis. *Am J Gastroenterol* 2004; **99**: 1497-1502 [PMID: 15307867 DOI: 10.1111/j.1572-0241.2004.30159.x]
- 48 **Yesilova Z**, Yaman H, Oktenli C, Ozcan A, Uygun A, Cakir E, Sanisoglu SY, Erdil A, Ates Y, Aslan M, Musabak U, Erbil MK, Karaeren N, Dagalp K. Systemic markers of lipid peroxidation and antioxidants in patients with nonalcoholic Fatty liver disease. *Am J Gastroenterol* 2005; **100**: 850-855 [PMID: 15784031]
- 49 **Narasimhan S**, Gokulakrishnan K, Sampathkumar R, Farooq S, Ravikumar R, Mohan V, Balasubramanyam M. Oxidative stress is independently associated with non-alcoholic fatty liver disease (NAFLD) in subjects with and without type 2 diabetes. *Clin Biochem* 2010; **43**: 815-821 [PMID: 20398645 DOI: 10.1016/j.clinbiochem]
- 50 **Nobili V**, Parola M, Alisi A, Marra F, Piemonte F, Mombello C, Sutti S, Povero D, Maina V, Novo E, Albano E. Oxidative stress parameters in paediatric non-alcoholic fatty liver disease. *Int J Mol Med* 2010; **26**: 471-476 [PMID: 20818484 DOI: 10.3892/ijmm.00000487]
- 51 **Irie M**, Sohda T, Iwata K, Kunimoto H, Fukunaga A, Kuno S, Yotsumoto K, Sakurai K, Iwashita H, Hirano G, Ueda SI, Yokoyama K, Morihara D, Nishizawa S, Anan A, Takeyama Y, Sakamoto M, Shakado S, Sakisaka S. Levels of the oxidative stress marker  $\gamma$ -glutamyltranspeptidase at different stages of nonalcoholic fatty liver disease. *J Int Med Res* 2012; **40**: 924-933 [PMID: 22906265 DOI: 10.1177/147323001204000311]
- 52 **Pirgon Ö**, Bilgin H, Çekmez F, Kurku H, Dündar BN. Association between insulin resistance and oxidative stress parameters in obese adolescents with non-alcoholic fatty liver disease. *J Clin Res Pediatr Endocrinol* 2013; **5**: 33-39 [PMID: 23367495 DOI: 10.4274/jcrpe.825]
- 53 **Seki S**, Kitada T, Yamada T, Sakaguchi H, Nakatani K, Wakasa K. In situ detection of lipid peroxidation and oxidative DNA damage in non-alcoholic fatty liver diseases. *J Hepatol* 2002; **37**: 56-62 [PMID: 12076862 DOI: 10.1016/S0168-8278(02)00073-9]
- 54 **Del Ben M**, Polimeni L, Baratta F, Bartimoccia S, Carnevale R, Loffredo L, Pignatelli P, Violi F, Angelico F. Serum Cytokeratin-18 Is Associated with NOX2-Generated Oxidative Stress in Patients with Nonalcoholic Fatty Liver. *Int J Hepatol* 2014; **2014**: 784985 [PMID: 24678423 DOI: 10.1155/2014/784985]
- 55 **Lavine JE**, Schwimmer JB, Van Natta ML, Molleston JP, Murray KF, Rosenthal P, Abrams SH, Scheimann AO, Santal AJ, Chalasani N, Tonascia J, Ünalp A, Clark JM, Brunt EM, Kleiner DE, Hoofnagle JH, Robuck PR. Effect of vitamin E or metformin for treatment of nonalcoholic fatty liver disease in children and adolescents: the TONIC randomized controlled trial. *JAMA* 2011; **305**: 1659-1668 [PMID: 21521847 DOI: 10.1001/jama.2011.520]
- 56 **Vivekananthan DP**, Penn MS, Sapp SK, Hsu A, Topol EJ. Use of antioxidant vitamins for the prevention of cardiovascular disease: meta-analysis of randomised trials. *Lancet* 2003; **361**: 2017-2023 [PMID: 12814711 DOI: 10.1016/S0140-6736(03)13637-9]
- 57 **Myung SK**, Ju W, Cho B, Oh SW, Park SM, Koo BK, Park BJ. Efficacy of vitamin and antioxidant supplements in prevention of cardiovascular disease: systematic review and meta-analysis of randomised controlled trials. *BMJ* 2013; **346**: f10 [PMID: 23335472 DOI: 10.1136/bmj.f10]
- 58 **Bjelakovic G**, Nikolova D, Gluud C. Antioxidant supplements to prevent mortality. *JAMA* 2013; **310**: 1178-1179 [PMID: 24045742 DOI: 10.1001/jama.2013.277028]
- 59 **Söderberg C**, Stål P, Askling J, Glaumann H, Lindberg G, Marmur J, Hulcrantz R. Decreased survival of subjects with elevated liver function tests during a 28-year follow-up. *Hepatology* 2010; **51**: 595-602 [PMID: 20014114 DOI: 10.1002/hep.23314]
- 60 **Zelber-Sagi S**, Lotan R, Shibolet O, Webb M, Buch A, Nitzan-Kaluski D, Halpern Z, Santo E, Oren R. Non-alcoholic fatty liver disease independently predicts prediabetes during a 7-year prospective follow-up. *Liver Int* 2013; **33**: 1406-1412 [PMID: 23656177 DOI: 10.1111/liv.12200]
- 61 **Del Ben M**, Baratta F, Polimeni L, Angelico F. Non-alcoholic fatty liver disease and cardiovascular disease: epidemiological, clinical and pathophysiological evidences. *Intern Emerg Med* 2012; **7** Suppl 3: S291-S296 [PMID: 23073870]
- 62 **Feldstein AE**, Wieckowska A, Lopez AR, Liu YC, Zein NN, McCullough AJ. Cytokeratin-18 fragment levels as noninvasive biomarkers for nonalcoholic steatohepatitis: a multicenter validation study. *Hepatology* 2009; **50**: 1072-1078 [PMID: 19585618 DOI: 10.1002/hep.23050]
- 63 **Feldstein AE**, Canbay A, Angulo P, Taniai M, Burgart LJ, Lindor KD, Gores GJ. Hepatocyte apoptosis and fas expression are prominent features of human nonalcoholic steatohepatitis. *Gastroenterology* 2003; **125**: 437-443 [PMID: 12891546 DOI: 10.1016/S0016-5085(03)00907-7]
- 64 **Musunuru K**. Atherogenic dyslipidemia: cardiovascular risk and dietary intervention. *Lipids* 2010; **45**: 907-914 [PMID: 20524075 DOI: 10.1007/s11745-010-3408-1]
- 65 **Grundy SM**. Small LDL, atherogenic dyslipidemia, and the metabolic syndrome. *Circulation* 1997; **95**: 1-4 [PMID: 8994405 DOI: 10.1161/01.CIR.95.1.1]
- 66 **Athyros VG**, Tziomalos K, Gossios TD, Griva T, Anagnostis P, Kargiotis K, Pagourelis ED, Theocharidou E, Karagiannis A, Mikhailidis DP. Safety and efficacy of long-term statin treatment for cardiovascular events in patients with coronary heart disease and abnormal liver tests in the Greek Atorvastatin and Coronary Heart Disease Evaluation (GREACE) Study: a post-hoc analysis. *Lancet* 2010; **376**: 1916-1922 [PMID: 21109302 DOI: 10.1016/S0140-6736(10)61272-X]
- 67 **Gómez-Domínguez E**, Gisbert JP, Moreno-Monteaudo JA, García-Buey L, Moreno-Otero R. A pilot study of atorvastatin treatment in dyslipemid, non-alcoholic fatty liver patients. *Aliment Pharmacol Ther* 2006; **23**: 1643-1647 [PMID: 16696815 DOI: 10.1111/j.1365-2036.2006.02926.x]
- 68 **Antonopoulos S**, Mikros S, Mylonopoulou M, Kokkoris S, Giannoulis G. Rosuvastatin as a novel treatment of non-alcoholic fatty liver disease in hyperlipidemic patients. *Atherosclerosis* 2006; **184**: 233-234 [PMID: 16168995 DOI: 10.1016/j.atherosclerosis.2005.08.021]
- 69 **Targher G**, Bertolini L, Scala L, Cigolini M, Zenari L, Falezza G, Arcaro G. Associations between serum 25-hydroxyvitamin D3 concentrations and liver histology in patients with non-alcoholic fatty liver disease. *Nutr Metab Cardiovasc Dis* 2007; **17**: 517-524 [PMID: 16928437 DOI: 10.1016/j.numecd.2006.04.002]
- 70 **Manco M**, Ciampalini P, Nobili V. Low levels of 25-hydroxyvitamin D(3) in children with biopsy-proven nonalcoholic fatty liver disease. *Hepatology* 2010; **51**: 2229; author reply 2230 [PMID: 20513013 DOI: 10.1002/hep.23724]
- 71 **Phillips RS**, Murphy DJ, Goldman L, Knaus WA. Patient characteristics in SUPPORT: disease specific clinical data. *J Clin Epidemiol* 1990; **43** Suppl: 41S-45S [PMID: 2174968 DOI: 10.1186/1741-7015-9-85]
- 72 **Holick MF**. Vitamin D deficiency. *N Engl J Med* 2007; **357**: 266-281 [PMID: 17634462 DOI: 10.1056/NEJMra070553]
- 73 **DeLuca HF**. Overview of general physiologic features and functions of vitamin D. *Am J Clin Nutr* 2004; **80**: 1689S-1696S [PMID: 15585789]
- 74 **Moore DD**, Kato S, Xie W, Mangelsdorf DJ, Schmidt DR, Xiao R, Kliewer SA. International Union of Pharmacology. LXII. The NR1H and NR1I receptors: constitutive androstane receptor, pregnane X receptor, farnesoid X receptor alpha, farnesoid X receptor beta, liver X receptor alpha, liver X receptor beta, and vitamin D receptor. *Pharmacol Rev* 2006; **58**: 742-759 [PMID: 17132852 DOI: 10.1124/pr.58.4.6]

- 75 **Barchetta I**, Carotti S, Labbadia G, Gentilucci UV, Muda AO, Angelico F, Silecchia G, Leonetti F, Fraioli A, Picardi A, Morini S, Cavallo MG. Liver vitamin D receptor, CYP2R1, and CYP27A1 expression: relationship with liver histology and vitamin D3 levels in patients with nonalcoholic steatohepatitis or hepatitis C virus. *Hepatology* 2012; **56**: 2180-2187 [PMID: 22753133 DOI: 10.1002/hep.25930]
- 76 **Zelber-Sagi S**, Nitzan-Kaluski D, Goldsmith R, Webb M, Blendis L, Halpern Z, Oren R. Long term nutritional intake and the risk for non-alcoholic fatty liver disease (NAFLD): a population based study. *J Hepatol* 2007; **47**: 711-717 [PMID: 17850914 DOI: 10.1016/j.jhep.2007.06.020]
- 77 **Janczyk W**, Socha P, Lebensztejn D, Wierzbicka A, Mazur A, Neuhoff-Murawska J, Matusik P. Omega-3 fatty acids for treatment of non-alcoholic fatty liver disease: design and rationale of randomized controlled trial. *BMC Pediatr* 2013; **13**: 85 [PMID: 23702094 DOI: 10.1186/1471-2431-13-85]
- 78 **Wu SD**, Li L, Wang JY. Ursodeoxycholic acid for nonalcoholic steatohepatitis. *Eur J Gastroenterol Hepatol* 2012; **24**: 1247-1253 [PMID: 22864259 DOI: 10.1097/MEG.0b013e3283572ec0]
- 79 **Ratziu V**, de Ledinghen V, Oberti F, Mathurin P, Wartelle-Bladou C, Renou C, Sogni P, Maynard M, Larrey D, Serfaty L, Bonnefont-Rousselot D, Bastard JP, Rivière M, Spénard J. A randomized controlled trial of high-dose ursodesoxycholic acid for nonalcoholic steatohepatitis. *J Hepatol* 2011; **54**: 1011-1019 [PMID: 21145828 DOI: 10.1016/j.jhep.2010.08.030]
- 80 **Cacciapuoti F**, Scognamiglio A, Palumbo R, Forte R, Cacciapuoti F. Silymarin in non alcoholic fatty liver disease. *World J Hepatol* 2013; **5**: 109-113 [PMID: 23556042 DOI: 10.4254/wjh.v5.i3.109]
- 81 **Loguercio C**, Festi D. Silybin and the liver: from basic research to clinical practice. *World J Gastroenterol* 2011; **17**: 2288-2301 [PMID: 21633595 DOI: 10.3748/wjg.v17.i18.2288]
- 82 **Hashemi SJ**, Hajiani E, Sardabi EH. A Placebo-Controlled Trial of Silymarin in Patients with Nonalcoholic Fatty Liver Disease. *Hepat Mon* 2009; **9**: 265-270
- 83 **Loguercio C**, Andreone P, Brisc C, Brisc MC, Bugianesi E, Chiamonte M, Cursaro C, Danila M, de Sio I, Floreani A, Freni MA, Grieco A, Groppo M, Lazzari R, Lobello S, Lorefice E, Margotti M, Miele L, Milani S, Okolicsanyi L, Palasciano G, Portincasa P, Saltarelli P, Smedile A, Somalvico F, Spadaro A, Sporea I, Sorrentino P, Vecchione R, Tuccillo C, Del Vecchio Blanco C, Federico A. Silybin combined with phosphatidylcholine and vitamin E in patients with non-alcoholic fatty liver disease: a randomized controlled trial. *Free Radic Biol Med* 2012; **52**: 1658-1665 [PMID: 22343419 DOI: 10.1016/j.freeradbiomed.2012.02.008]
- 84 **Kelishadi R**, Farajian S, Mirlohi M. Probiotics as a novel treatment for non-alcoholic Fatty liver disease; a systematic review on the current evidences. *Hepat Mon* 2013; **13**: e7233 [PMID: 23885277 DOI: 10.5812/hepatmon.7233]
- 85 **Liu Y**, Zhang L, Song H, Ji G. Update on berberine in non-alcoholic Fatty liver disease. *Evid Based Complement Alternat Med* 2013; **2013**: 308134 [PMID: 23843872 DOI: 10.1155/2013/308134]
- 86 **García-Ruiz C**, Baulies A, Mari M, Garcia-Rovés PM, Fernandez-Checa JC. Mitochondrial dysfunction in non-alcoholic fatty liver disease and insulin resistance: cause or consequence? *Free Radic Res* 2013; **47**: 854-868 [PMID: 23915028 DOI: 10.3109/10715762.2013.830717]
- 87 **Serviddio G**, Bellanti F, Vendemiale G, Altomare E. Mitochondrial dysfunction in nonalcoholic steatohepatitis. *Expert Rev Gastroenterol Hepatol* 2011; **5**: 233-244 [PMID: 21476918 DOI: 10.1586/egh.11.11]
- 88 **Ascensão A**, Martins MJ, Santos-Alves E, Gonçalves IO, Portincasa P, Oliveira PJ, Magalhães J. Modulation of hepatic redox status and mitochondrial metabolism by exercise: therapeutic strategy for liver diseases. *Mitochondrion* 2013; **13**: 862-870 [PMID: 23880173 DOI: 10.1016/j.mito.2013.07.002]
- 89 **Mukhopadhyay P**, Horváth B, Zsengeller Z, Bátkai S, Cao Z, Kechrid M, Holovac E, Erdélyi K, Tanchian G, Liaudet L, Stillman IE, Joseph J, Kalyanaraman B, Pacher P. Mitochondrial reactive oxygen species generation triggers inflammatory response and tissue injury associated with hepatic ischemia-reperfusion: therapeutic potential of mitochondrially targeted antioxidants. *Free Radic Biol Med* 2012; **53**: 1123-1138 [PMID: 22683818 DOI: 10.1016/j.freeradbiomed.2012.05.036]

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