

REVIEW-THEMED ISSUE

The role of nutraceuticals for the treatment of non-alcoholic fatty liver disease

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Non-alcoholic fatty liver disease (NAFLD) represents the most common chronic liver disease. It is characterized by a wide spectrum of hepatic changes, which may progress to liver fibrosis and to cirrhosis. NAFLD is considered as the hepatic component of the metabolic syndrome but mechanisms underlying the onset and progression of NAFLD are still under investigation. The traditional 'two hit hypothesis' has been developed within a more complex 'multiple parallel hit hypothesis' which comprises a wide spectrum of parallel hits. Many therapeutic approaches have been proposed so far and several types of nutraceuticals have been suggested for the treatment of NAFLD and non-alcoholic steatohepatitis (NASH), the most promising of which are those with antioxidant effects. In particular, vitamin E appears to be effective for the treatment of nondiabetic subjects with more advanced NASH, although the high suggested daily dosages are a matter of concern. Moreover, polyphenols reduce liver fat accumulation, mainly by inhibiting lipogenesis. At present, there are insufficient data to support the use of vitamin C supplements in patients with NAFLD. Data on polyunsaturated fatty acid (PUFA) supplementation are heterogeneous, and no well-designed randomized controlled studies (RCTs) of adequate size, with histological assessment of steatosis, have been conducted. Based on the available data, silymarin supplementation for the treatment of NAFLD seems to have a favourable effect. The results with anti-inflammatory agents, such as vitamin D and carnitine are uncertain. In conclusion, there are insufficient data either to support or refute the use of nutraceuticals for subjects with NAFLD. Further RCTs, with histological changes as an outcome measure, are needed.

Introduction

Non-alcoholic fatty liver disease (NAFLD) represents the most common chronic liver disease. It is characterized by a wide spectrum of hepatic changes, which may progress to liver fibrosis and to cirrhosis. NAFLD is considered as the hepatic component of the metabolic syndrome (MetS) [1], but mechanisms underlying its onset and progression are still under investigation. Many studies have demonstrated the presence of insulin resistance [2] and increased oxidative stress [3] in

patients with NAFLD/non-alcoholic steatohepatitis (NASH). Thus, according to the 'two-hit hypothesis', the first hit is represented by lipid accumulation in the hepatocytes, after which oxidative stress may lead to NASH [4]. This traditional view has been developed within a more complex 'multiple parallel-hit hypothesis' which comprises a wide spectrum of parallel hits, such as insulin resistance, oxidative stress, genetic and epigenetic mechanisms, environmental elements, cytokines and microbiota changes [5]. These factors may be variously associated, both in presence and absence of MetS.

Several therapeutic approaches have been proposed so far. However, the only proven effective strategy for NAFLD treatment is weight loss and vitamin E administration, the latter only for nondiabetic NASH patients. Notably, there is currently no medically approved treatment for NAFLD [6].

In recent years, the effects of nutraceuticals on NAFLD have received increasing attention and several types of these agents have been suggested for the treatment of NAFLD/NASH (Figure 1). For some of them, a number of clinical trials highlighted an improvement in liver function tests (LFTs) and a possible positive influence on liver histology.

The aim of the present review is to summarize the current evidence on the nutraceuticals proposed for the treatment of patients with NAFLD Table 1. For this purpose, we have divided nutraceuticals according to their anti-inflammatory or antioxidant properties.

Anti-inflammatory agents

Vitamin D

25-hydroxyvitamin D3 (vitamin D) is a fat-soluble vitamin which originates in the skin after irradiation of 7-dehydrocholesterol with ultraviolet light, generating active vitamin D. Its main biological effects include the regulation of mineral metabolism, modulation of the immune system, control of insulin secretion and differentiation of many cellular lines. Vitamin D may also have several favourable metabolic effects acting as an anti-inflammatory [10] and anti-fibrotic agent. These properties may be particularly useful in patients with NAFLD [8, 9]. It has been suggested that vitamin D deficiency could favour the development of insulin resistance and increase the risk of diabetes mellitus and MetS. Thus, many studies have reported an association between vitamin D deficiency and the presence of

NAFLD, with a prevalence of vitamin D deficiency in up to 75% of patients with MetS.

An association between low vitamin D levels and the histological severity of NASH was suggested in patients with chronic elevation of LFTs and hepatic steatosis detected by ultrasound (US) who underwent liver biopsy for suspected NASH [10]. Similar findings were obtained by Barchetta *et al.* [11], who found a strong association between NAFLD and low vitamin D levels in an adult population with normal LFTs. The same authors showed that the vitamin D receptor (VDR) is widely expressed in the liver, and that the vitamin D/VDR system may play a role in the progression of NASH [12]. Sharifi *et al.* [13] performed a double-blind, placebo-controlled study of 50 000 IU vitamin D3 supplementation vs. placebo, every 14 days for 4 months, in 53 patients. No significant differences in LFTs, the homeostatic model of insulin resistance (HOMA-IR) or grades of hepatic steatosis between the two groups were observed. Recently, high-dose oral vitamin D3 supplementation (25 000 IU week⁻¹) over 24 weeks was found to have no impact on liver histology, liver biochemistry, insulin resistance or adipocytokine profile in 12 patients with biopsy-proven NASH [14]. These negative results should be interpreted with caution, given the limited number of participants and the relatively short-term follow-up. Large interventional randomized controlled trials (RCTs) which evaluate directly the effect of vitamin D supplementation on disease progression in NAFLD patients are needed.

Carnitine

L-carnitine plays a critical role in a number of intracellular and metabolic functions, such as fatty acid transport into the mitochondria, stabilization of cell membranes and reduction of serum lipid levels [15]. Moreover, it is also important in the regulation of energy balance in tissues that derive much of their energy from fatty acid oxidation, and is able

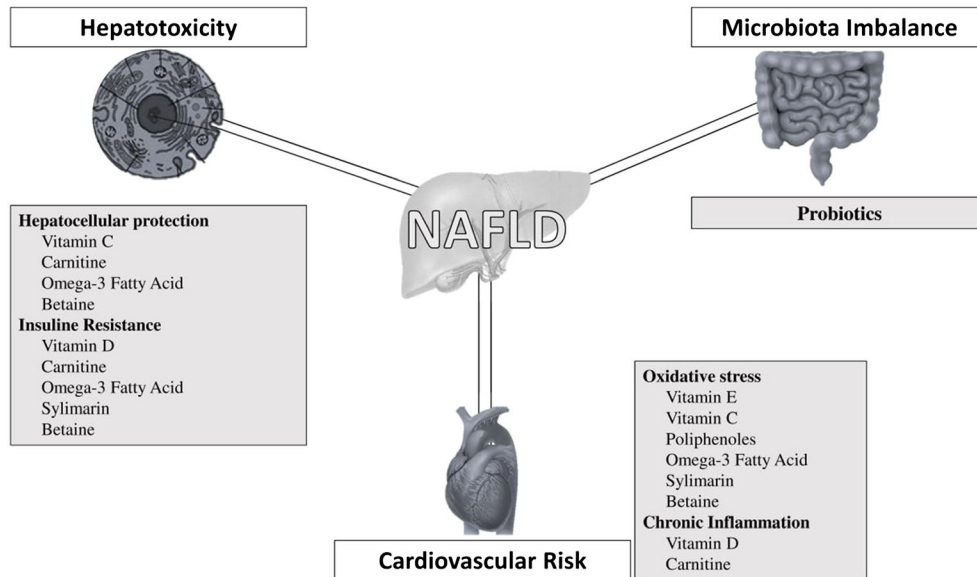


Figure 1

Targets for nutraceutical interventions in non-alcoholic fatty liver disease

Table 1

Principal nutraceuticals for the treatment of non-alcoholic fatty liver disease and possible targets

| Nutraceuticals | Target/mechanism of action | References |
|----------------------------|-----------------------------|---------------------------------|
| Vitamin D | • Chronic inflammation | Roth <i>et al.</i> [7] |
| | • Vitamin D deficiency | Barchetta <i>et al.</i> [11] |
| | • Insulin resistance | Targher <i>et al.</i> [10]. |
| Vitamin E | • Oxidative stress | Chalasan <i>et al.</i> [29] |
| | • Hepatocellular protection | |
| Carnitine | • Insulin resistance | Flanagan <i>et al.</i> [15] |
| | • Chronic inflammation | Malaguarnera <i>et al.</i> [16] |
| | • Hepatocellular protection | Somi <i>et al.</i> [17] |
| | • Atherogenic dyslipidaemia | |
| Vitamin C | • Oxidative stress | Duarte <i>et al.</i> [36] |
| | • Hepatocellular protection | Harrison <i>et al.</i> [40] |
| Omega-3 fatty acids | • Atherogenic dyslipidaemia | Hedengran <i>et al.</i> [66] |
| | • Cardiovascular risk | Endo <i>et al.</i> [42] |
| | • Oxidative stress | Jump <i>et al.</i> [67] |
| | • Hepatocellular protection | Parker <i>et al.</i> [48] |
| Silymarin | • Oxidative stress | Trappoliere <i>et al.</i> [51] |
| | • Chronic inflammation | Sorrentino <i>et al.</i> [54] |
| | • Insulin resistance | Feng <i>et al.</i> [68] |
| | • Hepatocellular protection | |
| Resveratrol | • Oxidative stress | Gambini <i>et al.</i> [57] |
| | • Insulin resistance | Faghihzadeh <i>et al.</i> [58] |
| | • Cardiovascular risk | Poulsen <i>et al.</i> [59] |
| | • Hepatocellular protection | |

to modulate the inflammatory response. L-carnitine supplementation appears to be beneficial in several conditions, such as obesity, type 2 diabetes and liver cirrhosis.

Malaguarnera *et al.* randomized 74 patients with NASH to receive either oral L-carnitine (2 g day⁻¹) or placebo for 24 weeks [16]. At the end of the study, subjects treated with L-carnitine showed significant improvements in LFTs, insulin resistance

and inflammatory biomarkers. Repeated liver biopsies showed a reduction in the NASH activity index (defined by steatosis, parenchymal inflammation and hepatocellular injury), and improvement in fibrosis scores. The authors concluded that L-carnitine supplementation, together with lifestyle changes, can have a positive effect in the treatment of NASH. More recently, data from an RCT of supplementation with L-carnitine (500 mg twice daily) for 1 year for the treatment of NAFLD showed no significant changes in LFTs and US grade [17].

Antioxidant agents

Vitamin E

Oxidative stress is one of key mediators of hepatic damage and, according to the two-hit hypothesis, is a major contributor to the progression from simple steatosis to steatohepatitis [18]. Thus, an increased generation of reactive oxygen species (ROS) can induce lipid peroxidation, leading to inflammation and fibrogenesis through the activation of stellate cells. Lipid peroxidation is increased in NAFLD and can promote inflammation and tissue damage [2].

Several studies have evaluated the pro-oxidant/antioxidant status in NAFLD [19, 20]. We found enhanced systemic oxidative stress in subjects with NAFLD, as assessed by increased levels of urinary 8-iso-prostaglandin F_{2α} (8-iso PGF_{2α}), a valid marker of *in vivo* oxidative stress, and of serum soluble NADPH oxidase 2 (NOX2)-derived peptide (sNOX2-dp), a marker of NOX2 activation which plays an important role in ROS production [21]. In our study, we also found a correlation between HOMA-IR, urinary 8-iso PGF_{2α} 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 (CK-18), a validated marker of liver damage, thus suggesting a possible role of oxidative stress in liver damage. More recently, we found reduced serum vitamin E levels in a cohort of 253 patients with steatosis [22]. Subjects with simple steatosis and those with biopsy-proven NASH had a similar reduction in cholesterol-adjusted serum levels of vitamin E, suggesting that oxidative stress imbalance could occur in the early stages of fatty liver. Increased oxidative stress is implicated in cardiovascular diseases [23, 24], and may well represent a possible link between NAFLD and cardiovascular events, and constitute an attractive target for antioxidant therapy.

Many clinical trials have evaluated the effects of vitamin E supplementation in NAFLD. In the Pioglitazone versus Vitamin E versus Placebo for the Treatment of Nondiabetic Patients with Nonalcoholic Steatohepatitis (PIVENS) trial, vitamin E therapy, as compared with placebo, was associated with a significant improvement in steatosis, inflammation, ballooning and resolution of steatohepatitis in adult patients with aggressive NASH who did not have diabetes or cirrhosis [25]. However, these findings could not be confirmed in 58 children and adolescents in whom vitamin E, supplied at 800 IU daily for 96 weeks, was found to be no better than placebo at either attaining a sustained reduction in alanine aminotransferase (ALT) levels or improving liver histology [26]. Similar negative results were reported by Nobili *et al.*, who showed that vitamin E

supplementation provided no benefit in a cohort of 53 children and adolescents with NAFLD [27]. Finally, in a recent meta-analysis conducted by Sato *et al.* [28], vitamin E treatment improved serum aspartate aminotransferase and ALT levels both in children and adults with NAFLD.

Based only on the results of the PIVENS trial [25], current guidelines recommend vitamin E, at a dose of 800 IU day⁻¹, as the first-line pharmacotherapy for nondiabetic and noncirrhotic adults with biopsy-proven NASH [29]. However, some meta-analyses have reported an increase in all-cause mortality with high-dose vitamin E [30], whereas others have failed to confirm such an association [31]. Moreover, vitamin E, given at a dose of 400 IU day⁻¹, increased the risk of prostate cancer in relatively healthy men [32].

Other homologues of vitamin E – namely, tocotrienols – exist as isomers designated as alpha, beta, gamma and delta. They are similar to tocopherols, except for the presence of an unsaturated phytyl chain with three unsaturation points compared with a saturated one in tocopherols [33]. Tocotrienols have potent anticancer activity and a protective effect in cardiovascular, cerebrovascular and neurodegenerative diseases. They distribute preferentially to the liver and are 40–60 times more potent than alpha-tocopherol against lipid peroxidation in rat liver microsomes. They attenuate triglyceride accumulation, leading to a reduction in hepatic inflammation and endoplasmic reticulum stress [34].

So far, the effect of tocotrienol supplementation in patients with NAFLD has been evaluated by only one RCT [35], which included hypercholesterolaemic adults with mild US-proven NAFLD who were randomized to receive 1 year of treatment with either mixed tocotrienols 200 mg twice daily (from palm oil) or placebo. A statistically significant advantage was seen for tocotrienols *vs.* placebo for normalization of the hepatic echogenic response. However, tocotrienols did not significantly affect lipid profile or liver function when compared with placebo.

Vitamin C

Vitamin C is a water-soluble antioxidant which acts as free radical scavenger [36]. It plays a role in the control of infections and is necessary for normal growth and development. Humans are unable to synthesize vitamin C, and must acquire it through diet, mostly from fruit and vegetables. Vitamin C deficiency affects around 5–10% of the population, with higher prevalence in subjects with an unhealthy lifestyle [37]. Low levels of vitamin C have been associated with a number of diseases, such as arterial hypertension, gallbladder disease, cancer, arteriosclerosis and an increased risk of stroke [38].

Some cross-sectional studies have investigated vitamin C levels in patients with NAFLD [39]. Some reported a low daily intake of vitamin C in NAFLD patients, while others found that dietary and/or plasma vitamin C levels were not different between patients with or without NAFLD. These conflicting results may be attributable to the fact that most of the studies evaluated only the estimated dietary intake of vitamin C and did not actually measure plasma concentrations. So far, the effect of vitamin C in patients with NAFLD has been evaluated in only two RCTs. After 6 months of treatment, vitamins C and E (1000 IU and 1 g daily, respectively) improved liver histology, but not LFTs, in 45 subjects with NASH [40]. In the St Francis Heart Study

[41], atorvastatin 20 mg combined with vitamin C 1 g and vitamin E 1000 IU was effective in reducing the odds of having hepatic steatosis, assessed by computed tomography scans, in individuals with NAFLD at baseline and after 4 years of therapy. However, in both trials [40, 41] it was not possible to detect whether this improvement was due to the combination treatment or to a single compound alone. Therefore, at present, there are insufficient data to support or refute the use of vitamin C supplements in patients with NAFLD.

Omega-3 fatty acids

The omega-3 fatty acids are essential polyunsaturated fatty acids (PUFAs) that cannot be synthesized *in vivo* and derive from alpha-linolenic acid. Docosahexaenoic acid (DHA) and eicosapentaenoic acid are the most represented omega-3 PUFAs. Fish oil is a convenient source of omega-3. Increased dietary intake of PUFAs from marine sources, such as two servings a week of fish, has been proposed to reduce the risk of heart disease [42]. The most important mechanism of action is the switching of cellular metabolism from lipogenesis and triacylglycerol accumulation to fatty acid oxidation. PUFAs modulate lipid metabolism in hepatocytes by promoting the restoration of peroxisome proliferator-activated receptor alpha (PPAR- α) and decreasing the expression of lipogenesis enzymes by depleting the sterol response element binding protein-1c (SREBP-1c), thus favouring fatty acid oxidation and reducing fatty liver. PUFAs may also be anti-inflammatory and improve insulin sensitivity.

Several clinical trials have assessed the efficacy of PUFAs in the treatment of NAFLD. In the first study, supplementation with PUFAs for 12 months reduced serum triglycerides and ALT, and improved US features of liver steatosis in 56 patients with NAFLD [43]. Similar results were found in an RCT performed in 40 subjects with NAFLD supplemented with PUFAs 2 g day⁻¹ for 6 months [44] and in a small study on 11 NAFLD subjects randomized to 6.5 ml olive oil enriched with PUFAs for 12 months or control diet [45]. Vega *et al.* [46] found that in 11 subjects with NAFLD, PUFAs (9 g d⁻¹ of fish oil) significantly reduced levels of plasma triglyceride by 46%, without a significant reduction in hepatic triglyceride content. Finally, Nobili *et al.* [47] performed an RCT with DHA supplementation (250 mg day⁻¹ and 500 mg day⁻¹) *vs.* placebo in 60 children with biopsy-proven NAFLD; children randomized to DHA showed less severe liver steatosis after treatment, a decrease in serum triglycerides and an improvement in insulin sensitivity, with no effect on ALT or body mass index. More recently, in a systematic meta-analysis including nine studies (five RCTs), involving 355 individuals, the pooled data suggested that PUFA supplementation may decrease LFTs and hepatic fat content, although the effect is generally small [48]. In conclusion, present data are heterogeneous, and there are no data available from well-designed RCTs of adequate size with a histological assessment of steatosis. Moreover, no information is available on the optimal dose of PUFA supplementation, the duration of therapy, and the long-term efficacy and safety.

Polyphenols

Polyphenols are members of a large family of plant-derived compounds [49] classified as flavonoids and non-flavonoids.

Non-flavonoids include stilbenes and phenolic acids. Polyphenols act as antioxidants, reducing liver fat accumulation, mainly by inhibiting lipogenesis. Epidemiological studies and meta-analyses have suggested that diets rich in plant polyphenols offer protection against cardiovascular and neurodegenerative diseases, diabetes, osteoporosis and cancer [50]. Polyphenols may present hepatoprotective effects because they increase fatty acid oxidation, and decrease oxidative stress, insulin resistance and inflammation, the main factors responsible for the progression from NAFLD to NASH.

Silymarin

Silymarin is a flavonoid polyphenol derived from the milk thistle plant which consists of silybin, isosilybinin, silicristin and other compounds. At the liver site, silymarin reduces inflammation and fibrogenesis, stimulates liver regeneration and interferes with leukotriene formation in Kupffer cell cultures, thus inhibiting hepatic stellate cell activation [51]. Silymarin exerts membrane-stabilizing and antioxidant activity. Hence, it has been proposed as complementary treatment for inflammatory liver conditions such as cirrhosis, hepatitis, alcoholic fatty liver disease and NAFLD.

Treatment with silybin plus phosphatidylcholine coformulated with vitamin E for 12 months was associated with an improvement in LFTs, insulin resistance and liver histology in 138 patients with histological NAFLD [52]. In a further study, food supplement containing vitamin E, L-glutathione, L-cysteine, L-methionine and *Silybum marianum*, given twice a day for 3 months in 72 subjects with NAFLD, reduced LFTs and hepatorenal brightness [53]. Similar results were obtained in a placebo-controlled trial of 180 mg day⁻¹ silymarin, in which treatment was associated with significant improvement of biometric parameters (reduction of abdominal circumference, body mass index, ultrasound measurement of right liver lobe), but not with a reduction of LFTs [54]. Recently, subjects with MetS and NAFLD were randomized to treatment with diet plus exercise, to silymarin plus vitamin E or to placebo for 3 months. The study suggested that, in subjects with NAFLD and MetS treated with diet and exercise, the addition of silymarin improved the efficacy of the lifestyle intervention [55]. We can conclude that silymarin supplementation for the treatment of NAFLD seems to have a favourable effect.

Resveratrol

Resveratrol belongs to stilbene family, which includes a series of compounds occurring in several plants, and is provided in the diet by various foodstuffs, such as grapes, berries, red wine and nuts [56]. Resveratrol improves insulin sensitivity and glucose tolerance, and reduces plasma lipids, inflammation and oxidative stress [57].

In an RCT including 50 NAFLD patients, 12 weeks' supplementation with 500 mg resveratrol reduced ALT and hepatic steatosis [58]. In a further RCT in 60 subjects with NAFLD, 600 mg resveratrol supplementation for 3 months reduced LFTs, HOMA-IR, and total and low-density lipoprotein cholesterol, with no improvement in US-diagnosed fatty liver [59]. By contrast, 8 weeks' administration of resveratrol did not significantly improve any features of NAFLD, compared

with placebo, but increased LFTs [60]. Moreover, in obese men supplemented with high doses of resveratrol, there was no effect on insulin sensitivity, lipid oxidation, ectopic or visceral fat content, or inflammatory and metabolic biomarkers [61]. These conflicting results raise doubt about the role of resveratrol in NAFLD, and future clinical studies will determine its safety and effectiveness in this setting.

Anthocyanins

Anthocyanins are water-soluble bioactive compounds which belong to the large family of flavonoids. Anthocyanin-rich foods improve hyperlipidaemia, counteract oxidative stress and ameliorate liver steatosis in experimental NASH. In a study carried out in subjects with borderline nonviral hepatitis, those randomized to purple sweet potato beverage rich in acylated anthocyanins had significantly decreased LFTs, particularly gamma-glutamyl transpeptidase [62]. Recently, improved insulin resistance and decreased plasma ALT, CK-18 M30 fragment and myeloperoxidase were found in 74 NAFLD patients randomized to a supplement of purified anthocyanin (320 mg day⁻¹) derived from bilberry and black currant (vs. placebo) for 12 weeks [63]. These findings suggest possible benefits of anthocyanin supplementation in subjects with NAFLD, although further studies using liver biopsy are clearly needed [64].

Betaine

Betaine is an important human nutrient obtained from a variety of foods. In the liver, betaine serves as a methyl donor to homocysteine to form methionine, resulting in decreased concentrations of homocysteine and increased concentrations of methionine.

The effect of betaine supplementation in subjects with NAFLD has been assessed in only one RCT, in which betaine did not improve LFTs or histology when compared with placebo [65].

Conclusions

NAFLD is a major challenge to healthcare systems worldwide. Most people with fatty liver do not develop severe liver disease but have an increased chance of developing cardiovascular diseases. Therefore, the treatment of NAFLD patients should be based on a global approach, not only addressing the treatment of insulin resistance and metabolic syndrome, but also including strategies focused on reducing oxidative stress, dyslipidaemia and cardiovascular risk. In this context, nutraceuticals may have an important role in the treatment of NAFLD, in combination with conventional treatments for cardio-metabolic risk factors.

Several nutraceutical supplements have shown promising results, especially those containing antioxidants and polyphenols. The results with vitamin D and carnitine supplementation are more uncertain. In particular, vitamin E appears to be effective for the treatment of nondiabetic subjects with more advanced NASH, although the high suggested daily dosages are a matter of concern. However, present information derives mostly from small trials with considerable heterogeneity with respect to inclusion criteria, sample size, type of experimental interventions and duration. At present,

there are insufficient data either to support or refute the use of nutraceuticals for subjects with NAFLD. Further RTCs, with histological changes as an outcome measure, are needed.

Competing Interests

All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: no support from any organisation for the submitted work; no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years; no other relationships or activities that could appear to have influenced the submitted work.

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