Role of Nutrition in the Pathogenesis and Prevention of Non-alcoholic Fatty Liver Disease: Recent Updates

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Abstract
Non-alcoholic fatty liver disease (NAFLD) is an acquired metabolic disease characterized by triglycerides (TGs) deposition in liver induced by other factors rather than alcohol consumption. NAFLD significantly contributes to liver diseases in children and adults. NAFLD pathogenesis is associated with age, gender, race and ethnicity. Insulin resistance, hyperinsulinemia, elevated plasma free fatty acids (FFAs), fatty liver, hepatocyte injury, liver inflammation, oxidative stress, mitochondrial dysfunction, imbalanced pro-inflammatory cytokines, and fibrosis are the characteristics of NAFLD. Factors including genetic and epigenetic pathways, sedentary lifestyle, sleep, and diet composition affect NAFLD pathogenesis. In this review, we discuss the aetiology, risk factors and pathogenesis of NAFLD. Special focus is given to macro and micro nutrition as causing factors and their role in the prevention of NAFLD pathogenesis.

Key words: nutrition, hepatic inflammation, NAFLD pathogenesis, prevention

Introduction
NAFLD is an acquired metabolic disease induced by metabolic stress and characterized by fat deposition in liver. NAFLD is associated with insulin resistance and genetic susceptibility and has overlapping histological characteristics with alcoholic liver [1]. NAFLD has four stages including hepatic fat deposition (hepatic steatosis), hepatic fat deposition with inflammation, fibrosis and cirrhosis (Fig. 1). The first stage is hepatic fat deposition also called non-alcoholic fatty liver (NAFL). The second stage is non-alcoholic steatohepatitis (NASH), characterized by excess hepatic fat deposition and inflammation. Persistent liver inflammation (hepatitis) causes scar tissue formation in the liver and this stage is called fibrosis. The fourth stage is cirrhosis, which is a severe form of NAFLD where fibrosis replaces most of the liver cells and therefore the structure and function of liver cells are compromised. Cirrhosis then leads to liver failure. Hepatocytes work well in fibrosis, but are compromised in cirrhosis. The prevalence of adult NAFLD in the general population is 5%-20%, whereas it is above 40% in obese and type 2 diabetic patients [2, 3]. The prevalence of NAFLD in children is 3% but it increases up to 50%-70% among obese children [4]. NAFLD prevalence is 16.9%-23.8% in boys and 16.2%-22.6% in girls in developed countries whereas it is 8.1%-12.9% in boys and 8.4%-13.4% in girls in developing countries [5]. These data suggest that NAFLD is one of the most important metabolic diseases arising parallel with other metabolic disorders including obesity, diabetes and cardiovascular disease.

Nutrition is the principal contributory factor effecting NAFLD pathogenesis. It has been reported that different diet components affect the progression and development of NAFLD [6-15]. Therefore, it is
very important to comprehensively discuss the role of nutrition in the progression, development and prevention of NAFLD. In this review, we discuss the risk factors and pathogenesis of NAFLD with a special focus on the role of nutrition in NAFLD pathogenesis and prevention.

**Risk factors and pathophysiology of NAFLD**

The development and progression of NAFLD were initially described by a “two hit” theory. The first hit, defined by “hepatic steatosis with or without hepatitis” is characterized by the presence of metabolic syndrome and TGs deposition in the liver. During the first hit, insulin resistance and hyperinsulinemia appear that alter hepatic pathways involved in the uptake, degradation, synthesis and secretion of free fatty acids (FFAs) that result in the deposition of fatty acids in the liver [16-18]. FFAs, Toll-like pattern recognition receptors (TLRs) and cytokines stimulate molecular pathways that block insulin signalling and thus induce insulin resistance. Insulin resistance is the result of overnutrition-induced inflammation that further supports NAFLD pathogenesis. The conditions of the first hit support hepatocyte injury and the pathogenic process of the second hit. Liver inflammation, oxidative stress, mitochondrial dysfunction, imbalanced pro-inflammatory cytokines, and fibrosis are the characteristics of the second hit that lead to the development of NASH and fibrogenesis (Fig. 1) [17-20]. Currently, “multiple hit” theory explains the progression of NAFLD where multiple hits act together to support liver inflammation. Many factors including diet, lifestyle, parental diet, genetic, epigenetic, environmental factors, insulin resistance and type 2 diabetes, etc. collectively contribute to obesity and widespread metabolic complications appear during NAFLD pathogenesis [16, 21, 22] (Fig. 2).

Importantly, the complex interplay of hepatic resident cells and activated immune cells, such as Kupffer cells, T cells, and hepatic stellate cells, with additional inflammatory factors and (TLRs) [23-26] contributes to the development and progression of NAFLD (Fig. 1). Additionally, the chronic activation of hepatic stellate cells and apoptosis are considered the principal mediators of hepatic fibrosis and cirrhosis. Similarly, hepatic progenitor cells (HPCs) associated with NASH and fibrosis, are the resident hepatic stem cells showing expansion during pediatric NAFLD and are involved in hepatic response to oxidative stress [27]. Furthermore, evidence suggests that Kupffer cells mediate different actions in NAFLD pathogenesis including immune tolerance and lipid homeostasis [24, 28]. In this regard, Stienstra et al. reported that Kupffer cells trigger TGs deposition and hepatic steatosis through interleukin-1 beta (IL-1β)-mediated suppression of peroxisome proliferator-activated receptor-α (PPAR-α) actions [29]. Similarly, De Vito et al. (2012) reported that deletion of Kupffer cells prevents hepatic steatosis and liver damage [24]. Likewise, altered functionality of peripheral T cell subpopulations has been reported in NASH [23]. Compared to CD4+ and CD20+, a high proportion of CD8+ T cell subpopulations have been reported in pediatric NAFLD. Additionally, these predominant CD8+ T cells in pediatric NASH were associated with up-regulated hepatic interferon-α, a high number of infiltrating neutrophils in association with reactive oxygen species (ROS) production in systemic neutrophils,

![Figure 1. Convergence of fats and carbohydrates induced key signalling molecules on NAFLD pathogenesis. Here, fats and carbohydrates mean a high intake or intake of harmful components of fats and carbohydrates supporting NAFLD pathogenesis. Fats and carbohydrates directly or indirectly induce metabolic stress and related signalling cascade that collectively induce insulin resistance and support overall NAFLD pathogenesis. FFA= free fatty acids, IKKβ= inhibitor of κB kinase-β, NF-κB= nuclear factor kappa B, Mac= macrophages, TNF-α= tumor necrosis factor-α, IL-6= interleukin 6, IL-1β= interleukin 1 β, ER= endoplasmic reticulum, IR= insulin resistance, TGs= triglycerides, NASH= non-alcoholic steatohepatitis, HSC= hepatic stellate cells, KC= Kupffer cells.](http://www.ijbs.com)
and altered phenotype and functionality of circulating lymphocytes and neutrophils. Similarly, in adult NASH, CD8+ cells were a minor component of natural killer cells [23, 30]. Furthermore, up-regulated autonomic receptors on HSC have been reported in adult NAFLD patients [31, 32].

On the basis of histology, NAFLD in children is similar to that in adults. However, the histologic distribution and progression of NASH in terms of inflammation and hepatocellular damage in children are different from those in adults [33, 34]. Liver biopsies from overweight and obese children (2-18 years of age) suffering from NAFLD revealed two different pathologic subtypes of NASH, subtype 1 (adult-type) and subtype 2 (pediatric-type) [35]. Histopathological features of NASH subtype 1 are steatosis, lobular inflammation, ballooning degeneration and presence or absence of perisinuoidal fibrosis, whereas the features of NASH subtype 2 are macrovesicular hepatocellular steatosis, portal inflammation, absence or minimal ballooning degeneration and presence or absence of portal fibrosis. Additionally, the prevalence of both subtypes differ with gender and race (for a review, see [36]). Although both subtypes overlap in 32% of patients, type2 NASH is the most predominant subtype [37]. Importantly, the etiopathogenesis, prognosis and differential response of both subtypes to treatments are still not clear and need further studies. NAFLD in children and adults has similarities and differences in the pathogenesis at the molecular and genetic level [38]. Similarly, the outcome also overlaps, however it is difficult to cover all of these in the current review; therefore interested readers can study the review article of Nobili et al. (2016) for detailed similarities and differences between NAFLD in children and adults [38]. The differences between NAFLD in adults and children are shown in Table 1.

### Table 1. Differences between Children and Adult NAFLD

| Type of parameter | Adults | Children |
|-------------------|--------|----------|
| Steatosis         | Typically mild to moderate | Typically moderate to severe |
| Ballooning        | Common | Uncommon |
| Inflammation      | Mainly lobular | Mainly portal |
| Fibrosis          | Pericellular chicken wire | Predominantly portal-periportal |
| Outcome           | Cirrhosis: 5-10% | 1-2% |
|                   | HCC: Strong clinical evidence | Rare |
|                   | Metabolic syndrome: Strong clinical evidence | Strong clinical evidence |
|                   | Cardiovascular disease: Strong clinical evidence | Increased risk |

### Contribution of macro and micro nutrients to the development of NAFLD

The key problem of NAFLD and NASH is overnutrition and, therefore, after NAFLD diagnosis, a first focus should be given to controlling over feeding and body weight. Weight loss (1kg/week) is recommended for overweight (BMI 25–30 kg/m²) and obese (BMI > 30 kg/m²) subjects and it seems unlikely that changes in the diet alone (without accompanying weight loss) might be sufficient to overcome NAFLD and NASH. However, it is important to note that rapid and uncontrolled weight loss is detrimental and even worsens NAFLD and NASH. We take two types of nutrients in our daily life: macronutrients and micronutrients.

### Macronutrients

The nutrients that we take in large amounts in our daily life are macronutrients. Carbohydrates, fats and proteins are the principal types of macronutrients. Various chemicals exist in macronutrients that either support or prevent NAFLD pathogenesis. The role of macronutrients in NAFLD pathogenesis and prevention is summarized in Fig. 3.

### Carbohydrates

Although the control of total caloric intake is very important to prevent metabolic complications including NAFLD induced by high energy intake, carbohydrates are also of great concern. NASH has been induced by a high carbohydrate diet in a desert gerbil [7]. Likewise, a carbohydrate rich diet is a major source of hepatic FFAs production in NAFLD subjects where it contributes to 30% FFAs production, whereas in normal subjects, high carbohydrate intake produces only 5% FFAs [39]. Diets with a low carbohydrate
content (<45% of carbohydrates/day) have been reported to be helpful in improving weight loss and the overall metabolic profile and reduce intrahepatic TGs content [40]. Low carbohydrate intake improves the condition of NAFL [41, 42]. Additionally, a low carbohydrate diet reduces TGs and increases HDL levels [43]. Paradoxically, consumption of a low carbohydrate diet over a long period stimulates NAFLD pathogenesis and glucose intolerance in an animal model [40] and increases total and low density lipoproteins (LDL) cholesterol in humans [43].

Interestingly, a low carbohydrate diet is helpful in weight loss if consumed for a short period (six months) but this weight loss cannot be maintained after one year. Importantly, the quick and rapid weight loss supports the disease progression in some NAFLD subjects [40]. Similarly, the composition of ingested carbohydrates is also important. Here, we will discuss the role of some special carbohydrate types in the pathogenesis and prevention of NAFLD.

**Fructose**

As a sweetener, fructose has been used in juices, jellies and jams. Fructose can easily induce metabolic complications in children with NAFLD compared to children without NAFLD [44]. Fructose enriched soft drinks induce insulin resistance and support NAFLD development. Fructose intake causes lipogenesis, TGs synthesis and studies in ducks and rats have shown that it also causes hepatic steatosis [45, 46]. Additionally, high fructose intake also inhibits leptin (fat derived anorexigenic hormone) secretion, and therefore satiety cannot be achieved [47]. Similarly, a high fructose diet reduces PPARα activity and hepatic lipid oxidation and stimulates NF-kB expression that leads to oxidative stress, hepatic steatosis and hepatic fibrosis in rats [48]. Fructose interacts with transcriptional factors and affects the gene expression involved in glycolysis and lipogenesis [6]. Soda, soda pop, Coca-Cola and tonic are soft drinks rich in fructose. A normo-caloric diet with a 3g fructose/kg of body weight per day increases hepatic fat deposition and serum TG levels and reduces insulin sensitivity in adult men [49]. Consumption of more than 1 soft drink (about 360ml)/day increases the risk for the development of metabolic syndrome, however this risk was not observed in subjects who were consuming less than 1 soft drink/day [45, 50].

**Fiber and glycemic index diet**

Fibers can be classified in two types: soluble and insoluble fibers. The examples of soluble fibers are pectin and gums. Pectin exists in fruits and gums exist in oat, beans, barley, lentils, peas, and chickpeas. Examples of insoluble fibers are cellulose (wheat), hemicellulose (grains) and lignin (vegetables). Insoluble fibers promote satiety (restrict caloric intake), whereas soluble fibers slow stomach emptying and glucose and cholesterol uptake. Soluble fibers from sources such as oat reduce TC levels [51, 52]. A recent meta-analysis study reviewed the effects of high fiber and low-glycemic index (GI) foods on glycemic response and cholesterol concentration [53] and suggested that such diet components might be beneficial for subjects who have an impaired insulin response. Low-GI foods or slow release carbohydrates induce a “second-meal effect” which is a reduced glucose response to the subsequent meal, and was described in early 1980 [54]. It was reported that a low-GI diet breakfast, a breakfast consisting of high-fiber indigestible and fermentable starch, induces the second-meal effect and reduces circulating FFAs and insulin levels after the subsequent meal in both men and women [55]. Of note, we cannot generalize the results of the above study to the long term effects of such diets on blood glucose, insulin and lipid profile but it does suggest that the inclusion of high-fiber indigestible and fermentable starch and low-GI carbohydrates might be helpful to maintain blood glucose, insulin and FFAs profile in subjects with insulin resistance and NASH.

From the above discussion it can be speculated that in the development of NAFLD and other hepatic complications, carbohydrate’s composition is also important. However, more pre-clinical and clinical trials are suggested to investigate the role of different components of carbohydrates in the progression and treatment of NAFLD.
Fat

A fat rich diet induces hepatic steatosis [9]. Fat accumulation triggers lipolysis in adipocytes and increases FFAs that result in reduced plasma adiponectin levels, attenuated lipid clearance from plasma and increased beta-oxidation in muscles [56]. In a previous study, we developed a rabbit model of pediatric NASH and found that HFD feeding increases body weight, liver weight and ALT, TG, IL-6, TNF-a levels, whereas it reduces serum adiponectin and IL-10 levels [57-60]. Similarly, in another study, we reported elevated levels of hyaluronic acid, serum ferritin, serum type III procollagen, ALT and aspartate aminotransferase (AST) in obese NAFLD and NASH children [61]. Fats are a diverse class of organic compounds; however, we will focus on some fat types involved in the pathogenesis and prevention of NAFLD.

Saturated fatty acids (SFAs)

SFAs are fatty acids that have only carbon to carbon single bonds (no double bond) in their hydrocarbon backbone. SFAs induce endoplasmic reticulum (ER) stress and hepatocyte injury in rats [62]. SFAs induce hypothalamic inflammation that leads to obesity onset and its related metabolic complications [63-65]. Dietary data revealed that NASH subjects took 14% of their total energy from SFAs, whereas it was 10% in control subjects. Intake of more than 10% of total energy from SFAs may support insulin resistance, whereas less than 10% of total energy from SFAs reduces plasma LDL and TG levels; however, less than 7% of total energy from SFAs does not further improve NASH but is even detrimental.

Monounsaturated fatty acids (MUFAs)

MUFAs are a class of fatty acids that have only one carbon to carbon double bond in their hydrocarbon backbone, with the rest of the bonds being single bonds. MUFAs exist in some foods including nuts, olive oil, and avocados. Diet driven MUFAs decrease oxidized LDL [66], LDL cholesterol [67], TC and TGs levels with no decrease in HDL [68-70]. Replacement of carbohydrates and saturated fats with MUFAs increases HDL and reduces glucose and blood pressure in diabetic subjects [71]. Additionally, another study reported that compared to a high-carbohydrate diet (28% of total energy intake), a diet rich in MUFAs (40% of total energy intake) extensively decreases VLDL triacylglycerol and VLDL cholesterol and is acceptable for T2D subjects [71]. Additionally, it has been reported that a diet composed of 20% MUFAs as an energy source of the total daily caloric intake increases fatty acid oxidation through activated PPARs activity and reduces lipogenesis through diminished sterol regulatory element binding protein (SREBP) activity in NAFLD men and women [72]. Regardless of physical exercise, a high MUFAs intake significantly reduced hepatic fat content in T2DM subjects [72]. The above evidence collectively suggests that replacement of a SFA and carbohydrate rich diet with a MUFAs rich diet might be beneficial in NAFLD treatment.

Polyunsaturated fatty acids (PUFAs)

PUFAs are a class of fatty acids that have two or more than two carbon to carbon double bonds in their hydrocarbon backbone. PUFAs are present in sea fish, green leafy vegetables, rapeseed oil and flax seeds, and are beneficial against NAFLD. Interestingly, 50 g/day intake of rapeseed/canola oil for four weeks has been shown to improve TC, LDL, and hepatic enzymes up to a healthier range in obese men compared to those who ingest 50 g/day of olive oil [73].

Omega-3 (ω-3) and omega-6 (ω-6) are important PUFAs and have a role in NAFLD pathogenesis. ω-3 fatty acids are beneficial, however ω-6 fatty acids should be avoided because of their ability to increase inflammatory markers [11]. It has been reported that patients with NASH have a high intake of ω-6 fatty acids and an abnormal ω-3/ω-6 fatty acids ratio [74]. Therefore the recommended ω-3 to ω-6 ratio should be 1 : 1 to 1 : 4. ω-3 fatty acids regulate gene expression involved in hepatic lipid metabolism, fatty acid oxidation and reduced expression of pro-inflammatory genes. Docosahexaenoic acid (DHA), linolenic acid (plant oil), and eicosapentaenoic acid (EPA) [fish oil] are examples of ω-3 fatty acids. It has been reported that ω-3 fatty acids have beneficial effects against hepatic steatosis and inflammatory markers and improve insulin sensitivity and cardiovascular disease [75-77]. Similarly, NAFLD is associated with reduced levels of dietary ω-3 fatty acids, elevated plasma and hepatic SFAs; therefore, reducing the daily dietary intake of SFAs and provision of ω-3 fatty acids improve NAFLD in children [78-80]. Interestingly, various studies reported that ω-3 fatty acid supplementation for 6 months in NAFLD children did not improve ALT levels [81-83]; however, Nobili et al. (2014) provided ω-3 fatty acids to NAFLD children for 18 months and a significant improvement in ALT levels was observed [80]. The observed effects of ω-3 fatty acids after an 18-month treatment on ALT levels suggest that ω-3 fatty acids are effective against ALT levels but needs prolonged treatment and therefore we don’t see the effects of ω-3 fatty acids in a 6-month treatment on serum ALT levels. Importantly, ω-3 fatty acids improve lipid profile, insulin
sensitivity and reduce plasma TG levels, hepatic steatosis and cytokines production [79]. Recently, it has been reported that DHA has the potential to reduce markers of NAFLD [84]. Likewise, ω-3 fatty acids improve gut mirobiota that are beneficial against genes involved in lipogenesis in high carbohydrate diet-induced steatotic mice [85]. DHA act on G protein-coupled receptor 120 (GPR-120), and has shown beneficial affects against NAFLD in children [80]. Additionally, reduced hepatic progenitor cells and macrophages and up-regulated GPR 120 expression after DHA supplementation [80] suggest that DHA modulate hepatic progenitor cells and macrophages through activated GPR-120 signalling. Nobili et al. (2011) reported that ω-3 improves insulin sensitivity and hepatic echogenicity in children suffering from NAFLD [86]. Although it has been suggested that it is premature to describe ω-3 as a treatment for NASH and NAFLD subjects, it should be the first line therapeutic agent to treat hypertriglyceridic NAFLD patients [78]. Further studies are required to confirm whether ω-3 is suitable for the treatment of NASH and NAFLD in humans.

Trans fatty acids

Trans fatty acids are produced in natural foods as a result of bacterial metabolism (dairy products) and hydrogenation (margarines) and may have a role in NAFLD pathogenesis. Trans fatty acids consist of many isomers that differentially regulate the human metabolism [10]. It has been reported that Cis-9, trans-11 conjugated linoleic acid and trans-11 oleic acid, derived from bacterial metabolism and existing in dairy products, have no adverse effects on lipoprotein levels [87]. Paradoxically, it has been reported that the intake of trans-10, cis-12 conjugated linoleic acid present in hydrogenated oils increases inflammatory markers in women [88], causes endothelial dysfunction [89], and adversely affects the plasma lipid profile by increasing LDL:HDL and total cholesterol (TC):HDL ratios in humans [90]. Although the exact role of trans fatty acids on the lipid profile and its mechanism of action is not yet clear, dietary recommendations in NAFLD suggest avoiding highly processed food products enriched with trans fatty acids [91]. Further pre-clinical and clinical studies are required to unveil the role of trans fats in NAFLD pathogenesis.

Proteins

The role of high protein intake in causing various complications including intrarenal capillary hypertension, glomerular sclerosis, and finally renal dysfunction in some susceptible individuals [92-96] has been studied; however, studies about the role of proteins in NAFLD pathogenesis are limited. Clinical studies have reported that protein intake affects the pathogenesis of NAFLD (for a review, see [74]). Malnutrition and protein deficiency induce hepatic steatosis and NASH [97, 98]. By inhibiting de novo lipogenesis, high protein and low carbohydrates diets improve carbohydrate metabolism and liver steatosis [74]. Dietary protein intake is important for hepatocyte regeneration and provides important amino acids that prevent fat deposition in the liver [98]. Arciero et al. (2008) proved that a moderate protein diet (25% energy from protein) has no side effects and reduces body fat content as much as a high protein diet does and, therefore, they recommended a moderate protein diet for NAFLD patients [99]. Likewise, recently it was reported that proteins that exist in dietary mung beans are beneficial against HFD-induced NAFLD in male mice [14]. Duarte et al. (2014) studied the effects of a high protein, low carbohydrate diet in adult NAFLD patients. They found no change in body weight, weight circumference and body fat. Interestingly the diet showed increased HDL-cholesterol and decreased total LDL and VLDL cholesterol, TGs, AST, gamma glutamyltransferase (GGT), alkaline phosphatase (AP) and fasting blood glucose [12]. This study suggests that independent of its effect on body weight and body fat, a high protein, low carbohydrate diet improves the lipid profile, insulin homeostasis and liver enzymes. Furthermore, soy protein has shown positive effects against NASH by reducing plasma cholesterol levels and fat deposition in body [100]. Additionally, soy protein also reduced TGs deposition in the liver and insulin sensitivity and antioxidant activities in rats [100]. To study the role of soy protein in NAFLD subjects, Kani et al. (2014) gave a low caloric, low carbohydrate soy containing diet to adult NAFLD patients. It was found that a soy containing diet reduced ALT levels, but the lipid profile was not affected [13].

Micronutrients

Micronutrients are different from macronutrients, as the body only requires them in very small amounts. Micronutrients consist of vitamins and minerals; however here, we will discuss the role of some important micronutrients in the prevention of NAFLD development (Fig. 3).

Vitamin E

Mitochondrial stress triggered by ROS is one of the etiological mechanisms of NAFLD pathogenesis (Fig. 1); therefore, antioxidants, for example, vitamin E, might have beneficial effects against NAFLD. Lavine was the first to propose vitamin E, a potent
fat-soluble antioxidant, as an adjunct therapy for NAFLD. He checked the effects of vitamin E against high levels of ALT and hepatic steatosis in children [101]. Subsequently, various studies have used vitamin E for NAFLD treatment. Many groups reported that vitamin E treatment is ineffective to improve ALT levels in biopsy proven [102, 103] and suspected NAFLD [104] children. Similarly, we studied the effects of vitamin E and lifestyle on ALT levels in Chinese obese children suffering from NAFLD [105]. Paradoxically, it was found that vitamin E is effective in reducing ALT levels in children; however the improvement was less compared to a summer camp lifestyle program. Therefore, these studies [103, 105] collectively suggest that lifestyle is more beneficial than vitamin E supplementation to improve ALT levels. The positive effects (opposite to other studies) of vitamin E in our study are not clear but it can be speculated that the different experimental design, food and ethnicity may have some effects on vitamin-induced changes in ALT levels; however further studies are required to confirm it. Additionally, vitamin E, DHA, and choline combination reduces ALT and glucose levels and improves steatosis in NASH children. As DHA, vitamin E and choline all have beneficial effects against NAFLD, it is difficult to say which component has improved the conditions. Additionally, reduced hepatic steatosis, inflammation, hepatocyte ballooning, and aminotransferase levels were reported after vitamin E supplementation in NASH patients [106]. Vitamin E is effective against steatohepatitis in NAFLD patients but is ineffective against hepatic fibrosis once it is established [107]. Furthermore, supplementation of vitamin E and ascorbic acid with a modified diet and physical exercise has improved liver function and glucose metabolism in children [103]. Additionally, vitamin E supplementation with a healthy lifestyle was effective against hepatocytes ballooning; however, aminotransferase levels were not affected but a significant improvement in NAS score and resolution of NASH was observed in both adults and children [102]. Importantly, one should be very careful during prescription of vitamin E as high doses of vitamin E induce complications including some cancer types [102].

Vitamin C

Being an antioxidant, vitamin C might be beneficial against NAFLD. In this regards, Oliveira et al. (2003) induced fatty liver disease in Wistar rats by feeding them with choline deficient diet [15]. To investigate the role of vitamin C (potent hydrosoluble antioxidant) against fatty liver disease, Oliveira et al. (2003) fed fatty liver rats (n=6) with vitamin C (30 mg/Kg/day) for four weeks. The control (n=6) rats were fed with a placebo. It was found that vitamin C treatment reduced oxidative stress and inhibited steatosis in choline-deficient diet induced fatty liver rats [15]. Therefore, the aforementioned [15] positive effects of vitamin C against oxidative stress and steatosis might be due to its antioxidant activity. However, another study reported that vitamin C treatment reduces plasma cholesterol and TG levels and suggests that the beneficial effects of vitamin C against fatty liver are because of its anti-atherogenic action [108]. Similarly, Harrison et al. (2003) performed a prospective, double-blind, randomized placebo-controlled study and included histology based NASH in men and women. They divided the subjects into two groups, either to receive vitamins E and C (1000 IU and1000 mg, respectively) or a placebo daily for 6 months [109]. It was found that vitamin E and vitamin C supplementations reduce hepatic fibrosis, especially in diabetic NASH patients. However, this therapy did not affect inflammation or ALT levels [109]. Although this study showed the positive effects of vitamins (E and C) in 6-month treatments on hepatic fibrosis, it remains unknown whether this therapy will be effective if applied for a longer time and that the resulted improvement in fibrosis affects the transition of NASH to cirrhosis and hepatocellular carcinoma. Therefore, further pre-clinical and clinical trials should be done to unveil the effects of long-term vitamin C treatment on fibrosis, cirrhosis, hepatocellular carcinoma, inflammation and ALT levels. It is also worthwhile investigating whether vitamin C treatment improves haptic complication through its antioxidant properties or any other action.

Vitamin D

Vitamin D deficiency is associated with NAFLD pathogenesis. Obese patients have been reported to be more deficient in vitamin D compared to normal weight and overweight subjects [106, 110-112]. Vitamin D regulates various genes widely distributed in the liver and some of them are involved in glucose and fat metabolism [106, 110]. Through the activation of TLRs, vitamin D deficiency exacerbates NAFLD and has association with hepatic inflammatory markers, oxidative stress and insulin resistance in rats.
[113]. Importantly, reduced plasma vitamin D levels have an association with insulin resistance and T2D and proper supplementation of vitamin D improves insulin sensitivity [114]. Similarly, independent of fat deposition and insulin resistance, reduced levels of plasma vitamin D has an association with NAFLD in adults [110]. Interestingly, vitamin D deficiency [115] and increased prevalence of NAFLD in obese children [4] link vitamin D deficiency and NAFLD occurrence. In this regard, Misra et al. (2008) studied the association between vitamin D and NAFLD in children. Vitamin D deficiency was reported in NAFLD patients, but vitamin D deficiency was not associated with NAFLD severity [115]. Similarly, Black et al. (2014) also reported vitamin D deficiency in NAFLD adult patients but the serum vitamin D levels were inversely proportional to NAFLD severity [110]. Additionally, the same inverse proportion between serum vitamin D levels and NAFLD severity has been reported in children with high ALT levels and a hyperchogenic liver [116]. The above studies clearly showed vitamin D deficiency in NAFLD patients and opened a new window for researchers to study the role of vitamin D deficiency in NAFLD pathogenesis and then to use vitamin D as an adjunct therapy for NAFLD treatment.

In this regard, one study used vitamin D supplementation against adult NAFLD subjects and reported reduced high-sensitive C-reactive protein (hs-CRP) and malondialdehyde (MDA) levels but ALT levels were not affected [117]. However, further pre-clinical and clinical studies are suggested to investigate the relation of vitamin D with insulin resistant, adiposity and NAFLD and whether supplementation of vitamin D is beneficial against NAFLD development and progression.

**Polyphenol**

It was reported that polyphenols have been used for NAFLD treatment (Aguirre et al., 2014). Resveratrol, a member of the polyphenols family has shown anti-steatotic, anti-inflammatory, and anti-oxidative effects (for a review, see [118]). In this regard, various studies have reported that resveratrol supplantations reduce liver enzymes and inflammatory cytokines [119, 120] and hepatic steatosis [121]. Similarly, Chen et al. (2015) randomized adult NAFLD subjects into two groups: (2 capsules with 150mg of resveratrol/day) as a treatment and (2 capsules of placebo/day) as a control. It was found that a treatment of 2 capsules with 150mg of resveratrol/day significantly reduced AST, ALT, LDL cholesterol and TC levels and improved the glucose profile compared to the control group [120]. Similarly, Faghihzadeh et al. (2014) also divided adult male and female NAFLD patients into two groups, control (placebo) and intervention (500 mg of resveratrol/day) with a balanced diet and healthy lifestyle. It was found that resveratrol treatment improves various parameters of NAFLD pathogenesis [119]. Various other members of the polyphenol family, including curcumin [122], quercetin [119], anthocyanin [122] and green tea polyphenols [123], also showed beneficial effects in the treatment of adult NAFLD subjects. The beneficial effects of polyphenols in adult NAFLD subjects hint at its beneficial effects in children but careful pre-clinical and clinical studies are required to test this hypothesis.

**Summary**

NAFLD is an acquired metabolic liver disease characterized by TGs deposition in more than 5% hepatocytes not induced by alcohol consumption and other etiologic factors causing liver diseases, including drugs, toxins, infectious diseases, etc.[124]. NAFLD ranges from simple steatosis to non-alcoholic steatohepatitis (NASH) that lead to liver fibrosis, cirrhosis, and hepatocellular carcinoma [125].

Various factors including genetic and epigenetic pathways, sedentary lifestyle, sleep, and high energy diets play a key role in NAFLD and NASH pathogenesis [33, 126, 127]. Oxidative stress, liver inflammation, mitochondrial dysfunction, imbalanced pro-inflammatory cytokines, fibrosis, insulin resistance, hyperinsulinemia, plasma FFAs, fatty liver, and hepatocyte injury are pathologies that support the development of NASH and fibrogenesis [16-20]. Nutrition plays a key role in the pathogenesis of NAFLD. Among macronutrients, fat and carbohydrate-rich diets support/prevent the pathogenesis of NAFLD [7, 39, 61, 128]. Fructose from carbohydrates [48, 49], as well as SFAs [62, 128], trans fatty acids [88] and ω-6 fatty acids [11] from fats, support NAFLD pathogenesis. Furthermore, fiber and low glycemic index diets from carbohydrates [53, 55], as well as MUFAs [72] and ω-3 fatty acids from fats, prevent NAFLD pathogenesis [80, 84]. Importantly, proteins have beneficial effects against NAFLD [99, 128]. Among micronutrients, vitamin C [109], vitamin E [102-104], vitamin D [117] and polyphenols [119, 120] prevent NAFLD pathogenesis. However, many research areas about NAFLD are still new and need further experimentations, including the role of vitamin C, vitamin D, ω-3/ ω-6 fatty acids and proteins in the pathogenesis/prevention of NAFLD. Similarly, further pre-clinical and clinical trials will be helpful to unveil the exact role and mode of action of high fiber and low glycemic diets in NAFLD pathogenesis.
Abbreviations

NAFLD: non-alcoholic fatty liver disease; TGs: triglycerides; NAFL: non-alcoholic fatty liver; FFAs: free fatty acids; TLRs: toll like receptors; NASH: non-alcoholic steatohepatitis; HPCs: hepatic progenitor cells; IL: interleukin, PPAR-α: peroxisome proliferator-activated receptor-α; ROS: reactive oxygen species; ALT: alanine aminotransferase; TNF-α: tumour necrosis factor alpha; AST: aspartate aminotransferase; SFD: saturated fatty acids; EPA: eicosapentaenoic acid; GPR-120: G protein-coupled receptor 120; EASL: European Association for the Study of the Liver; AASLD: American Association for the Study of Liver Diseases; MDA: malondialdehyde; DHA: docosahexaenoic acid; HDL: high density lipoprotein; SREBP: sterol regulatory element binding; GI: low-glycemic index; MUFAs: monounsaturated fatty acids; GGT: gamma glutamyltransferase, AP: alkaline phosphatase; BMI: body mass index; GL: low-glycemic index; MUFA: monounsaturated fatty acids; VLD: very low density lipoprotein; SREBP: sterol regulatory element binding protein; ω-3: omega 3; DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; GPR-120: G protein-coupled receptor 120; EASL: European Association for the Study of the Liver; AASLD: American Association for the Study of Liver Diseases; MDA: malondialdehyde; hs-CRP: high-sensitive C-reactive protein.

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Competing Interests

The authors have declared that no competing interest exists.

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