The Role of Vitamins in the Pathogenesis of Non-alcoholic Fatty Liver Disease

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ABSTRACT: The incidence of non-alcoholic fatty liver disease (NAFLD) is rising rapidly in parallel with obesity rates. The underlying pathogenesis of NAFLD remains an enigma but is largely influenced by individual lifestyle choices involving diet and exercise. Therefore, studies have highlighted the importance of calorie reduction and macronutrient composition (eg, carbohydrate and fat) in modifying disease outcomes. Micronutrients are also believed to play a role in disease progression. There are now an increasing number of studies linking vitamins with NAFLD, particularly vitamin E, and the supplementation of several different vitamins has been demonstrated as a promising therapeutic option in the treatment of NAFLD. This review provides a broad overview of the potential role of vitamins in NAFLD development and disease management.

KEYWORDS: fatty liver, metabolic syndrome, micronutrient, vitamin, oxidative stress

Introduction

Non-alcoholic fatty liver disease (NAFLD) is characterized by increased fat accumulation as triglycerides in the liver,[1] which can manifest as a wide spectrum of conditions: from hepatic steatosis to more severe steatohepatitis with fibrosis, cirrhosis, and potentially hepatocellular carcinoma.[2] Currently, NAFLD is the most common liver disease of the Western world, largely due to obesity induced by readily available high-energy food and sedentary lifestyle of the modern society. As a consequence, the incidence of NAFLD is rising drastically in tandem with the rising rates of obesity worldwide. Currently, the prevalence of NAFLD stands at 24.4% globally,[3] and it is found to be even higher in patients with a background of Type 2 diabetes mellitus (T2DM) and other metabolic syndrome features.[4] The mortality rate and the number of liver transplantations due to NAFLD are also on the rise, and non-alcoholic steatohepatitis (NASH) is now the second leading indication for liver transplantation in the US.[5,6] To prevent further exacerbation of this serious condition, it is necessary to find more effective interventions. Recently, in a large cross-sectional study of patients with liver biopsies, Dongiovanni et al.[7] showed a protection from liver damage in those patients who are at risk of non-alcoholic steatohepatitis after a statin treatment.[7] Lifestyle modification, involving exercise and dietary adjustment with reduced calorific content, continues to be the core therapeutic approach for NAFLD. However, several studies have highlighted that perhaps a reduction in energy alone is not sufficient for the alleviation and prevention of NAFLD; the dietary compositions, both macronutrient and micronutrient, may also play a crucial role in the manifestation and development of NAFLD. For example, recent studies have highlighted the importance of dietary vitamin composition and liver fat accumulation. Vitamins are essential micronutrients in the maintenance of health, which cannot be synthesized sufficiently from other molecules alone. Currently, there are 13 known vitamins: four lipid soluble (A, D, E, and K) and nine water soluble (groups B and C).[8] Until now, most review articles have summarized studies of a specific micronutrient/vitamin on hepatic metabolism, whereas the aim of this article was to provide a broader overview of the role of vitamins in NAFLD development and management.

Pathogenesis of NAFLD

To understand whether and how vitamins play a role in the mechanism of NAFLD, it is necessary to know how NAFLD is manifested. The *two-hits* hypothesis proposes that obesity or T2DM causes the liver to become vulnerable to further damage from reactive oxygen species (ROS) and proinflammatory cytokines.[9] This theory was further expanded into the *multi-hits* hypothesis, which suggests that NAFLD is a result of multiple factors, such as diet and maternal predisposition, rather than a definitive set of stimulators.[10] Moreover, new studies have also highlighted the role of epigenetics in

the pathogenesis of NAFLD.[11] This might also explain why maternal obesity can have an immediate effect on offspring.[12]

Genetically, studies have further supported the importance of the genetic background on NAFLD development: genome-wide association studies have identified the patatin-like phospholipase domain-containing 3 (PNPLA3) and the transmembrane 6 superfamily member 2 (TM6SF2) as genes related to the susceptibility of NASH.[7,13]

In addition, recent studies have shown that the alteration of innate immunity has an influential role in NAFLD. This is consistent with the fact that liver is often considered a major immune organ and has a wide repertoire of immune cells: dendritic cells (DCs), Kupffer cells (KCs), natural killer (NK) cells, and natural killer T (NKT) cells. Hepatic stellate cells, known to be responsible for collagen production, also exhibit immune properties by presenting antigens.[14] Other studies have indicated the involvement of immune system in the etiology or progression of NAFLD. Both NAFLD patients and animal models showed an increased number, but impaired phagocytic function of KCs.[15,16] NKT cell number was reported to be decreased in the animal models of NAFLD, accompanied by an increase in inflammatory cytokines.[17] Similar observations of NKT cells were also found in patients with NAFLD, suggesting a protective role of NKT cells in NAFLD.[18] Interestingly, there is a new hypothesis for the role of microbiota composition in NAFLD, based on the observation that patients with obesity have an altered ratio of Bacteroidetes to Firmicutes, intestinal bacteria concentration, and decreased richness of other gut bacteria.[19] It is believed that microbiota composition causes NAFLD either by increasing the endotoxin level or by affecting the integrity of the tight junctions in the gut.[20]

Taken together, the underlying pathogenesis of NAFLD is complex, and it might involve the interaction between innate immunity and the individual's microbiota composition. It is possible that the alteration of this axis is related to vitamins as they are known to have an immune modulatory role. Furthermore, the disruption of the gut integrity might affect the absorption and processing of the vitamins.

Vitamin A. Vitamin A is also known as retinoic acid, and its synthesis involves phospholipids and unsaturated fatty acids.[8] The first link between vitamin A and NAFLD is that the liver is the main storage site of vitamin A, and vitamin A has a close relationship with the control of adipose tissue. Furthermore, recently, a role of retinol as carriers of the PNPLA3 mutation has been demonstrated, which is a gene closely related to NAFLD.[21,22] The hepatic storage of vitamin A is mostly in the quiescent hepatic stellate cells (qHSC), and these cells are responsible for fibrogenesis. It is known that when qHSC gets activated to become profibrogenic HSC, the activated HSC lose their vitamin A content.[23] In addition, hepatocytes actively metabolize vitamin A and modify glucose and lipid metabolism in response to vitamin A metabolites.[23]

Several studies have highlighted the role of vitamin A in the pathogenesis of NAFLD. It has been demonstrated that retinoic acid effectively reduced adiposity in the liver and enhanced hepatic fat catabolism.[24] Furthermore, an *in vitro* study reported that retinoic acid was able to block adipogenesis of cultured adipocytes during the early stages of the differentiation process.[25] Similarly, mice treated with the active form of retinoic acid effectively reduced the body weight. In this study, although the liver weight was not significantly reduced, the hepatic content of both triglycerol and glycogen was decreased.[24] These changes were believed to be induced by the alteration of the lipid metabolism in the liver due to the upregulation of peroxisome proliferator-activated receptor alpha (PPARα), a key component in controlling fatty acid oxidation.[24] In a study involving patients with NAFLD or NASH, serum retinoic acid concentrations were reduced and retinoid X receptor α (vitamin A receptor) mRNA expression was inversely correlated with the extent of hepatic steatosis.[26] In addition, the same study found that circulating retinoic acid concentrations were associated with increased lipid metabolism and insulin resistance, which are the hallmarks of NAFLD. Moreover, the retinoic acid concentrations were negatively associated with liver injury and adiposity markers, body mass index, and waist circumference. Furthermore, hepatic ballooning and NAFLD activity scores (NAS) were also found to be associated with the serum concentration of retinoic acid.[26] Although the majority of the studies showed beneficial effects of vitamin A on obesity and obesity-related conditions, one study has shown that predisposing mice to vitamin A supplementation at a young age led to higher adiposity when the mice were then weaned onto high fat diet, thereby increasing the proliferative capacity in white adipose tissue.[27] This is perhaps due to the stage-dependent effects of vitamin A during development, which may be different from the effects seen in adults. Overall, the studies suggest that despite the obvious beneficial effects from vitamin A, precautions should be taken for the use of vitamin A supplementation in young children.

Vitamin B group. Vitamin B group has eight types of compounds: thiamine (B_1), riboflavin (B_2), niacin (B_3), pantothenic acid (B_5), pyridoxine (B_6), biotin (B_7), folic acid (B_9), and cyanocobalamin (B_{12}). This group of vitamins was originally believed to be one compound but was subsequently separated into distinctive identities. Only vitamins B_3 and B_{12} have been associated with NAFLD, whereas the evidence for the involvement of other vitamin B compounds is lacking. Vitamin B_3, commonly known as niacin, is a precursor of the coenzyme nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate (NADPH), which plays an important role in lipid metabolism.[8] It has been used for treating dyslipidemia and cardiovascular diseases.[28] In an obesogenic diet-induced rat model of NAFLD, vitamin B_3 supplementation increased redox potential, reduced the hepatic cholesterol content, and blocked the gain in liver weight. In addition, vitamin B_3 exerted a protective effect on

the preexisting hepatic steatosis, suggesting that vitamin B_3 can be used not only as a preventative measure but also as a potential treatment for already established NAFLD.[29] Ganji et al further demonstrated that treating human hepatoblastoma cells with niacin reduced hepatic lipid accumulation by 40%–60%, and the mechanism is likely through the inhibition of diglyceride acyltransferase 2 and NADPH oxidase, which are essential enzymes in lipid metabolism.[30] Similarly, niacin treatment on dyslipidemic patients reduced the plasma triglyceride concentration, the hepatic fat content, and improved liver enzymes. An improvement in hepatic transaminase concentration was also observed in this study.[31] Conversely, a random controlled trial involving 27 obese individuals with NAFLD given niacin failed to demonstrate a decrease in hepatic fat deposition. However, this supplementation was able to reduce serum triglyceride and very low-density lipoprotein as well as improve insulin sensitivity.[32] Perhaps, niacin does not have an effect on NAFLD in the short term; therefore, it would be interesting to follow these patients over a longer period to observe whether there are any significant differences. Other interventional studies have demonstrated that the long-term usage of niacin supplement can lead to insulin resistance.[33] Given that one prevailing theory in the pathogenesis of NAFLD involves insulin resistance and that the patients with NAFLD already have reduced insulin sensitivity, niacin may have adverse side effects, despite reducing the hepatic fat content.

Vitamin B_{12} plays an important role in DNA synthesis and repair.[34] In humans, vitamin B_{12} exists in two forms: methyl cobalamin and 5′-deoxyadenosylcobalamine. It is a cofactor for the mitochondrial enzyme, namely methyl malonyl CoA mutase, which is known to regulate the rate of long-chain fatty acyl-CoA transfer into the mitochondria, thereby influencing lipid metabolic pathways.[34] One prevailing theory of NAFLD is the change of mitochondrial environment, which leads to the overproduction of ROS.[35] Liver is an important storage site of vitamin B_{12}, which has been linked to acute hepatitis, cirrhosis, and hepatocellular carcinoma. Studies have demonstrated the generation effect of B_{12} deficiency on the lipid metabolism in animal models.[36] It has been reported that low vitamin B_{12} in maternal diet induced higher rates of adiposity and T2DM in the offspring, which was accompanied by a change in hepatic gene expression involved in lipid metabolism pathways. Furthermore, the reconstitution of B_{12} to the offspring was able to normalize the alterations.[36,37] In a study by Koplay et al, it was also observed that low serum B_{12} in patients with NAFLD was related to an elevated serum alanine aminotransferase (ALT) concentration.[38] In addition, vitamin B_{12} restriction in weaning female rats increased the body fat mass percentage, decreased fat-free mass, and impaired offspring capacity to secrete insulin.[39] Furthermore, offspring born to mothers with B_{12} deficiency had the dysregulation of pathways involved in fatty acid metabolism, amino acid metabolism, and glycolysis. The mechanism is believed to be due to the

alterations of PPARγ and PPARα hepatic expression. It was further shown that the enzymes involved in β-oxidation were downregulated, affecting the lipolysis process.[39] Similarly, another study showed that offspring subjected to maternal vitamin B_{12} deficiency increased the plasma total cholesterol, and offspring born to dams fed B_{12} supplemented diet had standard plasma total cholesterol levels.[37] However, the protective role of vitamin B_{12} in NAFLD could be controversial. One human study attempted to examine the relationship between serum level of vitamin B_{12} and NAFLD. The patients with NAFLD were matched with control subjects; however, no difference was observed between the two groups.[40] This disparity could be due to the limited number of subjects in the study, which only involved 30 patients with NAFLD, and the results are based on one center alone.

Vitamin D. Vitamin D and its role in NAFLD have been widely studied. Vitamin D is a fat-soluble vitamin with the active form calcitriol (also known as 1α-25-dihydroxy vitamin D_3 [1,25(OH)$_2$D$_3$]). The assessment of the vitamin D status is often based on either the calcidiol (precursor of the active form) level or calcitriol level. The association between vitamin D and NAFLD was first confirmed by Targher et al, who observed that the low level of serum calcitriol is linked with the biopsy-proven NAFLD.[41] The relationship between NAFLD and vitamin D was further supported by Jablonski et al, who reported that the low serum level of calcitriol is linked with NAFLD diagnosed by ultrasound.[42] In a cohort of 1,081 adults, serum calcidiol concentrations were negatively associated with waist circumference, fasting insulin, homeostatic model assessment-insulin resistance (HOMA-IR), plasma triglyceride levels, and visceral abdominal fat.[43] In addition, individuals with elevated serum ALT had lower calcidiol than those with normal ALT.[44] However, it is debatable whether vitamin D deficiency is the precursor or the consequence of liver diseases. Patients with vitamin D deficiency, measured by serum level of calcitriol, were also shown to have a higher grade of hepatic necroinflammation, more advanced fibrosis stage, and more rapid fibrosis progression. Finally, the hepatic expression of vitamin D receptors, CYP2R1 and CYP27A1, have been negatively correlated with the severity of steatosis inflammation and NAFLD scores in patients.[45] Conflicting results were also observed in a randomized control trial involving 60 patients with ultrasound-diagnosed NAFLD. Half of the participants were subjected to oral vitamin D_3 supplements fortnightly for four months. It was observed that although the supplement improved the insulin resistance status and lowered the high-sensitive C-reactive protein (a marker for cardiovascular diseases), the supplement showed insignificant effect on the liver transaminase levels and expression of inflammatory markers for NAFLD (tumor necrosis factor-alpha [TNF-α] and tumor growth factor-beta 1) in comparison with the control patients, suggesting that vitamin D supplement does not ameliorate NAFLD.[46] However, it could be argued

that the sample size of this study is not sufficiently large, and the study period is not long enough for vitamin D supplement to effect.

Vitamin D might also modulate the hepatic status via the immune pathway. Calcitriol is synthesized by both innate and adaptive immune cells.[47] *In vitro* evidence has shown that monocytes isolated from normal human peripheral blood readily synthesize calcitriol when treated with lipopolysaccharides, which are released from the gram-negative bacterial cell wall.[48] Additionally, vitamin D inhibits monocytes activation and expression of TNF-α and interleukin-1 (IL-1), the key inflammatory markers of NAFLD-related liver injury. NAFLD progression following a high-fat diet has been shown to be exacerbated by vitamin D deficiency, through the activation of toll-like receptor 2 (TLR2), TLR4, and TLR9, leading to more severe levels of hepatic inflammation correlated with an increase in inflammatory markers and markers of oxidative stress.[49] TLRs are pattern recognition receptors involved in innate immunity, with nuclear factor kappa B (NF-κB) functioning as a *master switch* to upregulate inflammatory mediators. In mice, increased NF-κB activity is associated with high-fat diet and activation of KC. Although there is no literature directly linking vitamin D with KC, vitamin D has been suggested to activate TLR on alveolar macrophage in the context of tuberculosis infection, leading to protective effects on lung fibrosis.[50] Therefore, it is very likely that the resident macrophage in the liver behaves the same way in response to vitamin D. It has been suggested that NK cells, another important component of the liver innate immunity system, are linked with NAFLD and also affected by vitamin D levels. Vitamin D also appears to promote an anti-inflammatory status. In addition, vitamin D enhances the secretion of IL-10 and decreases the secretion of IL-2 from dendritic cells. Furthermore, it inhibits Th17 cell development, which has recently been implicated in the manifestation of NAFLD.[51] In a mouse model of vitamin D deficiency with CYP27/B1 gene knock out, a gene coding for the 1α-hydroxylase enzyme is responsible for the conversion of calcidiol to calcitriol. It was observed that the hepatic iNKT, a type of NKT cells with an invariant T-cell receptor cells, were decreased. There were fewer mature iNKT cells, and higher percentage of iNKT cells went through apoptosis in the vitamin D-deficient mice in comparison to the control mice.[52]

Vitamin E. There are eight natural forms of vitamin E: four tocopherols (α, β, γ, δ) and four tocotrienols (α, β, γ, δ), with α-tocopherol being the most abundant and potent in inhibiting lipid oxidation. Despite there are no standard protocols for the treatment of NAFLD, the prescription of vitamin E supplement to patients with NAFLD is a common practice. According to the US practice guideline for the management of NAFLD, vitamin E is recommended at a daily dose of 800 IU/day.[53] This dosage was shown to improve liver histology in nondiabetic adults with biopsy-proven NASH.[54]

Patients with NAFLD have increased oxidative stress. It is possible that antioxidant deficiency may lead to increased lipid peroxidation and cell death due to mitochondrial compromise. Vitamin E is an antioxidant which may act as scavengers of hydroxyl, peroxyl, and superoxide radicals and protect against plasma lipid and low-density lipoprotein peroxidation. Therefore, higher vitamin E intake might be able to counteract the increase in oxidative stress found in patients with NAFLD. In addition to the role of being antioxidant, some studies have also proposed that vitamin E improves the liver integrity by downregulating hepatic cluster of differentiation 36 protein, which is a type of membrane transporter, responsible for the uptake of fatty acids into the liver.[55] An animal model for NASH, involving rats fed with methionine-deficient diet with vitamin E enrichment, has reported a decreased level in lipid peroxidation, but liver histological features were not improved.[56] Moreover, chickens fed high-oxidant diet with the supplementation of vitamin E were able to normalize elevated hepatic transaminases levels.[57] Furthermore, the study involving the supplementation of vitamin E in combination with ursodeoxycholic acid, a drug for decreasing cholesterol absorption, gave long-term benefits and better tolerance.[58]

The landmark PIVENS study, a random controlled trial involving 247 adults with NAFLD, showed that vitamin E treatment comparing with pioglitazone and placebo over two years improved liver histology, reduced steatosis and inflammation, and decreased liver transaminases.[59,60] Similar results were observed from pediatric populations. In children, Vos et al reported that the insufficiency in vitamin E consumption is related to higher grade of hepatic steatosis.[61] Similarly, Nobili et al studied in children with NAFLD receiving vitamin E treatment led to an improvement in transaminases and liver histology.[62] In a pediatric population of NAFLD diagnosed by biopsy, there was no significant improvement in ALT from daily 800 IU vitamin E after 96 weeks, although there was an improvement in hepatocellular ballooning score and NAS.[63] A possible explanation is that the treatment period was not sufficiently long enough to have an impact on the ALT level. In a separate random controlled trial involving 44 overweight/obese children aged 14–17 years, supplement with α-tocopherol and ascorbic acid was randomly given to the participants daily for four months. It was observed that the liver transaminases were moderately improved in the treatment group in comparison with the controls, further suggesting that vitamin E may have a beneficial role in NAFLD.[64] However, there is yet any study on the effect of vitamin E on children with biopsy-proven NAFLD.

Several studies have also highlighted the drawbacks of vitamin E. One study has shown that the daily administration of vitamin E increased the risk of developing prostate cancer in healthy men.[65] There are also contentious studies linking high-dose vitamin E supplementation with increased mortality.[66] Therefore, the effectiveness and safety of using vitamin E as a form of treatment require further study.

Table 1. Summary of human studies relating different vitamins to NAFLD.

	AUTHOR	POPULATION	STUDY DESIGN/METHOD	RESULTS/OBSERVATIONS
A	Liu et al 2015	45 Chinese with NAFLD, 38 with NASH.	One centre, cross sectional study	– Serum RA concentration is lower in NAFLD/NASH – RXRα mRNA expression is inversely related with steatosis
B₃	Hu et al 2012	37 out of 46 Chinese age 18 to 85	Interventional study with Niaspan® daily for 23 weeks	– Liver fat reduction – Decreased average weight – No significant changes in total body fat and abdominal fat
B₉ (folate), B₁₂	Polyzos et al 2012	30 patients with biopsy proven NAFLD; 24 matching control	One centre cross sectional study	– No between-group difference in serum vitamin B_{12} and folate level – B_{12} level not correlated with insulin, HOMA-IR – Serum folate correlated with waist circumference, GGT and total cholesterol levels
B₁₂	Koplay et al 2011	40 patients with NAFLD 30 healthy controls	Cross sectional study	– Lower vitamin B_{12} in NAFLD patients
D	Sharifi et al 2014	53 patients with NAFLD	Double-blind, placebo controlled study for 4 months	– Amelioration of in serum hs-CRP in patients
	Jablonski et al 2013	607 patients with NAFLD; 607 matching control	Case-control study	– Patients with NAFLD have significantly decreased 25(OH)D levels – Low serum 25(OH)D associated with increased odds of NAFLD
	Seo et al 2013	1081 adults	Population based cohort study	Low vitamin D status is associated with NAFLD
E	Pietu et al 2012	101 adult patients with biopsy proven NAFLD, and elevated serum AST, ALT or GGT	Retrospective, interventional study	Vitamin E in combination with UDCA improves the liver function tests.
	Lavine et al 2011	173 children with biopsy proven NAFLD	Randomised, double-blind, double-dummy, placebo controlled clinical trial, 96 weeks	No significant differences between the groups
	Sanyal et al 2010	247 adults with NAFLD	Randomised, placebo controlled trial	– Histologic improvement – Reduction of hepatic steatosis – Reduced transaminases

Other vitamins. Vitamin C is an antioxidant; therefore, it may function in the same manner as vitamin E by reducing the levels of oxidative stress in NAFLD. There is a lack of literature on the role of vitamin C on NAFLD. Many studies involved the combination of vitamins E and C; thus, it is unclear whether any effects are due to the individual micronutrient or a combined effect. There is evidence that by supplementing vitamin C to rats with glucose intolerance induced by dexamethasone, the animals had improved insulin sensitivity.[67] A study involving 149 pediatric patients with NAFLD demonstrated that a decrease in serum vitamin C is related to increased hepatic ballooning.[61] In contrary, a cross-sectional study demonstrated no difference between serum vitamin C level among control, NAFLD, and NASH subjects.[68]

Literature on the role of vitamin K in the NAFLD is very limited; so far there is little evidence that vitamin K is involved in lipid metabolism. One study has reported the storage of vitamin K in the adipocytes, and there appeared to be a positive relationship between adult obesity and vitamin K concentration in the adipocytes.[69] However, studies are lacking in how and whether this micronutrient has a role in NAFLD at all.

Conclusion

Although this review is focused on the effect and possible mechanisms of action on NAFLD by each different vitamins, it is also important to highlight that there can be interactions between different vitamins, as well as between vitamins and micro/macronutrients intake. For example, it has been recently demonstrated that supplementation with vitamin A can compensate vitamin D deficiency.[70] Furthermore, other studies focused on cancer have also shown the relation between these two vitamins,[71] and it has been also described that the high levels of vitamin A can antagonize the effects of vitamin D.[72] On the other hand, the interaction between vitamins and micronutrients intake can affect obesity-associated features such as disturbances in lipid profile, inflammatory status, and insulin resistance, all of the key factors in the development of NAFLD.[73] Therefore, vitamins and micronutrient mixes have

been also described as potential tools against the development of diet-induced NAFLD.[11]

In conclusion, NAFLD is a complex chronic liver condition, with a strong association with obesity, and multiple factors influencing its pathogenesis. The underlying mechanism involves the modification and interaction of innate immunity and microbiota composition, among other factors. Although the role of dietary macronutrients in NAFLD pathogenesis is now better characterized, micronutrients such as vitamins may also play a key role. The current evidence appears to suggest a positive role of vitamins A, B_3, B_{12}, D, and E as therapeutic targets (Table 1), but some of them may also be linked with several adverse outcomes. Further, well-designed studies are therefore required to better define the potential role of these micronutrients in the development and treatment of NAFLD.

Author Contributions

Wrote the first draft of the manuscript: JL, PC. Contributed to the writing of the manuscript: JL, PC, VN, JO. Agree with manuscript results and conclusions: JL, PC, VN, JO. Jointly developed the structure and arguments for the paper: JL, PC, VN, JO. Made critical revisions and approved final version: JL, PC, JO. All authors reviewed and approved of the final manuscript.

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