

REVIEW

Micronutrients in Nonalcoholic Fatty Liver Disease Pathogenesis



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SUMMARY

We review the contribution of micronutrients to nonalcoholic fatty liver disease pathology and therapeutics. We examine the relevant data on minerals, vitamins, and carotenoids in nonalcoholic fatty liver disease experimental and human studies and offer insight into their use (or nonuse) therapeutically.

Micronutrients include electrolytes, minerals, vitamins, and carotenoids, and are required in microgram or milligram quantities for cellular metabolism. The liver plays an important role in micronutrient metabolism and this metabolism often is altered in chronic liver diseases. Here, we review how the liver contributes to micronutrient metabolism; how impaired micronutrient metabolism may be involved in the pathogenesis of nonalcoholic fatty liver disease (NAFLD), a systemic disorder of energy, glucose, and lipid homeostasis; and how insights gained from micronutrient biology have informed NAFLD therapeutics. Finally, we highlight some of the challenges and opportunities that remain with investigating the contribution of micronutrients to NAFLD pathology and suggest strategies to incorporate our understanding into the care of NAFLD patients. (*Cell Mol Gastroenterol Hepatol* 2018;6:451-462; <https://doi.org/10.1016/j.jcmgh.2018.07.004>)

Keywords: NAFLD; Obesity; Micronutrients; Vitamins; minerals; carotenoids.

Nonalcoholic fatty liver disease (NAFLD) is a systemic disorder of energy, glucose, and lipid homeostasis with hepatic manifestations. Patients with NAFLD have perturbations of central signals involved in satiety and preference that result in excess consumption of largely obesogenic macronutrients (reviewed by Carr et al¹), as well as deranged energy balance that may result in part from polymorphisms in metabolic regulatory genes.²⁻⁴ The constellation of this dysregulation of energy balance ultimately results in the accumulation of lipids within hepatocellular lipid droplets, the onset of which predisposes patients to nonalcoholic steatohepatitis (NASH), NASH fibrosis, and cirrhosis. NAFLD differentially impacts racial/ethnic groups (with the highest prevalence observed in Hispanics⁵), and the burden of NAFLD parallels that of obesity (defined as having a body mass index [BMI] ≥ 30). Indeed, both NAFLD and obesity prevalence are estimated to affect at least 40% of US adults.^{6,7} Although nonobese patients can develop NAFLD,⁸⁻¹⁰ and, conversely, some

obese patients appear to be protected from NAFLD based on currently defined cut-off values for intrahepatic triglyceride levels,^{11,12} most data support an intersection between NAFLD and obesity pathogenesis. Specifically, the majority of patients with NAFLD and obesity have a relative deficit in energy expenditure and disturbances in lipid and glucose homeostasis (ie, insulin resistance) resulting from excess macronutrient intake. In NAFLD, some of these macronutrients (eg, sucrose and fructose) cause direct injury to the small intestinal wall epithelium.¹³ Such epithelial injury leads to translocation of bacterial products that increase portal plasma lipopolysaccharide concentrations.¹⁴ The resultant hepatic Toll-like receptor 4 activation leads to tumor necrosis factor- α up-regulation,^{15,16} steatosis, and inflammation.^{17,18} Coupled with the onset of an insulin-resistant state (including the dysregulation of the glucose homeostatic adipokines, adiponectin and leptin),¹⁹ these factors are considered key components of NAFLD pathogenesis.

Although the pathogenic role of macronutrients is well established in both NAFLD and obesity, the contribution of micronutrients to NAFLD pathogenesis has garnered less attention than with obesity.²⁰ Nevertheless, micronutrients in NAFLD play an important role. This review uses insights gained from obesity to explore the mechanisms by which micronutrients contribute to NAFLD pathogenesis and establish the basis for the therapeutic targeting of micronutrients in NAFLD patients.

Micronutrient Definition

Micronutrients are defined as nutrients that are needed in only microgram or milligram quantities for physiologic functions²¹ as defined by the World Health Organization.²² Micronutrients include electrolytes, minerals, vitamins, and carotenoids, and are required for enzymatic activity, intermediary metabolism, and metabolic response to illness.²³

Electrolytes (ie, sodium, chloride, and potassium) and minerals (eg, calcium, phosphorus, zinc, and so forth) are inorganic compounds required for tissue structure, pH regulation, neuronal signaling, muscle contraction, and

Abbreviations used in this paper: ALT, alanine aminotransferase; BMI, body mass index; FXR, farnesoid X receptor; IL, interleukin; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; ROS, reactive oxygen species; TGF, transforming growth factor.



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enzymatic activities. Electrolytes also can be associated with minerals to confer charge. Although the role of electrolyte homeostasis is not well established in NAFLD (with the exception of an epidemiologic association between high-sodium diets and NAFLD prevalence),²⁴ the contribution of several minerals, vitamins, and carotenoids to the pathogenesis of NAFLD increasingly is being appreciated.²⁵

Minerals are inorganic compounds that share many of the basic functions of electrolytes. Minerals form salts with other elements or bind to organic compounds while maintaining their own chemical identity. Minerals are classified into major or trace minerals depending on their tissue concentration of greater than or less than 5 g, respectively. Examples of major minerals include calcium, phosphorus, and magnesium; and some minor minerals include zinc, copper, iron, and iodine.²⁶

Vitamins are organic compounds that regulate cellular growth and metabolism, and their solubility into either lipids or water determines the mechanisms by which they are absorbed, transported, stored, and excreted. After small intestinal absorption, fat-soluble vitamins (vitamins A, D, E, and K) are transported through the lymphatic system via chylomicrons and stored in liver and adipose tissues, whereas water-soluble vitamins (thiamin, riboflavin, niacin, vitamin B₆, folate, vitamin B₁₂, pantothenic acid, biotin, and vitamin C) enter the bloodstream, existing only in trace amounts in tissues before being excreted in urine. These differences in tissue reserves between fat- and water-soluble vitamins translate to differences in daily consumption requirements to maintain physiologic levels. Namely, daily intake of fat-soluble vitamins is not required whereas daily consumption of water-soluble vitamins is essential.²⁶

Carotenoids are a large class of phytochemicals with anti-oxidant and anti-inflammatory activity.^{27,28} This class includes carotenes (eg, α -carotene, β -carotene, and lycopene) and xanthophylls (eg, lutein, zeaxanthin, β -cryptoxanthin, and astaxanthin). They largely are found in fruits and vegetables, but also are found in smaller concentrations in poultry.²⁹ Because several carotenoids can be converted to vitamin A, carotenoids have additional roles in cellular development, growth, and differentiation.³⁰ The majority of this conversion occurs in the intestine and is impaired in obese individuals,³¹ thus linking carotenoid metabolism indirectly with NAFLD pathogenesis.

The Liver and Micronutrient Metabolism

The liver plays a critical role in micronutrient metabolism. The liver is involved in the transport and storage of many micronutrients such as vitamin A, vitamin B₁₂, and copper. In fact, the majority of the body's vitamin A stores are found in the stellate cells of the liver.³²⁻³⁴ In addition, as the body's major site of protein synthesis, the liver produces binding, transport, and regulatory proteins that are required for micronutrient homeostasis. For example, zinc and other micronutrients are found bound to albumin.³⁵ Thus, zinc deficiency is linked directly to hypoalbuminemia, which occurs as a result of hepatic synthetic dysfunction. Furthermore, the protein metallothionein is in part

synthesized in the liver and functions as a metal chelator and transport protein.³⁶ The liver also synthesizes bile (a process regulated in part through the bile acid nuclear receptor farnesoid X receptor FXR).³⁷ Bile is needed for fat emulsification and absorption of fat-soluble vitamins, and, in turn, fat-soluble vitamins regulate their own absorption through regulating hepatic bile acid synthesis³⁸ (Figure 1).

Micronutrients in NAFLD

The aforementioned derangements in energy and nutrient homeostasis in NAFLD patients are sufficient to cause hepatic steatosis. However, the specific factors that promote progression from steatosis to advanced stages of NAFLD (ie, NASH, NASH fibrosis, and cirrhosis) remain unknown. Emerging data suggest that the hepatocellular accumulation of bioactive lipids causes hepatocellular lipotoxicity and oxidative stress through the accumulation of reactive oxygen species (ROS).³⁹ The ensuing inflammatory and fibrogenic responses from nonparenchymal cells exacerbate liver injury and impair hepatic function.⁴⁰ It is perhaps at this junction between the noninflammatory steatotic state and onset of the inflammatory cascade where the role of micronutrients in NAFLD is implicated.

The specific micronutrients relevant for NAFLD pathogenesis can be ascertained from what is known in the context of obesity because 60% of obese patients also have NAFLD.¹⁰ Indeed, bariatric surgery observational studies have shown zinc, iron, calcium, and vitamins A, B, D, and E deficiencies in morbidly obese patients awaiting bariatric surgery,⁴¹⁻⁴³ several of which are deficient in NAFLD patients as well^{44,45} (Table 1). Similarly, shared pathophysiologic pathways between insulin resistance and NAFLD can provide insight into the role of micronutrients in NAFLD. For example, observational and in vivo studies have linked adiponectin deficiency with obesity, insulin resistance, and NAFLD⁴⁶; and adiponectin modulation is impacted by micronutrient availability.^{47,48}

Zinc and NAFLD

Despite data suggesting mineral deficiencies in NAFLD patients, most data do not support insufficient mineral consumption as a possible mechanism for these deficiencies, except in the case of zinc deficiency.⁴⁹⁻⁵¹ Other than inadequate intake, the mechanism of zinc deficiency in NAFLD patients is unknown. In an obesogenic mouse model using sucrose feeding, mice fed a normal diet supplemented with sucrose have lower serum zinc levels than mice fed a regular diet. Compared with sucrose-fed mice, zinc-supplemented sucrose-fed mice have higher levels of the satiety and energy homeostatic adipokine leptin, despite similar body weight and fat mass.⁵² Compared with lean C57BL/6J mice, obese leptin-deficient ob/ob mice have higher plasma zinc levels but lower hepatic zinc content.⁵³ Together, these studies suggest an inverse relationship between hepatic zinc content and plasma zinc levels, and also a mechanistic (albeit ill-defined) link between zinc and leptin. Clues to this relationship may exist by understanding how the ratio of free to bound zinc relates to both leptin and

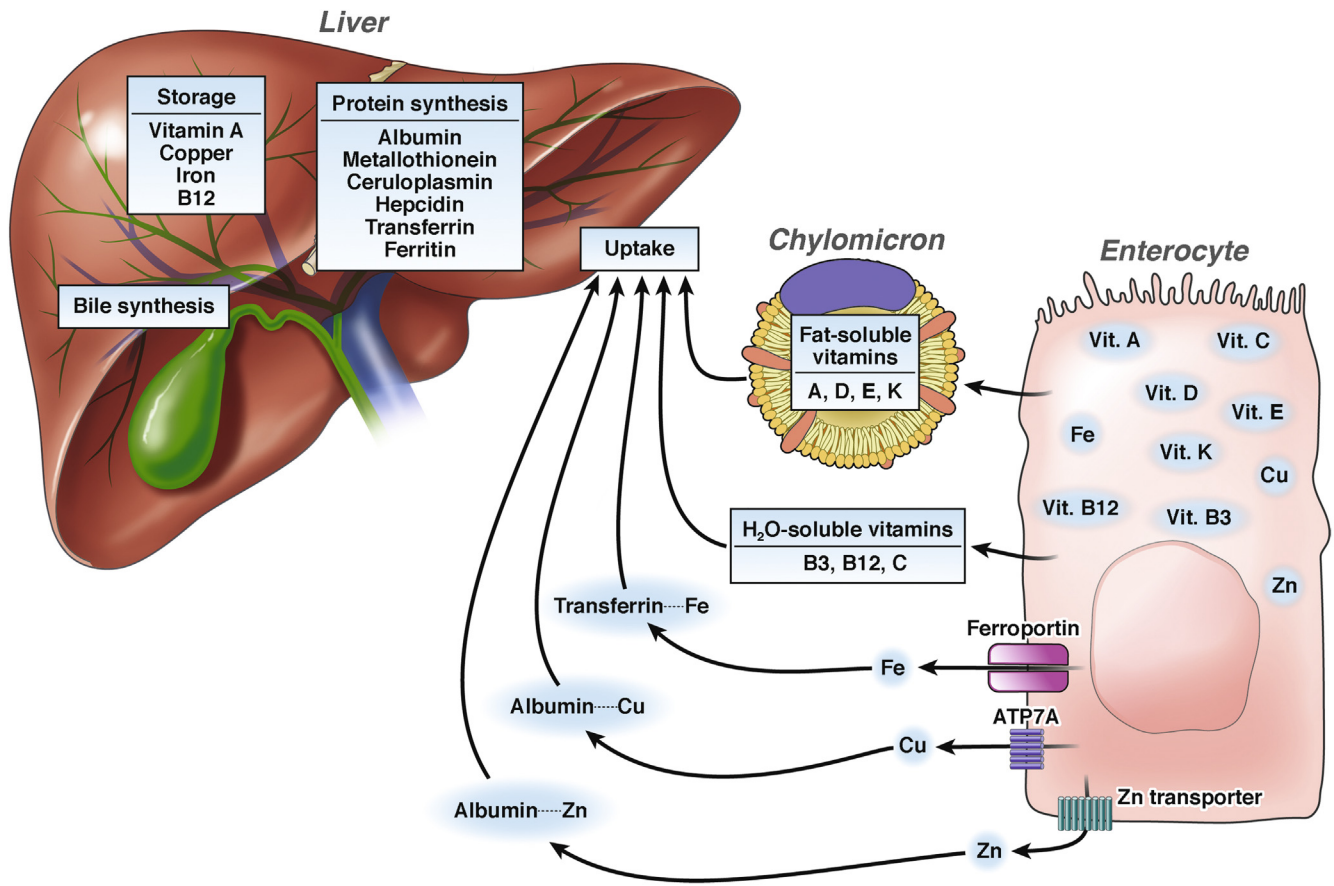


Figure 1. Micronutrients and the liver in NAFLD. Hepatic contribution to metabolism of the micronutrients involved in NAFLD pathogenesis. Vit., vitamin.

hepatic zinc levels and in further dissecting zinc’s known glucose lowering⁵⁴ and hepatic glucose regulatory effects.⁵⁵

Zinc deficiency may augment oxidative stress in NAFLD as seen in a rodent model of experimental alcoholic liver disease, a related condition of hepatic lipid dysregulation.⁵⁶ In this model, zinc reduces hepatic triglyceride accumulation and oxidative stress through enhanced very low density lipoprotein secretion and peroxisome proliferator activated receptor- α and hepatocyte nuclear factor-4 α -mediated

augmentation of fatty acid oxidation.⁵⁷ Such effects of zinc on lipotoxicity-induced oxidative stress are suggested further by the 20% reduction in malondialdehyde observed in high-fat diet-fed Sprague-Dawley rats co-supplemented with zinc and selenium.⁵⁸ In addition to the putative effects on oxidative stress, zinc deficiency also may exacerbate NAFLD fibrosis and cirrhosis. Cirrhosis can result in impaired ammonia clearance, a condition remedied by zinc supplementation in a carbon tetrachloride rat model of fibrosis.⁵⁹

Table 1. Putative Hepatocellular Mechanisms of Micronutrients in NAFLD

Micronutrient	Serum levels in NAFLD	Possible mechanism	NAFLD therapeutic	Referenced studies
Zinc	↓	A, L, F	X	56–59
Copper	↓	A, L	X	64, 66
Iron	↑	L, I	Phlebotomy ^{59,82,83}	70, 79, 80
Vitamin A	↓	L	X	85–87
Vitamin D	↓↑	A, L, F, I	X	106, 107
Vitamin E	↓	A, L, F	800 IU vitamin E daily ^{129,130}	110–116
Carotenoids	↓	A, L, F, I	X	122, 123, 125, 126

NOTE. Putative mechanisms of micronutrients in NAFLD pathogenesis and attempts at targeting micronutrients therapeutically in NAFLD. A, antioxidant; F, antifibrotic; I, immune effects; L, lipoprotective; X, no therapeutics exist.

Copper and NAFLD

The liver has a critical role in copper metabolism, including in the production of the copper transport protein ceruloplasmin. In Sprague–Dawley and leptin-receptor-deficient Zucker (fa/fa) rats fed a copper-depleted, copper-enriched, or normal diet for 8 weeks, copper deficiency caused severe hepatic steatosis and increased liver weight.⁶⁰ Serum ceruloplasmin levels mirror copper patterns^{60,61} and low ceruloplasmin levels are associated with advanced liver disease in children⁶² and adults.⁶⁰

Copper exerts a myriad of effects in the liver including on anti-oxidant and cellular respiratory systems.⁶³ In the liver, copper is a co-factor for several anti-oxidant enzymes.^{64,65} For example, in isolated Sprague–Dawley rat livers, copper deficiency causes oxidative stress and a presumed counter-regulatory transcriptional up-regulation of the anti-oxidant enzyme superoxide dismutase.⁶⁴ Furthermore, copper deficiency in Sprague–Dawley weanling rats promotes hepatic fatty acid synthesis and assembly into triacylglycerols and phospholipids.⁶⁶ Consequently, copper deficiency in NAFLD patients may exacerbate oxidative stress and lipotoxicity from both impaired mitochondrial function and up-regulation of triglyceride synthetic pathways. In NAFLD patients, serum and/or hepatic copper levels are up to 50% lower than in control patients or those with other liver diseases. Low hepatic copper is associated with more advanced liver disease, systemic metabolic disease, and diabetic status.⁶⁰ These studies must be considered in the context of both macronutrient (ie, fructose) inhibitory effects on copper absorption⁶⁷ and challenges in measuring hepatic copper in the steatotic liver.⁶⁸

Iron and NAFLD

The earlier-mentioned minerals have been implicated in NAFLD pathogenesis largely owing to serum and/or hepatic deficiencies, however, the contribution of iron to NAFLD is most widely accepted to be owing to iron excess.^{69,70} Excess iron correlates with hepatic lipid peroxidation⁷⁰ and NAFLD severity.^{70–72} The iron regulatory protein hepcidin is a hormone produced by the liver that regulates iron absorption from enterocytes by causing internalization of the basal membrane iron transporter ferroportin. Ferroportin is expressed in multiple tissues but the enterocyte ferroportin is considered the major driver of iron hemostasis.⁷³ Hepcidin, ferroportin, ferritin (the storage form of iron), and iron itself all have been associated with hepatic injury in NAFLD. In patients with NAFLD, genetic mutations and polymorphisms affecting hepcidin's regulation of ferroportin-mediated iron absorption may result in either increased hepatic iron content (in the case of genetic hemochromatosis)⁷⁴ or reduced hepatic iron content (in the case of the VV genotype of the transmembrane protease serine-6 gene).⁷⁵ Based on a large study involving patients who participated in studies of the NASH Clinical Research Network, hyperferritinemia is an independent predictor of NASH fibrosis.⁷² These results are consistent with data showing an association between hepatic iron content and NAFLD severity, albeit the specific hepatic iron depot is

debated.^{76,77} Excess hepatic iron in the liver may impair hepatic lipid homeostatic and immune functions. In leptin-resistant db/db mice fed an iron-rich diet, hepatocellular ballooning (the signature histologic feature of NASH diagnosis⁷⁸) was observed in 85% of the mice. In addition, iron-fed mice have increased levels of malonyldialdehyde, inflammasome and immune cell markers, and inflammatory cytokines.⁷⁹ These effects of iron on nonparenchymal cells are supported further by data by Maliken et al⁸⁰ showing an association between increased apoptosis and iron accumulation in hepatic reticuloendothelial cells in NAFLD patients. Whether iron is involved directly in the cascade of events that cause NAFLD progression remains unclear because results of phlebotomy trials in NAFLD patients are equivocal.^{81–84}

Vitamin A and NAFLD

Compared with both nondiabetic and diabetic non-NAFLD patients, patients with NAFLD have lower serum levels of retinoic acid, a metabolite of vitamin A. Retinoic acid deficiency worsens with progressive NAFLD, and the serum level of retinoic acid negatively correlates with both intrahepatic triglyceride content and transaminase levels.⁴⁵ This relationship between retinoic acid and hepatic triglyceride content suggests that vitamin A (most of which is stored in the liver) has a role in overall hepatic lipid metabolism, not only in stellate cell function. Indeed, retinoic acid binds the retinoid X receptor, a nuclear hormone receptor that heterodimerizes with peroxisome proliferator activated receptor- α , a key hepatic fatty acid β -oxidative regulator, and in so doing regulates hepatic lipid metabolism.^{85,86} In support of this link between retinoic acid deficiency and impaired hepatic lipid metabolism, transgenic mice that lack hepatic expression of retinoic acid develop steatohepatitis predominantly owing to defects in mitochondrial β -oxidation, effects that can be reversed by a high-retinoic-acid diet.⁸⁷

B Vitamins and NAFLD

There are 8 B vitamins, but only vitamins B₃ (niacin) and B₁₂ have been examined in NAFLD patients. Vitamin B₃ has roles in cellular lipid biology,⁸⁸ while B₁₂ has roles in DNA synthesis and modification and mitochondrial metabolism.⁸⁹ It is likely that initial investigations of vitamin B₃ in NAFLD stemmed from the known protective role of niacin supplementation in cardiovascular health⁸⁸ and cardiovascular oxidative stress.⁹⁰ In Sprague–Dawley rats fed a high-fat diet to induce NAFLD, addition of niacin after NAFLD induction significantly reduced both hepatic and serum triglyceride levels, ameliorated hepatic steatosis, and reduced hepatic lipid peroxidation as measured by thiobarbituric acid–reactive substances.⁹¹ Because this was not a model of steatohepatitis, the lack of a change in inflammatory markers by niacin is of unclear significance. The anti-inflammatory action of niacin has been established in vitro with palmitate-incubated Hep G2 cells and primary human hepatocytes. Co-incubation with niacin not only reduces lipid accumulation owing to down-regulation of the

triglyceride synthetic enzyme diacylglycerol acyltransferase 2, but also reduces ROS production, reduced nicotinamide adenine dinucleotide phosphate oxidase activity (a ROS enzyme), and interleukin (IL)8 inflammatory cytokine levels.⁹² These *in vitro* results are consistent with human data showing a diacylglycerol acyltransferase 2 polymorphism dose-dependent effect of niacin supplementation on hepatic fat, plasma triglycerides, and body weight reduction. Specifically, patients with the T allele experience an approximately 60% reduction in hepatic fat while those with 2 copies of the variant C allele have only a 25% reduction in hepatic fat.⁹³ Unlike NAFLD-relevant studies that show reduced B₃ levels, studies with vitamin B₁₂ are circumstantial at best because serum B₁₂ levels in NAFLD patients are either unchanged⁹⁴ or modestly reduced.⁹⁵

Vitamin C and NAFLD

Vitamin C and other anti-oxidants balance the effects of ROS in cells by scavenging free radicals. In NAFLD, this scavenging mechanism may protect cells from lipotoxicity-induced cellular oxidative stress. Low vitamin C levels are associated modestly with biopsy-proven NASH in children,⁹⁶ but in adults there appears to be no association.^{51,97} Besides attempts to use vitamin C therapeutically in NAFLD,⁹⁸ there are no experimental studies in NAFLD directed toward understanding how vitamin C deficiency promotes hepatic injury in NAFLD.

Vitamin D and NAFLD

Vitamin D intake is estimated to be 50% lower in NAFLD patients than in non-NAFLD patients,⁴⁹ and low vitamin D levels are associated with incident liver disease risk (some of whom develop NAFLD).⁹⁹ Levels of vitamin D have been associated inconsistently with NAFLD, NAFLD severity, and insulin resistance,^{25,100–105} a condition commonly shared by both obese and NAFLD patients. In addition, several genes involved in vitamin D metabolism have shown no differential regulation in NAFLD patients compared with controls.¹⁰² Nevertheless, the putative effects of vitamin D on hepatic biology are provocative. Namely, vitamin D deficiency exacerbates experimental NASH. Sprague–Dawley rats fed a Western diet deficient in vitamin D have pronounced steatohepatitis compared with dietary controls. In addition, pathways involved in oxidative stress (eg, heme oxygenase) and inflammation (eg, IL6, IL4, and IL1 β) by way of Toll-like receptor signaling are up-regulated in the absence of vitamin D,¹⁰⁶ thus linking vitamin D metabolism with both NAFLD lipotoxicity and the liver's pathogenic response to gut translocation of microbial products.

Vitamin D also reduces secretion of fibrogenic growth factors (including transforming growth factor [TGF] β and α -smooth muscle actin) from primary human hepatic stellate cells. The antifibrotic effect of vitamin D is dependent on both the genotype of the vitamin D receptor and vitamin D receptor levels because vitamin D fails to inhibit the TGF β -induced fibrogenic program in vitamin D-receptor-deficient stellate cells.¹⁰⁷ It is perhaps this effect of vitamin D-receptor polymorphisms that partially explains the

inconsistent results regarding the relationship of vitamin D levels to NAFLD severity.

Vitamin E and NAFLD

Plasma levels of vitamin E are reduced in patients with biopsy-proven NASH compared with healthy patients.¹⁰⁸ There is also a trend toward lower hepatic vitamin E levels in NAFLD patients compared with control patients.¹⁰⁹ In experimental models, vitamin E has antisteatotic, anti-inflammatory, and antifibrotic effects. Effects on steatosis may in part be owing to vitamin E's inhibitory effect on hepatocyte fatty acid uptake. Namely, in guinea pigs fed an obesogenic diet, vitamin E prevents the up-regulation of the hepatic fatty acid receptor CD36.¹¹⁰ The reduced hepatocellular uptake of circulating lipids reduces the intracellular pool of lipids available for lipid peroxidation. By using mice fed a methionine-choline-deficient diet (a steatohepatitis experimental model), Nan et al¹¹¹ showed that the reduction in liver enzyme levels, steatosis, and inflammation was associated with down-regulation of genes involved in lipid peroxidation and inflammation. Although not measured directly in their study, it is likely that this down-regulation lead to reduced concentrations of hepatic malonyldialdehyde (a product of phospholipid peroxidation), as shown in high-fat diet-fed Sprague–Dawley rats¹¹² and high-carbohydrate diet-fed ob/ob mice¹¹³ supplemented with vitamin E.

In addition to protective effects on lipid peroxidation, vitamin E has antifibrotic effects.

In a model of advanced fibrosis (albeit not a NAFLD model), rats subjected to intraperitoneal carbon tetrachloride injections had reduced lipid peroxidation, inflammation, and fibrosis when co-administered vitamin E.¹¹⁴ Congruent with those findings was the observation that vitamin E down-regulates Col1A2 expression in cultured rat hepatic stellate cells that overexpress Cyp2E1.¹¹⁵ These beneficial effects of vitamin E on remodeling are partially through the down-regulation of hepatic TGF β 1 and pro-collagen genes.¹¹⁶ This constellation of hepatoprotective effects of vitamin E established the basis for its therapeutic use in NASH.¹¹⁷

Carotenoids and NAFLD

Plasma levels of several carotene and xanthophyll carotenoids are lower in biopsy-proven NASH patients than in controls independent of BMI and liver enzyme levels.¹⁰⁸ In addition, not only are serum carotenoid levels associated inversely with NAFLD prevalence¹¹⁸ and liver enzyme levels,^{119,120} but also a large prospective study of 2687 NAFLD subjects showed that serum carotenoid levels are associated with NAFLD improvement,¹²¹ thus positioning carotenoids as potential therapeutic targets.

The potential of carotenoids as NAFLD targets is supported by evidence in high-fat diet-fed rats in whom β -carotene supplementation reduced liver weight and liver enzyme levels.¹²² β -carotene also reduced lipid peroxidation as measured by thiobarbituric acid-reactive substances and reduced oxidative stress by increasing levels of the anti-

oxidant superoxide dismutase.¹²² In addition, in a mouse model of diet-induced NASH, β -cryptoxanthin supplementation reduced hepatic steatosis, steatohepatitis, lipid peroxidation, and fibrosis, likely owing to the down-regulation of lipid synthetic genes established by DNA microarray analysis.¹²³ In fact, β -cryptoxanthin supplementation modulates the expression of more than 500 genes, including the down-regulation of genes involved in immune cell trafficking and tumor necrosis factor- α signalling,¹²³ the latter of which is a key mediator of NASH.^{15,16} In a follow-up study by the same investigators,¹²⁴ β -cryptoxanthin was found to accumulate predominantly in the liver and reduce steatosis, steatohepatitis, and fibrosis via down-regulation of lipogenic and fibrogenic genes, enhanced lipolysis, and reduced infiltration and activation of Kupffer cells. Furthermore, β -cryptoxanthin supplementation ameliorated NASH progression and improved glucose tolerance and insulin resistance.¹²⁴

Similar to β -cryptoxanthin, astaxanthin exerts anti-steatotic effects on the liver in both genetic and diet-induced mouse models of NAFLD.¹²⁵ In female ddY mice fed a high-fat diet, astaxanthin reduced liver triglyceride levels and liver weight in a dose-dependent manner. These changes in part were owing to increased fat utilization as shown by a lower respiratory exchange ratio in the astaxanthin-supplemented mice as compared with control mice.¹²⁶ In a cholate-rich dietary model of NASH, astaxanthin reduced lipid peroxidation and expression of lipogenic genes and improved glucose tolerance and insulin sensitivity.¹²⁵

Micronutrients as Therapeutic Targets

Micronutrient biology only modestly has informed NAFLD therapeutics. For example, carotenoid supplementation has not been trialed in NAFLD patients despite some evidence of alanine aminotransferase (ALT) improvement in runners supplemented with the carotenoid-containing pequi fruit pulp oil.¹²⁷ In addition, the majority of NASH patients fail to normalize vitamin D₃ levels or liver histology in response to 6 months of a daily dose of 2000 IU of vitamin D₃ supplementation.¹²⁸ Perhaps more promising is the combination of daily 1000 mg vitamin C and vitamin E 1000 IU for 6 months, which modestly improved fibrosis scores in biopsy-proven NAFLD patients. Unfortunately, these results are tempered by the statistical weighting toward diabetic patients with fibrosis in the intervention group.⁹⁸ Finally, to mitigate iron excess, phlebotomy has been used as a potential therapeutic strategy, but these small studies have shown either no⁸⁴ or only mild benefit.⁸¹⁻⁸³

In contrast to the aforementioned micronutrient therapeutic attempts, vitamin E supplementation is a successful example of how micronutrients can be used to modulate NAFLD. The Pioglitazone versus Vitamin E versus Placebo for the Treatment of Nondiabetic Patients with Non-alcoholic Steatohepatitis trial included 247 adult patients with biopsy-proven NASH who were randomized to receive 800 IU vitamin E, 40 mg pioglitazone, or placebo daily for 96 weeks. The primary end point was end-of-study histologic improvement. Compared with placebo, vitamin E supplementation improved liver enzyme levels and body

weight. Pioglitazone versus Vitamin E versus Placebo for the Treatment of Nondiabetic Patients with Non-alcoholic Steatohepatitis additionally established that vitamin E improved NASH steatosis and/or lobular inflammation in 43% of patients compared with 19% of patients who received placebo. Despite the proposed antifibrotic mode of action of vitamin E, there was no benefit of vitamin E supplementation in hepatic fibrosis reduction in adults.¹²⁹ In children, 800 IU vitamin E daily similarly improved NASH histology.¹³⁰ The treatment of nonalcoholic fatty liver disease in children (TONIC) trial was a study of 173 children with biopsy-proven NAFLD who were randomized to receive 800 IU vitamin E, 1000 mg metformin, or placebo daily for 96 weeks. The primary outcome was a sustained reduction of ALT (a largely inconsistent end point in NAFLD patients¹³¹). Fifty-eight percent of patients achieved NASH histologic resolution owing to improvement in inflammation scores. As seen with adults, there was no antifibrotic effect of vitamin E in children.¹³⁰ Vitamin E supplementation now is approved for the clinical management of adult patients with NASH,¹³¹ but not for children because the TONIC study failed to meet its primary end point of ALT reduction.¹³⁰

Conclusions

Although excessive macronutrient intake contributes to both tissue injury and perturbations of energy homeostasis in NAFLD patients, micronutrients (either their deficiency or excess) may compound these insults by deregulating lipid homeostatic and anti-oxidant pathways. Understanding the role of micronutrients in NAFLD even may help improve our understanding of nonobese NAFLD. Dissecting the specific contributions of micronutrients, however, remains challenging because human diets are complex and fail to replicate experimental dietary models. In addition, serum levels of micronutrients reflect an intricate *in vivo* physiology that involves multiple organs, hormonal signals, and varied volumes of distribution. Consequently, serum laboratory values are only surrogate markers of hepatic micronutrient exposure. Nevertheless, the interaction of these nutrients with the hepatic intracellular and extracellular environments ultimately may prove critical to our understanding of how NAFLD patients advance from steatosis to steatohepatitis and fibrosis.

Reliable noninvasive biomarkers to establish NAFLD stage are elusive^{131,132} and, here too, micronutrients have a niche. For example, levels of ferritin and ceruloplasmin help prognosticate NAFLD disease severity,^{60,62,72} while vitamin A and D deficiencies suggest advanced liver disease in cirrhotic patients.¹³³ We even eventually may uncover that circulating levels of these and other fat-soluble vitamins predict the efficacy of novel NAFLD therapies that target bile acid signaling, as in the case of FXR agonists.^{37,134,135} Could some of the metabolic benefit derived from FXR agonism result from alterations in micronutrient absorption and secondarily hepatic uptake? These and other questions represent new areas of investigation in micronutrient NAFLD pathogenesis.

The dearth of data related to micronutrients in NAFLD patients as compared with our current understanding of

how macronutrients promote disease means that routine serum and hepatic measurement of micronutrients cannot be recommended (yet). Neither can hepatologists, gastroenterologists, and other providers recommend diets with specific micronutrient compositions. Still, we remain optimistic and predict that the current armamentarium of anti-obesity modalities¹³⁶ will serve as a window for the future of NAFLD nutritional management. For now, it is our view that the best advice for NAFLD patients is to consume a nutritionally balanced but relatively energy-restricted diet that avoids exposure to processed foods and fructose-containing beverages,¹³² akin to a sodium-reduced version of a Mediterranean-style diet, which is high in fiber, rich in anti-oxidants, and improves BMI, insulin resistance, and hepatic steatosis.^{132,137–139}

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Octavia Pickett-Blakely, Kimberly Young, and Rotonya M. Carr reviewed the literature, drafted the manuscript, and critically revised the manuscript; and Rotonya M. Carr provided funding support.

Conflicts of interest

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