

Carotenoids and non-alcoholic fatty liver disease

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Abstract: Non-alcoholic fatty liver disease (NAFLD) is a growing health problem around the world, especially in developed countries. NAFLD includes all cases of fatty liver disease from simple steatosis to cirrhosis, without excessive alcohol intake, use of steatogenic medication or hereditary disorders. Pathogenesis is associated with dietary high fat intake, decreased free fatty acid (FFA) oxidation, increased hepatic lipogenesis and lipolysis from the adipose tissue. These metabolic alterations contribute to the hepatic fat accumulation. Consequently, stimulated oxidative stress and inflammation play a major role in hepatocellular damage. Therefore, antioxidant and anti-inflammatory agents may have a role in the prevention of this disease. Carotenoids are potent antioxidant and anti-inflammatory micronutrients, which have been investigated in the prevention and treatment of NAFLD. The main sources of the carotenoids are fruits and vegetables. In this article we review the potential role and possible molecular mechanism of carotenoids in NAFLD.

Keywords: Fatty liver; steatosis; non-alcoholic steatohepatitis; carotenoid; oxidative stress; antioxidant

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Introduction

The results of recent investigations suggest that dietary interventions may modulate the expression of some genes that may affect many chronic diseases such as cancer, cardiovascular disease, diabetes, hypertension and neurodegenerative diseases. Carotenoids are among the most investigated dietary micronutrients in this regard.

Carotenoids are fat-soluble pigments that give the yellow, red and orange color to fruits and vegetables. Although at least 600 carotenoids were defined in the nature, approximately forty of these are consumed in the human diet. Furthermore, fourteen of these carotenoids and some of their metabolites are identified in blood and tissues (1,2). They can only be synthesized by plants and microorganisms, therefore their sources are mostly from fruits and vegetables. β -carotene, lycopene, α -carotene, β -cryptoxanthin, lutein and zeaxanthin are among the most studied carotenoids (3-5). Carotenoids can be separated

into provitamin A carotenoids (e.g., β -carotene α -carotene and β -cryptoxanthin) and non-provitamin A carotenoids (lycopene, lutein and zeaxanthin). These compounds play an important role in human physiology due to their remarkable biological activities such as their effects on oxidative stress and inflammation (3). Carotenoids can act as potent antioxidants and protect the cells against oxidative damage induced by reactive oxygen species (ROS) (4).

Nonalcoholic fatty liver disease (NAFLD) is the broad spectrum of diseases from simple hepatic steatosis to cirrhosis, without excessive alcohol intake and use of steatogenic medication or hereditary disorders. Hepatic steatosis must be proven by imaging or histopathology. According to the NAFLD diagnosis and management guidelines of the American Association for the Study of Liver Diseases (AASLD), nonalcoholic fatty liver (NAFL) is defined as the presence of hepatic steatosis without ballooning degeneration and inflammation in the hepatocytes. Non-alcoholic steatohepatitis (NASH) is

defined as the presence of diffuse fatty infiltration in the liver and characterized by ballooning hepatocyte injury and inflammation in the hepatocytes with or without fibrosis (6-8). NAFL is mostly benign and risk of progression to cirrhosis and liver failure is minimal, whereas NASH can lead to cirrhosis, liver failure and hepatocellular carcinoma (HCC) (8,9).

There are limited and inconsistent data regarding the incidence rate of NAFLD. Inconsistency is related to different age, ethnic groups, and geographic groups (8). Although a study from England has shown an incidence of 29 cases per 100,000 person-years (10), Japanese researchers have reported rates of 31 and 86 suspected cases per 1,000 person-years in two different studies (11,12). NAFLD has become the most common cause of chronic liver disease with increasing prevalence and is reported to affect up to 20-40% of the general adult population worldwide (13). Increased prevalence of the disease has nearly doubled from 1988 to 2008 in the US and it is responsible for over 75% of the chronic liver diseases (14,15). The primary reason of the increased rate in the US population is obesity. Together with obesity, it is commonly associated with diabetes mellitus type 2, metabolic syndrome and hyperlipidemia (7,14).

In this paper, we review the underlying mechanisms of NAFLD pathogenesis, including oxidative stress and inflammation, and discuss the potential preventive and therapeutic effects of carotenoids in NAFLD.

NAFLD pathogenesis

The pathogenesis of NAFLD is a complicated process and is not fully understood. Assessment of the hepatic molecular changes, metabolic signaling networks and other pathogenetic factors could be helpful in understanding the process and developing appropriate preventive or therapeutic interventions. In 1998, a model was proposed to describe the pathogenetic process from simple steatosis NAFL to NASH. According to this widely adopted “two hits” pathophysiological model, first hit refers to hepatic fat accumulation and subsequently the second hit is the increased oxidative stress and initiation of lipid peroxidation. Recently, this model gave place to “multiple parallel hits” hypothesis, which has been suggested by Tilg and Moschen, and involved obesity, insulin resistance, oxidative stress, and proinflammatory processes (16).

Obesity is defined as excessive fat accumulation in adipose tissue and especially visceral adipose tissue, which is

more important than subcutaneous adipose tissue in terms of NAFLD. Visceral obesity is associated with hepatic steatosis, hepatic inflammation and fibrosis (6,17,18). It has also been associated with increased insulin resistance, metabolic syndrome, lipogenesis, and lipolysis (19,20).

Hepatic fat accumulation, i.e., steatosis, is primarily associated with increased intake of dietary fat and/or increased free fatty acids (FFA) due to increased lipolysis from adipose tissue. Furthermore decreased FFA oxidation, decreased hepatic very low-density lipoprotein (VLDL), triglyceride secretion and increased hepatic lipogenesis contribute to the steatosis (16,21). FFAs are responsible for approximately two-thirds of accumulated lipid in liver (22).

Insulin resistance has been related to metabolic syndrome and type 2 diabetes (23). Hepatic insulin resistance is characterized by hyperinsulinemia, hyperglycemia and an increase in VLDL production. Accordingly the hepatic excessive VLDL leads to a low high-density lipoprotein (HDL) concentration and hypertriglyceridemia (9,24,25).

Oxidative stress and inflammatory process in NAFLD

Oxidative stress refers to an imbalance between the ROS and the antioxidant molecules (26). Oxidative stress stimulated by ROS could generate oxidative damage and lead to chronic diseases. ROS are highly reactive and thus may interact with cellular membranes, proteins and nucleic acids (27,28). Oxidative stress may result from hepatic overloading of FFA which induces mitochondrial β -oxidation or microsomal enzymes such as cytochrome P4502E1 (CYP2E1). Increased CYP-P450 activity has been observed in obesity and NAFLD in both humans and rodents (29). Increased fatty acid oxidation in mitochondria and CYP2E1 have enhanced NADPH oxidase activity resulting in increased production of the ROS superoxide and hydrogen peroxide by redox cycling of endogenous and exogenous substrates (30-32). ROS overproduction causes cellular damage, which induces lipid peroxidation and consequently mitochondrial dysfunction that contributes to hepatocellular damage. In response to the state of oxidative stress, superoxide dismutase, glutathione (GSH) peroxidase, and catalase activities are increased in NAFLD (33).

ROS production has also indirect hepatotoxic effect via mitochondrial β -oxidation. The peroxisome proliferator activated receptors (PPAR) regulate the fatty acid metabolism and storage. FFA-induced PPAR- α up-regulates carnitine palmitoyltransferase-1 (CPT-1) expression and

increases the mitochondrial β -oxidation, and regulates the uptake and clearance of fatty acids (14,34,35). Knockout models of PPAR- α have been associated with steatosis, implicating a possible role for PPAR- α in NAFLD (36). Moreover, PPAR- γ is involved in insulin sensitivity and triglyceride storage and is connected with NAFLD. PPAR- γ levels have been found to be increased in the livers of NAFLD mice (14,37).

Tumor necrosis factor- α (TNF- α) and IL-6 are two important pro-inflammatory cytokines produced by injured hepatocytes, immune cells, and activated Kupffer cells and play an important role in inflammation. Both of TNF- α and IL-6 are increased in patients with NASH and their levels are correlated with the severity of inflammation, fibrosis and histological changes in the liver (38-40). Increased TNF- α activates c-Jun N-terminal kinase (JNK) signaling pathway resulting in hepatocyte apoptosis (41).

As mentioned above, NASH is characterized by steatosis with inflammation and hepatocyte ballooning with or without fibrosis (6,8). Leading feature of the NASH is the presence of inflammation and fibrosis. Hepatic stellate cells synthesize several growth factors and extracellular matrix materials such as collagen (42,43). Proliferation of these cells and collagen synthesis induced by cytokines such as TGF- β by Kupffer cells and oxidative stress play a key role in hepatic fibrosis (44,45).

Carotenoids and NAFLD

Due to increasing prevalence and incidence, and lack of established therapeutic intervention, NAFLD has become one of the most important health problems in the world. Obesity and metabolic disorders related to excessive fat intake play an important role in the pathogenesis of NAFLD. Epidemiological studies suggest that lifestyle modifications, such as altered diet with reduced caloric intake, weight loss, and physical activity are safe and effective interventions for improving obesity-mediated insulin resistance and NAFLD (6,46). Therefore, many natural dietary compounds have been studied for prevention and treatment of NAFLD. Carotenoids are among the most studied dietary compounds and their sources are mostly fruits and vegetables. Among the carotenoids, lycopene and β -carotene are the most studied compounds. The exact mechanisms of the protective effects of carotenoids in NAFLD is unclear, but there is evidence from various experimental studies that carotenoids may work through multiple mechanisms, including antioxidant and anti-

inflammatory effects (3,4).

In a human study which investigated the association of NAFL and serum levels of carotenoids, serum β -carotene was decreased with fat accumulation in the liver, but the levels of lycopene, α -carotene, β -cryptoxanthin, and lutein were not decreased (47). Another human study reported that plasma levels of carotenoids (lutein, zeaxanthin, β -cryptoxanthin, lycopene, α -carotene, and β -carotene) were significantly decreased in patients with NASH compared to control subjects (48).

Lycopene and NAFLD

Lycopene is a carotenoid that gives tomato and watermelon their red color. It has no vitamin A activity and is classified as a non-provitamin A carotenoid (27,49). Lycopene is a fat-soluble hydrocarbon with 40 carbon atoms and 56 hydrogen atoms ($C_{40}H_{56}$) (49). It is predominantly concentrated in fatty tissues like adrenal glands, liver, testis and has also been found in different human tissues such as lung, skin, cervix, ciliary body and retinal pigment epithelium (50,51). Major protective effect of lycopene is its antioxidant effect through inactivation of ROS and quenching of free radicals (52). As a potent antioxidant, it has been studied as a potential protective agent in NAFLD. Studies investigating lycopene in NAFLD are listed on *Table 1*.

A study examining the preventive role of lycopene in NASH was conducted on rats fed a high-fat diet (HFD) (53). Supplementation with lycopene lowered serum malondialdehyde (MDA) and TNF- α levels and elevated liver GSH level ($P < 0.001$). Additionally, a decreased CYP2E1 protein with increasing lycopene dose was observed in rats fed a HFD. Steatosis and inflammation were significantly lower in lycopene-fed rats. They found that lycopene administration had a preventive effect on experimental NASH induced by HFD in the rat model and lycopene could reduce HFD-induced oxidative stress.

Several studies reported that intake of tomato juice appeared to have a protective effect on NAFLD in rats that are hypercholesterolemic and fed a HFD (24,58). Rats fed tomato juice supplemented diet had lower TG level in plasma and isoprostane content in urine indicating alleviation of oxidative stress. They also had enhanced activity of enzymes involved in mitochondrial and peroxisomal β -oxidation of long-chain fatty acids in NAFLD. Lycopene accumulation in the liver was observed in tomato juice supplemented rats. Others have reported significantly improved absorption of carotenoids linked to

Table 1 Studies of NAFLD with lycopene

Reference	Model	Agent	Main effect	Main conclusion
Bahcecioglu et al. 2010 (53)	Rat: HFD induced NASH	Lycopene	Decreased CYP2E1 protein, MDA and TNF- α levels Elevated GSH level	Steatosis and inflammation were significantly lower in lycopene fed rats
Wang et al. 2010 (54)	Rat: HFD induced NASH promoted DEN-initiated hepatocarcinoma	Lycopene and tomato extract	Lycopene increased nuclear Nrf2- and heme-oxygenase-1 proteins Tomato extract decreased CYP2E1 and mRNA expression of TNF- α , IL-1 β and IL-12	Both lycopene and tomato extract reduced HFD-induced lipid peroxidation in the liver Lycopene and tomato extract inhibit NASH-promoted hepatocarcinogenesis
Ahn et al. 2012 (55)	Mouse: HFD induced NAFLD	Lycopene	Up-regulation of miRNA-21 expression Inhibition of FABP7 expression	Blocked stearic acid-induced intracellular lipid accumulation Lycopene may be a useful functional compound for treating NAFLD
Chung et al. 2012 (56)	Mouse: DEN-initiated, HFD-promoted hepatic tumorigenesis and inflammation	APO10LA	Increased SIRT1 gene expression and activity Decreased hepatic fat accumulation	APO10LA (lycopene metabolite) protects against the development of hepatosteatosis
Ip et al. 2013 (57)	Mouse: DEN-initiated, HFD-promoted hepatic tumorigenesis and inflammation	APO10LA	Reduce in TNF- α , IL-6, NF- κ B p65 protein expression and STAT-3 activity by increased SIRT1 gene expression and activity Increased AMPK- α phosphorylation	APO10LA can effectively inhibit hepatic tumorigenesis and reduce hepatic inflammation by stimulating SIRT1 signaling
Bernal et al. 2013 (58)	Rat: hypercholesterolemic and HFD induced NAFLD	Tomato juice	Decreased TG plasma levels and isoprostanol urine levels Increased liver lycopene accumulation Alleviation of aminoacid depletion Recovery of the redox balance Incrementing L-carnitine levels	Lycopene seems to have a protective effect in NAFLD
Martin-Pozuelo et al. 2014 (24)	Rat: HFD induced NAFLD	Tomato juice	Enhance the activity of mitochondrial and peroxisomal β -oxidation Reduced hallmark of steatosis	Tomato products should be taken into consideration in the treatment of NAFLD

NASH, nonalcoholic steatohepatitis; NAFLD, nonalcoholic fatty liver disease; HFD, high-fat diet; CYP2E1, cytochrome P4502E1; MDA, malondialdehyde; TNF- α , tumor necrosis factor α ; GSH, glutathione; DEN, diethylnitrosamine; Nrf2, nuclear factor-E2-related factor 2; FABP7, fatty acid-binding protein 7; APO10LA, Apo-10'-lycopenoic acid; NF- κ B, nuclear factor- κ B; STAT-3, signal transducers and activators of transcription 3; SIRT, Sirtuin1; TG, triglyceride.

the intake of dietary fat (59).

Wang *et al.* (54) investigated the protective effect of lycopene and tomato extract supplementation on NASH-promoted hepatocarcinogenesis in rats. HFD-induced NASH-promoted diethylnitrosamine (DEN)-initiated hepatocarcinoma rat model was used in the study. Lycopene and tomato extract could inhibit NASH-promoted hepatocarcinogenesis through reduced oxidative stress but with different mechanisms. They observed significantly decreased cytochrome P450 2E1, inflammatory foci and mRNA expression of proinflammatory cytokines (TNF- α , IL-1 β and IL-12) in the tomato extract fed group, but increased nuclear NF-E2-related factor-2 and heme oxygenase-1 proteins in the lycopene fed group.

Carotene-15,15'-monooxygenase (CMO-I) and carotene-9',10'-monooxygenase (CMO-II) are primary mammalian carotenoid cleavage enzymes. CMO-I cleaves β -carotene to two molecules of retinal, whereas CMO-II preferentially cleaves non-provitamin A carotenoids such as lycopene but also has affinity to other carotenoids such as β -carotene (60-63). CMO-I has been identified as a cytoplasmic enzyme, whereas CMO-II has been identified as a mitochondrial enzyme. These enzymes convert the carotenoids to biologically active metabolites (60). Lycopene may also interfere with the β -carotene and retinoid metabolism. It has been found that lycopene supplementation decreased the expression of CMO-I and PPAR- γ in the kidney and adrenal tissues of rats (64). Apo-10'-lycopenoic acid (APO10LA) a cleavage metabolite of lycopene, generated by CMO-II and had significant biological activities (56,62,65).

APO10LA supplementation significantly reduced DEN-initiated, HFD-promoted hepatic tumorigenesis and inflammation in C57BL/6J mice (57). Hepatic pro-inflammatory biomarkers including TNF- α , IL-6, NF- κ B p65 protein expression, caspase-1 cleavage and activation of the oncogenic transcription factor STAT3 were significantly reduced in the liver tissue by APO10LA supplementation. These effects of APO10LA were associated with increased hepatic Sirtuin1 (SIRT1), protein and deacetylation of SIRT1 targets (NF- κ B p65 and FoxO1) and AMP-activated protein kinase (AMPK) phosphorylation. Another study suggested significantly decreased hepatosteatosis together with increased SIRT1 gene expression and activity by APO10LA supplementation in the ob/ob mice with HFD induced hepatosteatosis model (56). SIRT1, an NAD⁺-dependent protein deacetylase, is expressed in various tissues including the liver (66) and plays an important role in the regulation of lipid metabolism (67) and deacetylation

of many proteins such as p53, NF- κ B, FOXO, liver X receptor (LXR) (68). Protective effect of overexpression of SIRT1 was found in HFD-induced fatty liver disease (69).

Ahn *et al.* (55) reported that HFD induced downregulation of miRNA-21 expression was reversed by lycopene. As a post-transcriptional regulator of gene expression, up-regulating miRNA-21 was achieved by lycopene through targeting the fatty acid-binding protein 7 (FABP7). FABPs are most active proteins in long chain fatty acid uptake and metabolism in the hepatocytes. They found that lycopene up-regulated the miRNA-21 and inhibited FABP7 expression and blocked stearic acid (SA) induced intracellular lipid accumulation. They also observed that miRNA could have an important role in hepatic function and its expression was changed by HFD in liver tissues in an animal model (55,70). Ahn *et al.* also reported that miR-21 expression was decreased in HFD-induced NASH and stearic acid treated Hepa 1-6 cells. NASH was associated with downregulation of miRNA-21 and upregulation of FABP7. FABPs are most active proteins in long chain fatty acid uptake and metabolism in the hepatocytes (71). FABP7 was one of the targets of miRNA-21, and it was directly and inversely associated with miR-21 (55). Lycopene normalized the effects of HFD and regulated the hepatic lipid metabolism in this model. It downregulated PPAR γ and fatty acid synthase (FASN) and upregulated CPT1- α , LCAD, PPAR- α and ApoA4 in HFD induced NASH mouse model (55).

β -carotene and NAFLD

β -carotene is the most widely distributed carotenoid in yellow-orange and dark green fruits and vegetables (72). β -carotene is also the most abundant carotenoid in the liver (42). Among provitamin A carotenoids, β -carotene has the highest provitamin A activity because it is partly converted to vitamin A (73). β -carotene has a strong antioxidant effect through scavenging free radicals and physically quenching singlet oxygen (74). Major source of β -carotene in human diet is primarily green leafy vegetables, carrots, apricots, sweet potatoes, red palm oil, mature squashes, pumpkins, and mangoes (73,75,76). As a potent antioxidant, β -carotene has been studied as a potential protective agent in NAFLD. The studies reporting the effects of β -carotene and other carotenoids on NAFLD are summarized in *Table 2*.

In vivo and *in vitro* experimental studies have shown potential preventive and therapeutic effects of β -carotene

Table 2 Studies of NAFLD with carotenoids other than lycopene

Reference	Model	Agent	Main effect	Main conclusion
Ikeuchi <i>et al.</i> 2007 (77)	Mouse: HFD induced obesity	Astaxanthin	Inhibition of increased body weight, adipose tissue weight Reduced liver weight and triglycerides Reduced plasma triglyceride and total cholesterol	Astaxanthin prevented obesity and fatty liver disease
Harari <i>et al.</i> 2008 (78)	Mouse: HFD fed, LDL-receptor knockout mouse	9-cis β -carotene enriched alga Dunaliella bardawil	NF- κ B associated decreased mRNA levels of VCAM-1, MCP-1, INF- γ	Carotenoid reduced plasma cholesterol and atherogenesis; reduced fat accumulation and inflammation in liver
Ozturk <i>et al.</i> 2009 (79)	Rat: carbon tetrachloride induced hepatic steatosis and damage	Apricot (β -carotene rich)	Decreased malondialdehyde Increased total glutathione levels and catalase, superoxide dismutase, glutathione peroxidase activities	Apricot decreased oxidative stress Apricot decreased hepatosteatosis and liver damage
Tainaka <i>et al.</i> 2011 (80)	Zebrafish: diet induced obesity	Campari tomato (lycopene and β -carotene rich)	Decreased srebf1 mRNA Increased of foxo1 gene expression	Tomato decreased diet-induced obesity, dyslipidemia and hepatosteatosis
Kim <i>et al.</i> 2012 (81)	Guinea pig: hypercholesterolemic diet induced NAFLD	Lutein	Decreased hepatic malondialdehyde, TNF- α and free cholesterol	Lutein can be protective in NAFLD by antioxidant effect
Her <i>et al.</i> 2013 (82)	Zebrafish: ubiquitous transcription factor YY1 induced liver steatosis	Astaxanthin	Decreased PPAR- γ and CHOP-10 expression	Preventive effect on hepatosteatosis and triglyceride accumulation in liver
Ha and Kim 2013 (83)	Rat: HFD induced NASH	Fucoxanthin	Increased CPT1, CYP7A1 expression	Fucoxanthin can be effective in improving lipid metabolism
Kobori <i>et al.</i> 2014 (84)	Mouse: high-cholesterol and HFD induced NASH	β -cryptoxanthin	Inhibition of inflammatory gene expression in NASH Reduced inflammatory response	β -cryptoxanthin reduced hepatosteatosis by reducing oxidative stress
Xiao <i>et al.</i> 2014 (85)	Rat: HFD induced NASH	Lycium barbarum polysaccharides (β -carotene rich)	NF- κ B, MAPK pathways modulated Decreased hepatic fat accumulation, hepatic inflammatory response, fibrosis and oxidative stress	These carotenoids were effective in the prevention of NASH

NAFLD, nonalcoholic fatty liver disease; HFD, high-fat diet; NF- κ B, nuclear factor- κ B; VCAM-1, vascular cell adhesion molecule-1; MCP-1, monocyte chemoattractant protein-1; INF- γ , interferon- γ ; FoxO1, Forkhead box O1; PPAR- γ , peroxisome proliferator activated receptor gamma; CHOP-10, C/EBP homologous protein 10; TNF- α , tumor necrosis factor α ; CPT1, carnitine palmitoyltransferase-1; CYP7A1, cholesterol 7 α -hydroxylase¹.

on hepatic inflammation, fibrosis (86) and cirrhosis (87). Another study reported that β -carotene could decrease hepatitis C virus induced-hepatosteatosis via inhibition of HCV RNA replication (88). Dietary β -carotene supplementation has been found to have a protective effect on liver damage. In rats with monocrotaline-induced steatosis, fat accumulation and hemorrhages decreased in the liver with β -carotene supplementation (89).

9-cis β -carotene is an isomer of β -carotene. Harari *et al.* (78) showed that 9-cis β -carotene supplementation reduced plasma cholesterol concentrations and atherogenesis, and inhibited fat accumulation and inflammation in the livers of mice fed a HFD. This could be due to reduced mRNA levels of inflammatory genes such as vascular cell adhesion molecule-1 (VCAM-1), IL-1 α , monocyte chemoattractant protein-1 (MCP-1), interferon- γ (INF- γ).

Hepatic protective effects of some β -carotene rich products have been shown in experimental studies. Administration of an herbal derivative, *Lycium barbarum* polysaccharides has been shown to have ameliorative effects on hepatic fibrosis, oxidative stress and inflammatory response in HFD induced NASH and cellular steatosis rat model (85).

A study conducted by Ozturk *et al.* (79) demonstrated that dietary intake of apricot could reduce the risk of hepatic steatosis and damage caused by free radicals. Apricot is a fruit that has a high content of carotenoids, largely β -carotene. Markers of oxidative stress MDA, total GSH levels, catalase, superoxide dismutase and GSH peroxidase activities were significantly altered in carbon tetrachloride induced hepatic steatosis and damage in Wistar rats. Oxidative stress was decreased and hepatic steatosis and damage were ameliorated in rats by β -carotene rich apricot feeding.

In another study, Campari tomato, which contains more β -carotene and lycopene than regular tomato, ameliorates diet-induced obesity, dyslipidemia and hepatosteatosis via downregulation of gene expression related to lipogenesis in the zebra fish model. Campari tomato decreased sterol regulatory element-binding transcription factor 1 (srebf1) mRNA by increase of forkhead box O1 (foxo1) gene expression, which may depend on high contents of β -carotene in this tomato strain (80).

In a human study, researchers found that NAFLD had inverse relationship with vitamin A nutritional status in individuals with class III obesity (90). Retinol and β -carotene serum levels were evaluated as a biochemical indicator. The researchers observed low retinol and β -carotene serum

levels in the presence of the NAFLD. They also reported significant association between insulin resistance with retinol and β -carotene levels.

Other carotenoids and NAFLD

Other carotenoids such as astaxanthin, lutein, β -cryptoxanthin, and fucoxanthin have also shown a protective effect in NAFLD.

Hypolipidemic and antioxidant effects of astaxanthin supplementation have been observed in human clinical trials (91,92). Astaxanthin treatment prevented triglyceride accumulation and liver steatosis by inhibiting PPAR- γ in ubiquitous transcription factor YY1 induced zebrafish liver steatosis (82). In another study, astaxanthin prevented the development of hepatic steatosis and lowered plasma total cholesterol and triglyceride in obese mice fed a HFD (77).

A study was performed with lutein administration in hypercholesterolemic diet fed guinea pigs, which showed that hepatic free cholesterol, hepatic MDA and TNF- α were decreased in the lutein supplemented group. The lutein group also had lower NF- κ B DNA binding activity. These antioxidant effects of lutein could be protective in NAFLD (81).

An experimental study evaluated the effect of β -cryptoxanthin in mice with high-cholesterol and HFD induced NASH. Comprehensive gene expression analysis was performed in the livers of the mice. β -cryptoxanthin reduced steatosis through alteration in the expression of genes associated with cell death, inflammatory responses, infiltration and activation of macrophages and other leukocytes, quantity of T cells, and free radical scavenging (84).

Supplementation with fucoxanthin reduced body weight, body and liver fat content, and improved liver function tests in obese premenopausal women with NAFLD (93). Consumption of fucoxanthin was effective in improving lipid and cholesterol metabolism by increasing CPT1, cholesterol 7 α -hydroxylase1 (CYP7A1) in rats with a HFD. These results suggest that fucoxanthin could be helpful in preventing NAFLD (83).

Conclusions

NAFLD has become one of the most important chronic liver diseases in the developed countries with consistently increased prevalence. Its association with obesity, type 2 diabetes mellitus, insulin resistance, metabolic syndrome and progression to cirrhosis and HCC increases its

clinical importance. Pathogenesis of the NAFLD is a very complex process and may have many mechanisms. Understanding the pathogenetic mechanisms may assist in developing new preventive and therapeutic strategies. Oxidative stress and proinflammatory processes play an important role in the pathogenesis. Carotenoids, which are antioxidant natural compounds, appear to have beneficial effects in the prevention and treatment of NAFLD. Antioxidant and anti-inflammatory properties are the leading mechanisms of actions of carotenoids. These effects modulate intracellular signaling pathways influencing gene expression and protein translation. Recent studies by Ip *et al.* have provided additional potential mechanisms of lycopene and its metabolites (94). They found that lycopene supplementation in beta-carotene-9',10'-oxygenase (BCO₂)-knockout mice suppressed oncogenic signals, including Met mRNA, β -catenin protein, and mTOR complex 1 activation, which was associated with increased hepatic microRNA (miR)-199a/b and miR214 levels. These results provide novel experimental evidence that dietary lycopene can prevent HFD-promoted HCC incidence and multiplicity in mice, and may elicit different mechanisms depending on BCO₂ expression. Future investigations are warranted to understand the precise mechanisms as well as potential preventive and therapeutic effects of carotenoids in NAFLD and HCC.

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