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Review

Health Benefits of Carotenoids: A Role of Carotenoids in the Prevention of Non-Alcoholic Fatty Liver Disease

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ABSTRACT: Non-alcoholic fatty liver disease (NAFLD) is one of the most common chronic liver diseases with a prevalence of ~25% worldwide. NAFLD includes simple hepatic steatosis, non-alcoholic steatohepatitis, fibrosis, and cirrhosis, which can further progress to hepatocellular carcinoma. Therefore, effective strategies for the prevention of NAFLD are needed. The pathogenesis of NAFLD is complicated due to diverse injury insults, such as fat accumulation, oxidative stress, inflammation, lipotoxicity, and apoptosis, which may act synergistically. Studies have shown that carotenoids, a natural group of isoprenoid pigments, prevent the development of NAFLD by exerting antioxidant, lipid-lowering, anti-inflammatory, anti-fibrotic, and insulin-sensitizing properties. This review summarizes the protective action of carotenoids, with primary focuses on astaxanthin, lycopene, β-carotene, β-cryptoxanthin, lutein, fucoxanthin, and crocetin, against the development and progression of NAFLD.

Keywords: non-alcocholic fatty liver disease, carotenoids, astaxanthin, lycopene, β-carotene

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) encompasses a spectrum of chronic liver disease, which includes simple fatty liver, non-alcoholic steatohepatitis (NASH), fibrosis, and cirrhosis (Younossi et al., 2018). The prevalence of NAFLD has been increasing in the past years, and the global prevalence of NAFLD was estimated to be ~25% in 2016 (Younossi et al., 2016). NAFLD can progress to hepatocellular carcinoma, which is the second-leading cause of cancer-related death worldwide (Younossi et al., 2018; Younossi et al., 2016). According to the "two-hit hypothesis", fat accumulation, the first hit, sensitizes the liver to the second hits, i.e., oxidative stress, inflammation, lipotoxicity, and apoptosis, triggering liver injury while they act synergistically (Day and James, 1998; Buzzetti et al., 2016). Therefore, dietary factors that can inhibit one or more of the disease insults may be beneficial to prevent the development of NAFLD.

Carotenoids are yellow to red isoprenoid pigments abundant in the organisms with a capability of photosynthesis, such as plants, algae, and some of the bacteria (Fiedor and Burda, 2014; Khoo et al., 2011). Carotenoids are categorized into two classes, i.e., oxygen-containing xanthophylls and unoxygenated carotenes, depending on the presence of an oxygen molecule therein (Pallet and Young, 2017). Xanthophylls include lutein, astaxanthin, fucoxanthin, zeaxanthin, β-cryptoxanthin, and crocetin, while lycopene and β-carotene are carotenes. As both xanthophylls and carotenes contain conjugated double bonds in their structure, they have a high reducing capability by transferring electrons (Vershinin, 1999), which confers an antioxidant property to carotenoids. Moreover, it is well known that carotenoids exert anti-inflammatory responses (Ip et al., 2013; Ni et al., 2015b). Therefore, the consumption of carotenoids may prevent the development of NAFLD. This review provides the current understanding of the pathogenesis of NAFLD, and how carotenoids impact the pathogenic processes for the prevention of NAFLD.

PATHOGENESIS OF NAFLD

Liver steatosis

Fat accumulation in the liver is a hallmark of NAFLD. In the healthy liver, hepatocytes maintain lipid homeostasis through the regulation of the influx of adipocyte-derived

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free fatty acids (FFAs), *de novo* lipogenesis, the uptake of dietary lipids from chylomicron remnants, fatty acid β-oxidation, and lipoprotein formation and secretion (Dowman et al., 2009). However, failure to maintain lipid homeostasis in hepatocytes can result in excessive accumulation of fat, primarily triglycerides (TG) (Liu et al., 2010). When more than 5% of hepatocytes have steatosis, it is considered as "liver steatosis" (Bedossa, 2017).

In the patients with NAFLD, adipose tissue-derived FFAs, *de novo* lipogenesis, and dietary lipids derived from chylomicron remnants account for 59%, 26%, and 15% of total hepatic FFA pool, respectively (Donnelly et al., 2005). In insulin-sensitive adipose tissue, insulin increases TG accumulation by promoting fatty acid uptake via the activation of lipoprotein lipase and the induction of fatty acid transporter protein (FATP) 1 while repressing lipolysis by inhibiting the activity of hormone-sensitive lipase (Dimitriadis et al., 2011; Czech et al., 2013). However, when the adipose tissue is insulin-resistant, an excessive amount of FFAs are released from adipocytes due to dysregulated lipolysis (Pappachan et al., 2017; Karpe et al., 2011). FFAs are taken up by the liver, specifically hepatocytes, via FATPs and CD36 (Kawano et al., 2013). FATP2 and FATP5 are highly expressed in hepatocytes (Hirsch et al., 1998). Falcon et al. (2010) demonstrated that adeno-associated virus-mediated liver-specific FATP2 knockdown prevents high-fat diet (HFD)-induced hepatic steatosis in mice. Also, adeno-associated virus-mediated FATP5 knockdown markedly reduced hepatic lipid contents in mice on a HFD (Doege et al., 2008). The studies demonstrate the critical contribution of adipose tissue-derived FFAs to the development of NAFLD when insulin resistance exists.

In healthy humans, *de novo* lipogenesis occurs in response to excessive intake of carbohydrates to convert carbohydrates to fats for storage. Carbohydrate response element binding protein (ChREBP) and sterol regulatory element binding protein 1c (SREBP-1c), which are activated by glucose and insulin, respectively, are primary transcription factors that regulate lipogenesis by increasing the transcription of lipogenic genes, such as acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), and stearoyl-CoA desaturase 1 (SCD1) (Kawano et al., 2013; Ferré and Foufelle, 2010). Obese subjects with liver steatosis have ~3-fold higher rates of *de novo* lipogenesis than those without liver steatosis (Lambert et al., 2014). Also, when *de novo* lipogenesis was inhibited by decreasing the expression of FAS and SCD1 using microRNA 27a (miR-27a), liver steatosis was attenuated in mice fed a high-carbohydrate diet (Zhang et al., 2017).

Dietary lipids delivered to the liver via chylomicron remnants contribute to the development of steatosis in the liver. Hepatic uptake of chylomicron remnants is primarily mediated through low-density lipoprotein (LDL) receptor-related protein, LDL receptor, and lipolysis stimulated lipoprotein receptor (Cooper, 1997; Yen et al., 2008). Evidence suggests that suppression of chylomicron remnant uptake to the liver prevents steatosis in mice. Specifically, lactoferrin is shown to prevent liver steatosis in HFD-fed mouse (Lee et al., 2018), which is attributable to its capacity of binding to lipolysis stimulated lipoprotein receptor (Ahmad et al., 2012), thereby inhibiting hepatic uptake of chylomicron remnant.

Fatty acids hydrolyzed from TG in hepatocytes are directed to mitochondrial β-oxidation for energy in an energy-deprived state. Studies have reported contradicting roles of hepatic β-oxidation on the development of liver steatosis. Miele et al. (2003) showed increases in hepatic mitochondrial β-oxidation in patients with NASH using ^{13}C-octanoate breath test, which could be a source of mitochondrial reactive oxygen species (ROS). However, Pérez-Carreras et al. (2003) reported that the activity of hepatic carnitine palmitoyl transferase (CPT) 1, a rate-limiting enzyme of β-oxidation, was not altered in patients with NASH, indicating that β-oxidation is not likely altered in NASH. On the other hand, in rats on methionine-choline deficient (MCD) diet for the induction of NASH, primary hepatocytes and isolated hepatic mitochondria showed lower activities of CPT1 and 3-hydroxy-acyl-CoA dehydrogenase, a key enzyme of β-oxidation, compared to control mice (Serviddio et al., 2011). Similarly, deletion of HADHA, a gene encoding α-subunit of mitochondrial trifunctional protein, to induce a defect in its role in mitochondrial β-oxidation, is shown to exacerbate HFD-induced hepatic steatosis in mice (Nassir et al., 2018). As the role of hepatic mitochondrial β-oxidation in the development of liver steatosis is not consistent across studies, further studies are warranted for a comprehensive understanding of its role in NAFLD development. Also, the differences between murine and human model should be taken into consideration.

Hepatic lipotoxicity

Excessive accumulation of fatty acids and lipids in the liver can induce the production of toxic lipid intermediates, such as diacylglycerols, ceramides, acylcarnitines, and lysophosphatidylcholine, which can trigger oxidative stress, endoplasmic reticulum (ER) stress, and insulin resistance, resulting in hepatocyte dysfunction and apoptosis (Alkhouri et al., 2009; Cazanave et al., 2010). Therefore, lipotoxicity promotes the release of damage-associated molecular pattern molecules, including high mobility group box-1, heat-shock proteins, and uric acid, consequently activating macrophages for the progression of NAFLD (Magee et al., 2016; Martin-Murphy et al., 2010).

Hepatic oxidative stress

Oxidative stress is defined as a disturbance caused by the imbalance between the production and removal of ROS. In hepatocytes, ROS are primarily produced in mitochondria, peroxisomes, and ER (Ashraf and Sheikh, 2015). ROS levels within cells are regulated by the action of antioxidant enzymes, e.g., superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), and non-enzymatic antioxidants including vitamin E, β-carotene, and ascorbate (Cichoż-Lach and Michalak, 2014). However, excessive production and decreased removal of ROS lead to their accumulation, triggering lipid peroxidation through free radical chain reactions (Polimeni et al., 2015). Lipid peroxides damage proteins and nucleic acids, and disrupt the integrity of cellular membranes, eventually causing apoptosis and necrosis of hepatocytes (Leung and Nieto, 2013; Sharma and John, 2017). Studies have demonstrated that patients with NAFLD have increased oxidative stress. For instance, circulating levels of 8-isoprostane, a lipid peroxidation product, are higher in patients with NAFLD than healthy subjects (Konishi et al., 2006). Also, NAFLD patients have decreased antioxidant defense as demonstrated by reduced activities of antioxidants, such as coenzyme Q10, SOD, and CAT (Yesilova et al., 2005).

Excessive ROS production is closely associated with dysfunctions in the mitochondrial electron transport chain (ETC) because mitochondrial ROS, i.e., superoxide and hydrogen peroxide, are produced during the mitochondrial oxidative phosphorylation (Paschos and Paletas, 2009). The impairment of any complex in the ETC increases ROS production (Wei et al., 2008). For example, selective inhibition of complex III with antimycin A in the mitochondria isolated from the rat liver increases the production of hydrogen peroxide (García-Ruiz et al., 1995). Similarly, the mitochondria isolated from rats fed a choline-deficient diet showed decreased complex I activity with a concomitant increase in hydrogen peroxide (Hensley et al., 2000). Also, the induction of hepatic cytochrome P450 2E1 (CYP2E1) increases ROS production during the oxidation of polyunsaturated fatty acids (Leung and Nieto, 2013; Aljomah et al., 2015). CYP2E1 metabolizes polyunsaturated fatty acids, producing ω-hydroxylated fatty acids, which are further metabolized into toxic lipid metabolites at high concentrations (Leung and Nieto, 2013). Evidence demonstrates that hepatic CYP2E1 levels were markedly increased in patients with steatohepatitis compared to those of the healthy liver (Weltman et al., 1998). The induction of hepatic CYP2E1 level was also observed in a high-fat emulsion-induced rat model of NASH (Zou et al., 2006). Specifically, hepatocyte-specific overexpression of CYP2E1 induced liver injury with increased hepatic oxidative stress in mice (Kathirvel et al., 2010).

Hepatic inflammation

ROS activate inflammatory c-Jun N-terminal kinases (JNK) and nuclear factor κB (NF-κB) pathways in hepatocytes, promoting the production of pro-inflammatory cytokines and chemokines, such as interleukin (IL)-1β, IL-6, tumor necrosis factor α (TNFα), C-C motif chemokine ligand 2 (CCL2), and C-X-C motif chemokine ligand 1 (CXCL1) (Magee et al., 2016; Zhou et al., 2016). Secretion of the pro-inflammatory cytokines activates hepatic resident macrophages, i.e., Kupffer cells, resulting in increased production of cytokines and chemokines (Vonghia et al., 2013). In turn, TNFα further stimulates Kupffer cells to produce chemokines that can promote inflammatory cell recruitment (Tilg and Diehl, 2000). Increased serum concentrations of TNFα were observed with NASH patients compared to patients with simple liver steatosis, substantiating the role of TNFα in the progression of NASH (Hui et al., 2004). Moreover, TNFα initiates apoptotic signals in hepatocytes through TNF receptor 1 (TNFR1), which leads to hepatocyte apoptosis, another hallmark of NAFLD progression (Malhi and Gores, 2008).

CCL2 and CXCL1 play crucial roles in the recruitment of circulating monocytes and neutrophils to the liver, respectively (Zhou et al., 2016; Vonghia et al., 2013), exacerbating hepatic inflammation. In particular, CCL2 mediates recruitment of $CD11b^{int}Ly6C^{hi}$ monocytes, a subset of pro-inflammatory monocytes, which differentiate into pro-inflammatory M1 macrophages in the liver (Miura et al., 2012; Italiani and Boraschi, 2014). In a mouse model of MCD diet-induced NASH, Kupffer cells produce TNFα at the initial stage of NASH development, followed by infiltration of $CD11b^{int}Ly6C^{hi}$ monocytes, and therefore inflammatory milieu in the liver become perpetuated (Tosello-Trampont et al., 2012). Depletion of Kupffer cells using clodronate liposomes abrogated the MCD diet-induced TNFα production and attenuated monocyte recruitment, further supporting a critical role of Kupffer cells in the initiation of liver inflammation (Tosello-Trampont et al., 2012).

Hepatocyte apoptosis

Apoptosis of hepatocytes, a critical feature occurring during NAFLD progression, is regulated by extrinsic and intrinsic pathways (Alkhouri et al., 2011). The extrinsic pathway includes the activation of death receptors, such as Fas, TNFR1, and TNF-related apoptosis-inducing ligand receptors, activated caspases, and proteolytic enzymes (Alkhouri et al., 2011). Specifically, activated Kupffer cells produce Fas ligand and TNFα, a ligand for death receptor Fas and TNFR1, respectively (Malhi et al., 2010). Patients with NASH have markedly increased hepatocyte apoptosis with increased Fas receptor in hepatocytes compared with healthy subjects and patients with sim-

ple steatosis (Feldstein et al., 2003).

On the other hand, the intrinsic pathway of hepatocyte apoptosis is initiated by ER stress and mitochondrial dysfunctions, which can activate caspases and B-cell lymphoma-2-associated X protein, a pro-apoptotic protein (Alkhouri et al., 2011). This intrinsic pathway is mainly caused by excessive ROS, FFAs, and toxic lipid intermediates in NASH (Alkhouri et al., 2011). In addition to triggering pro-apoptotic pathways, ROS can also induce apoptosis by decreasing levels of anti-apoptotic proteins, such as B-cell lymphoma-extra large and myeloid cell leukemia sequence 1, in hepatocytes (Herrera et al., 2001).

Liver fibrosis

One of the features of advanced NASH is liver fibrosis (Stål, 2015). Liver fibrosis results from the excessive deposition of extracellular matrix (ECM) due to increased production and decreased breakdown of ECM proteins (Bae et al., 2017). Hepatic stellate cells (HSCs) play a crucial role in the development of liver fibrosis as they are primary scar tissue-producing cells in the liver (Moreira, 2007). HSCs are resident nonparenchymal cells, located in the perisinusoidal space of Disse (Puche et al., 2011). In the healthy liver, HSCs remain in a quiescent state characterized by cytoplasmic lipid droplets containing largely retinyl esters, serving as the primary storage depot for vitamin A in the body (Moreira, 2007; Puche et al., 2011). However, in the NASH liver, HSCs become activated with a loss of retinoid-laden lipid droplets by multiple insults, such as transforming growth factor β (TGF-β), TNFα, platelet-derived growth factor (PDGF), IL-1, and fibronectin, etc. (Bae et al., 2017; Li et al., 2008). These growth factors and cytokines are produced by hepatocytes, immune cells, platelets, and endothelial cells when they are damaged or activated upon liver injury.

TGFβ1, a potent pro-fibrotic cytokine, activates HSCs when it binds to its cell surface receptors, which subsequently induces the phosphorylation of small mother against decapentaplegic (SMAD) 2/3 (Zi et al., 2012). The phosphorylated SMAD2/3 binds to SMAD4, and the complex then translocates to the nucleus, causing the expression of fibrogenic genes in HSCs. Activated HSCs are proliferative due to their high expression of PDGF receptor (Bae et al., 2017; Puche et al., 2011). Also, activated HSCs recruit immune cells, including neutrophils, monocytes, and natural killer T cells, to the liver by producing cell adhesion molecules, e.g., intercellular adhesion molecule 1 and vascular cell adhesion protein 1 (VCAM-1), and chemokines, such as CCL2, CCL5, and CXCL10 (Puche et al., 2011).

Importantly, activated HSCs produce ECM proteins, such as collagen type I, as a result of the activation of TGFβ1/SMAD signaling (Bae et al., 2017; Puche et al., 2011). Also, upon HSC activation, ECM degradation is inhibited by increased production of tissue inhibitor of metalloproteinases (Li et al., 2008). HSC activation scores were significantly increased by the progression of fibrosis in NAFLD patients, determined by histological analysis of alpha-smooth muscle actin (α-SMA) immunostaining (Feldstein et al., 2005). Furthermore, it has been demonstrated that senescence or removal of activated HSCs limits fibrosis in the liver (Melhem et al., 2006; Krizhanovsky et al., 2008).

PROTECTIVE ACTION OF CAROTENOIDS AGAINST NAFLD DEVELOPMENT

Astaxanthin

Astaxanthin (ASTX), a non-provitamin A carotenoid, is found in a variety of marine organisms, such as salmon, shrimps, and crabs. Studies have shown that consumption of ASTX prevents NAFLD development. Bhuvaneswari et al. (2010) reported that daily administration of 6 mg/kg body weight of ASTX for 60 days attenuated diet-induced obesity, hepatic steatosis and hepatic TGFβ1 protein levels in mice. Also, ASTX attenuated high-fat (HF)/high-sucrose (HS) diet-induced hepatic lipid droplet formation, macrophage infiltration, inflammation, and fibrosis in mice (Kim et al., 2017). Oral administration of ASTX alleviated hepatic oxidative stress and inflammation induced by streptozotocin in rats (Park et al., 2015).

ASTX is a potent antioxidant, therefore, it protects DNA, cell membranes, and lipids from oxidative damages (Chen et al., 2016a; Ambati et al., 2014). ASTX possesses a superior quenching capacity toward hydroperoxyl radicals than other carotenoids such as α-carotene, β-carotene, lutein, and lycopene (Naguib, 2000). This may be due to its unique molecular structure that contains hydroxyl and keto moieties in its each ionone ring (Liu et al., 2007). Oral administration of ASTX reduced biomarkers of oxidative stress, i.e., malondialdehyde (MDA), nitric oxide, and advanced protein oxidation product, in mouse brain by increasing activities of antioxidant enzymes including GPx, SOD, and CAT in both young and old mice (Al-Amin et al., 2015). We also previously reported that ASTX supplementation induced the expression of antioxidant enzymes in the liver of apolipoprotein E knockout mice fed a HF/high-cholesterol (HC) diet by increasing the expression of nuclear factor E2 related factor 2 (Yang et al., 2011).

ASTX supplementation has been reported to reduce plasma/hepatic total cholesterol (TC), TG, and non-esterified fatty acids (NEFA) in mice fed high-fat, cholesterol, and cholate diet (Ni et al., 2015b). Decreased expressions of hepatic lipogenic genes such as FAS and SCD1 may be responsible for the lipid-lowering effect of

ASTX (Ni et al., 2015b). Interestingly, ASTX decreased respiratory exchange ratio of diet-induced obese (DIO) mice, indicating it may increase fatty acid utilization as energy sources (Ikeuchi et al., 2007). The induction of CPT1α and acyl-coenzyme A oxidase 1, rate-limiting enzymes for mitochondrial and peroxisomal β-oxidation, respectively, in the skeletal muscle of ASTX-fed DIO mice supports this speculation (Kim et al., 2017).

Ni et al. (2015b) reported that ASTX supplementation remarkably reduced the numbers of F4/80-positive cells and the expression of pro-inflammatory cytokines in the liver of mice fed a HF/HC diet. Also, ASTX treatment decreased the percentages of pro-inflammatory M1-type macrophages while increasing those of anti-inflammatory M2-type macrophages in the liver (Ni et al., 2015b). Similarly, splenocytes isolated from ASTX-fed mice showed a decrease in IL-6 expression upon lipopolysaccharides (LPS) challenge, indicating that ASTX reduced the sensitivity of splenic monocytes to LPS (Yang et al., 2014). Therefore, ASTX may alleviate hepatic inflammation and the progression of NAFLD by altering the number and phenotypes of monocytes recruited to the liver.

A varying degree of liver fibrosis may develop in patients with NASH (Stål, 2015). ASTX treatment alleviated hepatic fibrosis induced by a HF/HS/HC diet (Kim et al., 2017). Moreover, by repressing the activation of SMAD3 pathway, ASTX inhibited the expression of profibrogenic genes induced by TGFβ1 in LX-2 cells, a human HSC cell line, and primary mouse HSCs (Yang et al., 2015). Interestingly, LX-2 cells treated with ASTX showed a decrease in mRNA and protein levels of histone deacetylase 9 (HDAC9) with a concomitant reduction in the expression of myocyte enhancer factor 2 (MEF2), a known transcriptional regulator of HDAC9 (Yang et al., 2017). Knock-down of HDAC9 decreased TGFβ1-induced fibrogenic gene expression, such as α-SMA and collagen type I alpha 1 chain (Yang et al., 2017). The studies suggest that inhibition of HDAC9 by ASTX may be, at least in part, responsible for its anti-fibrotic effect in the liver.

Lycopene

Lycopene, a red color non-provitamin A carotenoid, is rich in tomatoes, guava, pink grapefruit, and watermelon. While the liver is one of the major lycopene depots, lycopene is also found in other tissues including the adrenals and reproductive tissues (Ganesh et al., 2016). Patients with NASH have significantly lower serum lycopene levels than healthy subjects (Erhardt et al., 2011). Also, in rats, lycopene consumption prevented the development of NAFLD induced by a high-fat or a HF/HC diet (Piña et al., 2014; Piña-Zentella et al., 2016; Jiang et al., 2016). In DIO mice, lycopene lowered serum concentrations of TG and NEFA and attenuated liver steatosis with decreased hepatic expression of lipogenic genes, such as ACC1, FAS, and SREBP-1c (Fenni et al., 2017).

In addition to the lipid-lowering effect, lycopene exerts antioxidant and anti-inflammatory properties to prevent the development of NAFLD. Lycopene is a potent antioxidant because it possesses 11 conjugated and 2 unconjugated double bonds, which can quench singlet oxygens (Di Mascio et al., 1989; Rao and Rao, 2007). Also, lycopene enhances the activities of antioxidant enzymes in the liver, such as GPx (Moreira et al., 2005). It has been reported that lycopene can upregulate the expression of genes whose promoters contain the antioxidant response element (ARE) in HepG2 and MCF-7 (Ben-Dor et al., 2005) and LNCaP cells (Goo et al., 2007). Consequently, lycopene induces the expression of cellular antioxidant enzymes, such as SOD1 and CAT (Goo et al., 2007). Oral administration of lycopene lowered MDA level while increasing SOD activity in the rat liver with HFD-induced NAFLD (Jiang et al., 2016). Lycopene also inhibited the generation of ROS by attenuating CYP2E1 expression in rats with NASH (Bahcecioglu et al., 2010).

Lycopene consumption reduced the production of pro-inflammatory cytokines, such as TNFα and IL-1β, in the rat liver with HFD/methotrexate-induced liver injury (Jiang et al., 2016; Yucel et al., 2017). It is likely that lycopene inhibits inhibitor of κB kinase, which is essential for NF-κB activation, consequently suppressing the NF-κB pathway in MDA-MB-231 cells, a human breast cancer cell line (Assar et al., 2015). Also, apo-10'-lycopenoic acid (ALA), a major metabolite of lycopene, is known to suppress hepatic steatosis and inflammation by stimulating sirtuin 1 (SIRT1). ALA supplementation attenuated the development of liver steatosis in *ob/ob* mice fed a HFD (Chung et al., 2012). Ip et al. (2013) also reported that ALA supplementation increased hepatic SIRT1 protein with a concomitant increase in deacetylation of NF-κB p65 in the liver of HFD-fed mice, which in turn decreased hepatic protein levels of IL-6 and TNFα. Mechanisms by which ALA regulates SIRT1 expression are not well understood. Therefore, future studies are warranted for a better understanding of how this lycopene metabolite protects against the development of NAFLD.

β-Carotene

β-Carotene, the most abundant carotenoid in the liver (Vitaglione et al., 2004), is rich in tomato, red watermelon, guava, grapefruit, mango, papaya, yellow pepper, pineapple, pumpkin, and banana (Maiani et al., 2009). Patients with class III obesity (body mass index≥40) and NAFLD had significantly lower serum β-carotene levels than those without NAFLD (Villaça Chaves et al., 2008). Moreover, there was a significant association between insulin resistance and β-carotene/retinol deficiency, indicating β-carotene may play a preventive role in the development of NAFLD (Villaça Chaves et al., 2008).

Studies have shown that β-carotene prevents liver injury. For instance, β-carotene prevents *tert*-butyl hydroperoxide-induced oxidative damage in HepG2 cells (Martin et al., 1996). Also, oral administration of β-carotene attenuated CCL_4-induced liver fibrosis (Seifert et al., 1995) and thioacetamide-induced cirrhosis (Wardi et al., 2001) in rats. Consumption of apricot rich in β-carotene reduced serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), hepatic MDA level and centrilobular necrosis while increasing antioxidant enzyme activities in the rat liver with CCL_4-induced injury (Ozturk et al., 2009).

Metabolites and isomers of β-carotene also have beneficial effects against NAFLD. β-Carotene possesses the highest provitamin A activity among provitamin A carotenoids (Yilmaz et al., 2015). Retinoic acid (RA), a metabolite of vitamin A, has been reported to significantly up-regulate the expression of genes related to fatty acid oxidation, e.g., PPARα and CPT1α, and brown adipocyte-specific uncoupling protein 1 (UCP1) in white adipose tissue (WAT), indicating that RA promotes browning of WAT (Mercader et al., 2006). As a result, RA injection significantly reduced body and serum TG levels in mice (Mercader et al., 2006). The effects of 9-cis β-carotene, an isomer of β-carotene (Yilmaz et al., 2015), on NAFLD have also been studied. 9-cis β-carotene-enriched HF/HC diet reduced hepatic TG levels and the expression of inflammatory genes, including toll-like receptor 2, E-selectin, and VCAM-1, in LDL receptor knockout mice fed a HFD (Harari et al., 2008). Also, β-carotene prevented hepatic oxidative damage and fibrosis.

β-Cryptoxanthin

β-Cryptoxanthin, a carotenoid specifically found in Satsuma mandarins (*Citrus unshiu*), is the only xanthophyll carotenoid that has a pro-vitamin A activity in mammals (Latowski et al., 2014). Along with lutein, zeaxanthin, and β-carotene, β-cryptoxanthin is abundantly present in human plasma (Ni et al., 2016; Sugiura et al., 2009). β-Cryptoxanthin has a similar structure to β-carotene except an additional hydroxyl group on one of the β-ionone rings. When Wistar rats were fed a diet containing β-cryptoxanthin or β-carotene for 4 weeks, β-cryptoxanthin was found at higher concentrations in the liver, kidney, spleen, brain, uterus, and heart than β-carotene, indicating bioavailability of β-cryptoxanthin is likely higher than β-carotene (Sugiura et al., 2014).

β-Cryptoxanthin has potent antioxidant properties, preventing oxidative DNA damage. β-Cryptoxanthin has a higher singlet oxygen quenching activity than β-carotene in mouse cell line C3H/10T1/2 clone 8 (Stahl et al., 1997). Moreover, oral consumption of β-cryptoxanthin 37.5 μg/kg/d reduced 8-OHdG positive cell numbers, a marker of DNA oxidation damage, in ferrets exposed to a cigarette (Liu et al., 2011). Also, incubation with 1 and 4 μM of β-cryptoxanthin reduced half-life of H_2O_2-induced DNA breaks and radiation-derived 8-oxo-7,8-dihydroguanine, an oxidized DNA base group, in HeLa cells (Lorenzo et al., 2008). Also, serum β-cryptoxanthin levels in women are inversely associated with levels of lymphocyte 8-Hydroxy-2'-deoxyguanosine and urinary 8-epiprostaglandin F2α, indices of oxidative DNA damage and lipid peroxidation, respectively (Haegele et al., 2000). The above data suggest that β-cryptoxanthin can protect hepatic cells from oxidative stress in NAFLD.

In addition, β-cryptoxanthin can protect hepatic cells from oxidative damage by inducing antioxidant enzyme expressions. β-Cryptoxanthin increased intracellular glutathione (GSH) level in RAW264 macrophages (Katsuura et al., 2009). In humans, consumption of a beverage containing β-cryptoxanthin reduced serum levels of liver enzymes, i.e., ALT, AST, and γ-glutamyltransferase, with a concomitant increase in serum SOD levels in NAFLD patients (Matsuura et al., 2017). This result suggests that β-cryptoxanthin ameliorated hepatic cell damage in NAFLD patients, which may be attributable to the induction of SOD.

β-Cryptoxanthin shows anti-inflammatory and anti-fibrogenic properties in mouse liver. Compared to the control group, 0.003% β-cryptoxanthin supplementation inhibited hepatic lipid accumulation, fibrogenesis induced by a HF/HC diet in mice (Kobori et al., 2014). Moreover, diet-induced increases in hepatic F4/80-positive cells, expression of TNFα inducible macrophage genes, e.g., cytochrome b, interferon gamma receptor 1 and VCAM-1, and expression of major histocompatibility complex class II molecules and T cell markers, i.e., CD3, CD4, and CD8, were reduced by β-cryptoxanthin supplementation (Kobori et al., 2014). The results indicate that β-cryptoxanthin exerts its anti-inflammatory and anti-fibrogenic properties by suppressing recruitment of macrophages and T cells. Also, in mice with diet-induced NASH, β-cryptoxanthin treatment attenuated hepatic steatosis and fibrosis, inhibited pro-inflammatory cytokine expression in Kupffer cells as well as in LPS challenged mouse peritoneal macrophages (Ni et al., 2015a). These facts indicate that β-cryptoxanthin may inhibit hepatic inflammation and subsequent fibrosis by repression of macrophage differentiation to their pro-inflammatory phenotype (Ni et al., 2015a). These data suggest that the anti-inflammatory properties of β-cryptoxanthin may be responsible for its protective effects against NAFLD.

Other carotenoids

Other carotenoids, including lutein, fucoxanthin, and crocetin, have also been shown to prevent the development of NAFLD. Lutein is abundant in corn products and egg yolks (Perry et al., 2009). In Chinese adults, serum

levels of lutein were significantly and negatively associated with the degree of NAFLD (Cao et al., 2015). Lutein supplementation reduced free cholesterol accumulation and TNFα level in the liver of guinea pigs fed a high-cholesterol diet, which was attributed to a reduction in DNA binding activity of NF-κB (Kim et al., 2012). Also, lutein supplementation reduced hepatic TC and TG contents in rats on a HFD with concomitant increases in PPARα protein level, which can enhance fatty acid oxidation (Qiu et al., 2015). Therefore, evidence exists that lutein alleviates hepatic lipid accumulation and inflammation.

Fucoxanthin is a carotenoid primarily found in brown seaweed (Maeda et al., 2005). Potential protective functions of fucoxanthin against the development of NAFLD have recently been recognized. Consumption of 600 mg of Xanthigen (containing 2.4 mg of pure fucoxanthin) for 16 weeks decreased liver fat content and serum concentrations of TG and C-reactive protein in obese premenopausal women with NAFLD (Abidov et al., 2010). Several animal studies have supported the protective role of fucoxanthin against NAFLD as well. Park et al. (2011) demonstrated that supplementation of fucoxanthin-rich *Undaria* ethanol extract or pure fucoxanthin prevented insulin resistance and hepatic fat accumulation induced by a HFD in mice. The effects of fucoxanthin were attributed to increased activity of enzymes for fatty acid β-oxidation while decreasing lipogenic enzyme activities (Park et al., 2011). Compared to the control group, mice fed a diet containing 2% fucoxanthin-rich *Undaria* extracts had lower abdominal white adipose tissue weight and higher UCP1 in white adipose tissue (Maeda et al., 2005). Interestingly, dietary fucoxanthin increased hepatic docosahexaenoic acid (DHA) levels in obese mice (Tsukui et al., 2007). As DHA is the precursor of anti-inflammatory and pro-resolution lipid mediators, such as resolving D1 and protectin D1 (Serhan et al., 2008), fucoxanthin may promote the production of anti-inflammatory lipid mediators, suppressing hepatic inflammation and NAFLD development.

Crocetin is a carotenoid found in *Gardenia jaminoides* Ellis and saffron with a short carbon chain. Crocetin is known to be absorbed more rapidly than β-carotene, lutein, and lycopene in humans (Umigai et al., 2011). Similar to fucoxanthin, crocetin is an emerging therapeutic agent for NAFLD. By exerting its antioxidant activity, crocetin decreased lipid peroxidation and protected primary rat hepatocytes from oxidative DNA damage (Tseng et al., 1995). Oral administration of crocetin and its derivatives, crocin-1 and total crocins, restored hepatic GSH levels and antioxidant enzyme activities that were diminished in CCl_4-treated mice (Chen et al., 2016b). Also, crocetin ameliorated CCl_4-induced liver injury, supporting that the antioxidant properties of crocetin may be responsible for its protective effects on liver injury (Chen et al., 2016b). Administration of crocetin reduced serum NEFA and TG levels and improved glucose tolerance in diabetic rats (Xi et al., 2005). Furthermore, crocetin increased insulin sensitivity, hepatic NEFA uptake and oxidation, and TG clearance from the circulation, while reducing the accumulation of hepatic diacylglyceride and long-chain acyl CoA in rats with HFD-induced diabetes (Sheng et al., 2008). The beneficial effects of crocetin may be attributed to the increased lipoprotein lipase activity, β-oxidation rate, and CPT-1 activity in the liver (Sheng et al., 2008). Insulin resistance and chronic low-grade inflammation in diabetes contribute to NAFLD progression (Bugianesi et al., 2010). Therefore, crocetin may protect against NAFLD by lowering circulating lipid levels and increasing insulin sensitivity.

CONCLUSION

NAFLD is one of the common chronic liver diseases, reaching 25% of prevalence worldwide. During the development of NAFLD, various injury insults, i.e., lipid accumulation, insulin resistance, oxidative stress, inflammation, lipotoxicity, hepatocyte apoptosis and fibrosis, trigger liver damage. Studies have demonstrated the inhibition of one or more of the insults prevents the development of NAFLD. In this review, we summarized the protective action of astaxanthin, lycopene, β-carotene, β-cryptoxanthin, lutein, fucoxanthin, and crocetin against the development of NAFLD. Evidence shows that the protective effects of the carotenoids on NAFLD development is mediated through their antioxidant, lipid-lowering, anti-inflammatory, anti-fibrotic, and insulin-sensitizing properties. As the exercise and dietary approaches are critical strategies to prevent NAFLD due to the lack of Food and Drug Administration-approved NAFLD treatments, the consumption of the carotenoids with proven protective effects on NAFLD development may be recommended to obtain their health benefits.

AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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