Lead Article

Biological activity of lycopene metabolites: implications for cancer prevention

Jonathan R Mein, Fuzhi Lian, and Xiang-Dong Wang

While early studies focused on the potential roles in health and disease of provitamin A carotenoids, such as β-carotene, research over the past decade has provided a framework for our understanding of the functions of non-provitamin A carotenoids such as lycopene, especially in regards to its association with a reduced risk of a number of chronic diseases, including cancer. Recent data suggests that lycopene metabolites may possess specific biological activities on several important cellular signaling pathways and molecular targets. Carotenoid metabolites may have more important biological roles than their parent compounds in human health and disease. This notion has been reinforced by the observation of both beneficial and detrimental effects of carotenoid metabolites in cancer prevention.

INTRODUCTION

Considerable interest and research efforts have been expended in an effort to uncover the potential roles of carotenoids in human health and disease. While early studies focused on pro-vitamin A carotenoids, more recent research efforts have focused on the potential roles in health and disease of the non-provitamin A carotenoids, such as lycopene. Lycopene has been implicated as having a potential beneficial impact in a number of chronic diseases including cancer. Although evidence from epidemiological and animal studies supports a potential chemopreventive role of lycopene,[1–5] the biochemical mechanisms behind such beneficial effects have, as of yet, not been well defined. Several reports have demonstrated potential beneficial effects of lycopene, especially in respect to the following: antioxidant function; enhanced cellular gap junction communication; induction of phase II enzymes through activation of the antioxidant response element (ARE) transcription system; suppression of insulin-like growth factor-1-stimulated cell proliferation by induced insulin-like growth factor binding protein; anti-angiogenesis and inhibition of cell proliferation; and induction of apoptosis (Figure 1). With the cloning and characterization of two distinct carotenoid cleaving enzymes, recent research has focused on the metabolic fate of lycopene and the subsequent metabolites created. Several reports, including our own, suggest that the biological activities of lycopene may be mediated, in part, by lycopene metabolites. Lycopene metabolites and carotenoid metabolites in general, can possess either more or less activity than the parent compound or can have an entirely independent function. This review highlights the chemical and biological metabolism of lycopene and the potential actions of lycopene and its metabolites on chemoprevention.

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Abbreviations: ACR, acyclo-retinoic acid; ARE, antioxidant response element; CMO1, beta-carotene 15,15′-oxygenase; CMO2, carotene-9′,10′-oxygenase; Cx43, connexin 43; GJC, Gap Junction Communication; IGF, insulin-like growth factor; IGFBP, insulin-like growth factor binding protein; RAR, retinoic acid receptor; RARE, retinoic acid response element; RXR, retinoid X receptor.

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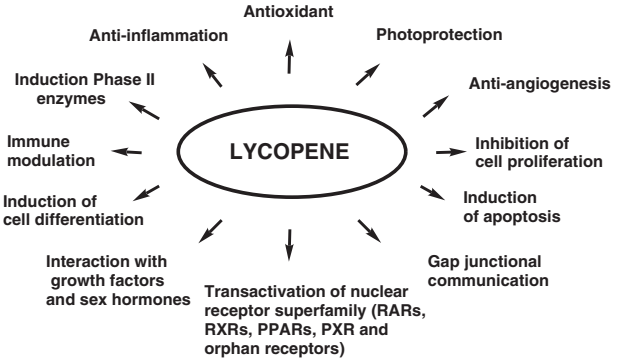

Figure 1 **Possible biological functions of lycopene.**

LYCOPENE METABOLISM

Carotenoids are a class of lipophilic compounds with a polyisoprenoid structure. Most carotenoids contain a series of conjugated double bonds, which are sensitive to oxidative modification and *cis-trans* isomerization. There are six major carotenoids (β-carotene, α-carotene, lycopene, β-cryptoxanthin, lutein, and zeaxanthin) that can be found routinely in human plasma and tissues. Among them, β-carotene has been the most extensively studied. More recently, lycopene has attracted considerable attention due to its association with a decreased risk of a number of chronic diseases, including cancers. Considerable efforts have been expended in order to identify its biological and physiochemical properties. Relative to β-carotene, lycopene has the same molecular mass and chemical formula, yet lycopene is an open-polyene chain lacking the β-ionone ring structure. While the metabolism of β-carotene has been studied extensively, the metabolism of lycopene remains poorly understood.

Chemical oxidation of lycopene

There have been a number of reports studying the formation of lycopene metabolites and oxidation products *in vitro*. Many of these studies have utilized various oxidizing systems and have identified several unique metabolites. Kim et al.[6] identified oxidative products after incubation of lycopene at 37°C for 72 h under atmospheric oxygen. Using three separate solubilization schemes (toluene, aqueous Tween 40, and liposomal suspension) eight oxidative products were identified: 3,7,11-trimethyl-2, 4, 6, 10-dodecatetraen-1-al, 6,10,14-trimethyl-3,5,7,9,13-pentadecapentaen-2-one, acycloretinal, apo-14′-lycopenal, apo-12′-lycopenal, apo-10′-lycopenal, apo-8′-lycopenal, and apo-6′-lycopenal. It was subsequently demonstrated that *acyclo*-retinal could be oxidized to the corresponding *acyclo*-retinoic acid when incubated with pig liver homogenate,[6] indicating potential *in vivo* formation. Incubation of deuterated lycopene with rat intestinal post-mitochondrial fractions and soy lipoxygenase led to the identification of several additional lycopene metabolites and oxidative products.[7] The cleavage products identified were 3-keto-apo-13-lycopenone and 3,4-dehydro-5, 6-dihydro-15,15′-apo-lycopenal, while the four oxidative metabolites identified were: 2-apo-5,8-lycopenal-furanoxide, lycopene-5,6,5′,6′-diepoxide, lycopene-5,8-furanoxide, and 3-keto-lycopene-5′,8′-furanoxide. Zhang et al.[8] isolated and identified a cleavage product of lycopene from an autooxidation mixture of lycopene products. Lycopene was oxidized by exposure to atmospheric oxygen and perfusion of ozone. A lycopene oxidative product was tentatively identified as (E, E, E)-4-methyl-8-oxo-2,4,6-nonatrienal.[8] Using a seperate oxidizing system, Aust et al.[9] identified an additional lycopene oxidative metabolite. Lycopene was completely oxidized using a combination of hydrogen peroxide and osmium tetroxide. The oxidized lycopene mixture was separated and a new metabolite was tentatively identified as 2,7,11-trimethyl-tetradecahexaene-1, 14-dial.[9] Utilizing yet another oxidizing system, Caris-Veyrat et al.[10] identified an extensive number of lycopene oxidative products. Oxidation of lycopene by potassium permanganate produced eight apo-lycopenals and three apo-lycopenones, as detected by HPLC-DAD-MS, including *acyclo*-retinal and apo-10′-lycopenal, which are the predicted central and excentric enzymatic cleavage products. Six apo-carotendials were also detected. Although a number of putative lycopene oxidative metabolites have been identified and characterized *in vitro*, few products identified in these reports have been identified *in vivo*. However, taken together, the results from these studies suggest that the susceptibility of carbonyl compounds to cleavage by autooxidation, radical-mediated oxidation, and singlet oxygen occurs in carotenoids with a long chain of conjugated double bonds. These products may be produced *in vivo* if the tissues are exposed to oxidative stress such as smoking and drinking. While oxidative metabolites are inevitably produced *in vivo*, the importance of such metabolites remains poorly understood.

ENZYMATIC CLEAVAGE OF LYCOPENE

Carotene-15, 15′-oxygenase

For provitamin A carotenoids, such as β-carotene, α-carotene, and β-cryptoxanthin, central cleavage is a major pathway leading to vitamin A formation (Figure 2).[11–13] The carotene 15,15′-oxygenase (CMO1) gene, which is responsible for central cleavage at the 15, 15′ double bond,[14,15] has been cloned and characterized in a number of species including the human and mouse.[16–20]

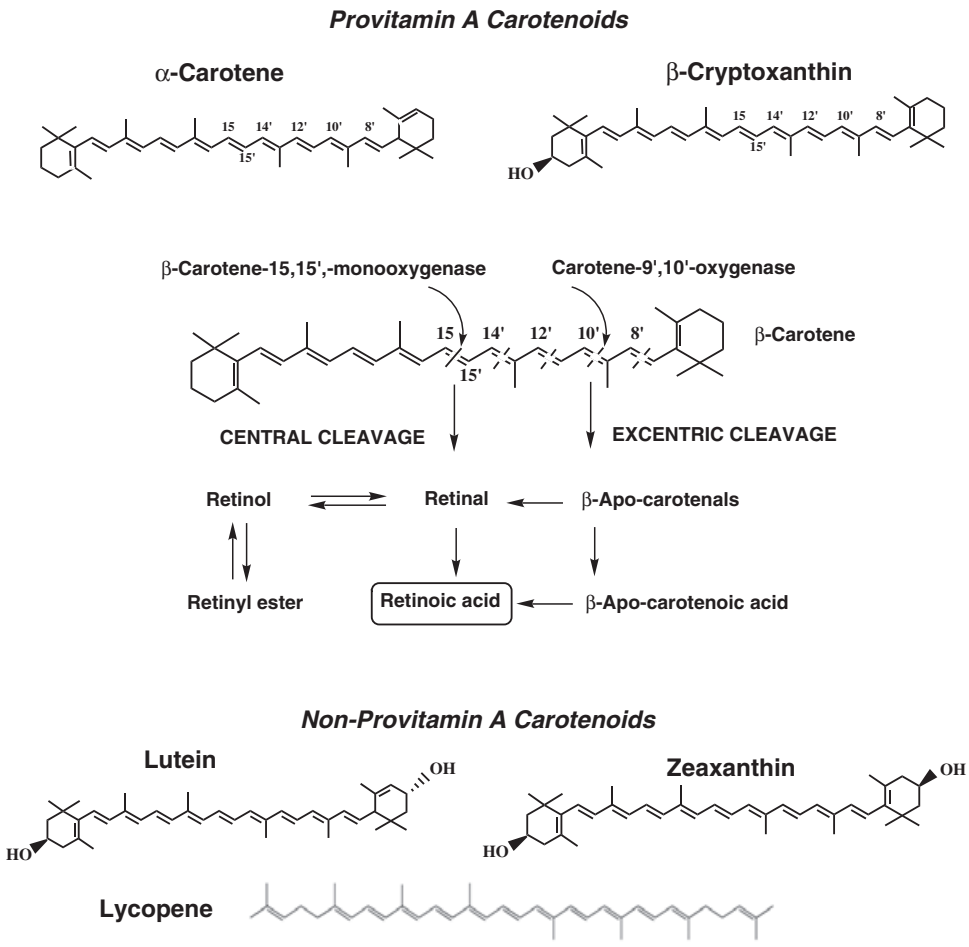

Figure 2 **Metabolic pathway of β-carotene, and chemical structures of provitamin A carotenoids (β-carotene, α-carotene, and β-cryptoxanthin) and non-provitamin A carotenoids (lutein, zeaxanthin, and lycopene).** Adapted from Merntiz et al. (2007).[11]

With the molecular characterization of CMO1, it has been definitively shown that CMO1 catalyzes the central cleavage of β-carotene to yield two molecules of retinal, thus contributing to vitamin A stores.[21] While many reports have focused exclusively on the ability of CMO1 to cleave β-carotene, few have explored other carotenoids such as lycopene as potential substrates, and those that have, have had mixed results. Using the murine CMO1, Redmond et al.[18] demonstrated cleavage activity towards lycopene. Using lycopene-accumulating *Escherichia coli*-expressing mouse CMO1, they observed a distinct bleaching of color when induced with L-arabinose, suggesting cleavage of lycopene. In addition, purified recombinant mouse CMO1 displayed cleavage activity towards lycopene. However, *acyclo*-retinal was only detected when the lycopene concentrations used were 2.5–3 times higher than the observed K_m for β-carotene ($K_m = 6$ μmol). In contrast, Yan et al.[20] observed no detectable activity of human retinal pigment epithelium CMO1 towards lycopene or lutein. Their report supported the data of previous reports. For example, when lycopene was incubated with the *Drosohpila* homologue of CMO1[14] and crude preparations of rat liver and intestine,[22] no lycopene cleavage products were detected. Additionally, Lindqvist and Andersson[16], using a purified recombinant CMO1 isolated from a human liver cDNA library, demonstrated cleavage activity towards both β-carotene and β-crytoxanthin but no activity towards lycopene or zeaxanthin. Although CMO1 was shown to cleave β-cryptoxanthin, analysis of the apparent K_m revealed an approximate fourfold lower affinity towards β-cryptoxanthin ($K_m = 30.0 \pm 3.8$ μM) than towards β-carotene ($K_m = 7.1 \pm 1.8$ μM).[16] These authors concluded that the presence of at least one unsubstituted β-ionone ring appears to be sufficient for catalytic cleavage of the central carbon 15,15′ double bond. Taken together, these studies suggest that lycopene is a poor substrate for CMO1. Yet, this fact has not hindered research into the potential biological activity of *acyclo*-retinoids.

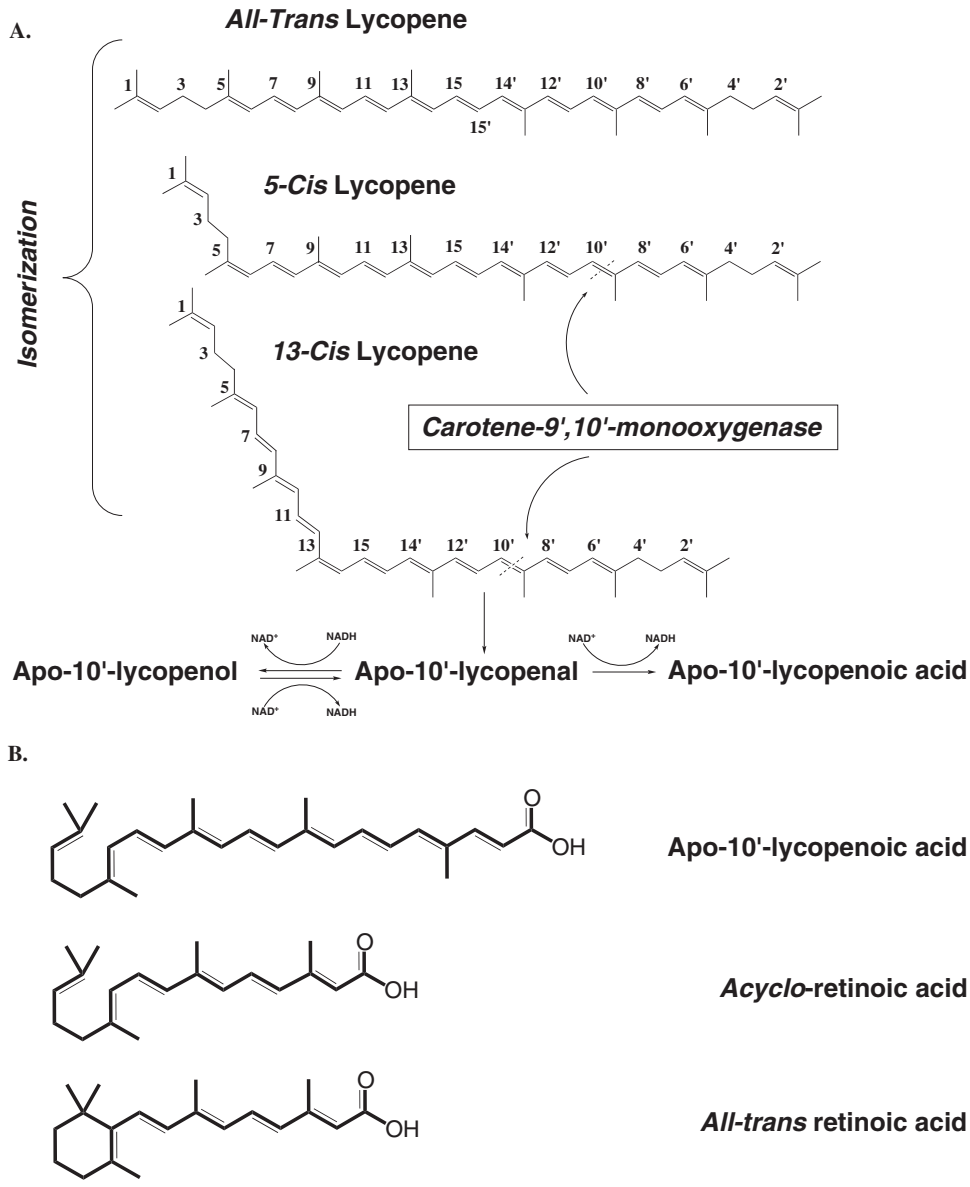

Figure 3 **Schematic illustration of lycopene metabolic pathway by CMO2.** (**A**) 5-cis and 13-cis lycopene are preferentially cleaved by CMO2 at 9′10′-double bond. The cleavage product, apo-10′-lycopenal, can be further oxidized to apo-10′-lycopenol or reduced to apo-10′-lycopenoic acid, dependent on the presence of NADH. (**B**) Chemical structures of apo-10′-lycopenoic acid, acycloretinoic acid, and *all-trans* retinoic acid.
Adapted from Hu et al. (2006).[23]

Although previous observations provide little evidence for lycopene as a substrate for CMO1, the previous observation that *cis*-lycopene isomers were superior substrates for CMO2 compared to all-*trans* lycopene[23] brings about an important question. Does CMO1 cleave *cis*-lycopene isomers to *acyclo*-retinoids? Previous *in vivo* reports using all-*trans* lycopene as a supplement observed dramatic increases in the *cis*-isomers of lycopene, including 5-*cis*, 13-*cis*, and 9-*cis* isomers.[24–27] From the previous *in vitro* kinetic analyses,[16,18,20] it is unclear if all-*trans* lycopene was used as the substrate in determination of CMO1 activity towards lycopene. The chemical structures of *cis*-lycopene isomers could mimic the unsubstituted β-ionone ring structures of other carotenoid molecules and fit into the enzyme-substrate pocket enabling central cleavage (Figure 3). More understanding of the significance of the metabolism of *cis*-lycopene will help further elucidate the biological functions of lycopene.

Carotene-9′, 10′-oxygenase

In addition to the central cleavage pathway, an alternative pathway for carotenoid metabolism in mammals, termed

the excentric cleavage pathway, remained a controversial issue for several decades. The controversy centered on the existence of a dedicated enzyme responsible for excentric carotenoid metabolism. We had previously demonstrated the random cleavage of β-carotene and identified a series of homologous carbonyl cleavage products, including β-apo-14′-, 12′-, 10′-, and 8′-carotenals, β-apo-13-carotenone, and retinoic acid in tissue homogenates of humans, ferrets, and rats.[28–30] This controversy was put to rest with the cloning and characterization of the murine carotene-9′, 10′-oxygenase by Kiefer et al.,[31] thus confirming the existence of the asymmetric cleavage pathway of carotenoids. Keifer et al.[31] isolated the murine CMO2 and demonstrated enzymatic cleavage of both β-carotene and lycopene. Using β-carotene as the substrate, HPLC analysis revealed that no retinoid compounds were formed, yet a peak matching the retention time of the β-apo-10′-carotenal standard was detected. Cleavage of β-carotene was further demonstrated using beta-carotene synthesizing and accumulating *Escherichia coli* strains expressing the mouse CMO2. A similar *E. coli* model, which synthesizes and accumulates lycopene, was used to demonstrate cleavage of lycopene by CMO2. When CMO2 was induced, a distinct color shift from red to white occurred, indicating cleavage. HPLC analysis revealed the formation of several putative apo-lycopenals. Although the authors state that significant amounts of apo-lycopenals were detected and tentative identifications were made based upon UV-visible spectra, no data was shown to accompany these observations.[31] Given the putative observations regarding lycopene and cleavage by CMO2, we sought to further characterize the relationship of lycopene as a possible substrate for CMO2.

Because ferrets (*Mustela putorius furo*) and humans are similar in terms of carotenoid absorption, tissue distribution and concentrations, and metabolism,[32,33] we cloned and characterized the ferret CMO2 gene.[23] Using the reported cDNA sequence for a carotene excentric cleavage enzyme from humans, we cloned a full-length carotene-9′, 10′-oxygenase in ferrets that encodes a protein of 540 amino acids and has 82% identity with human carotene, 9′, 10′-oxygenase. Further analysis revealed that the enzyme is expressed in the testis, liver, lung, prostate, intestine, stomach, and kidneys of ferrets, similar to the expression pattern of human CMO2.[34] Using the recombinant ferret CMO2 expressed in *Spodoptera frugiperda* (Sf9) insect cells for kinetic analysis, we found that the cleavage of carotenoids by the ferret CMO2 occurs in a pH, incubation time, protein dose, and substrate dose-dependent manner.[23] Notably, the optimum pH for CMO2 is 8.5, which differs from the optimum pH (7.7) for the activity of CMO1, the central cleavage enzyme for carotenoids.[16] This difference in optimum pH between these two carotenoid cleavage enzymes may indicate different roles of the two pathways in carotenoid metabolism or different functions in various pathophysiological conditions, which needs further investigation. Nonetheless, similar to the CMO1, we found that the cleavage activity of ferret CMO2 for both β-carotene and lycopene was iron-dependent, indicating that iron is an essential cofactor for the enzymatic cleavage activity of carotenoids. This is supported by the existence of four conserved histidine residues in the ferret CMO2.[23] These data are in agreement with previous observations demonstrating these conserved histidines act as putative iron-binding residues for iron coordination in apocarotenoid 15,15′-oxygenase[35] and CMO1,[36] supporting the notion that the entire superfamily of oxygenases shares a common structure.[36]

Interestingly, we demonstrated that the recombinant ferret CMO2 catalyzes the excentric cleavage of *all-trans* β-carotene and *cis*-lycopene isomers effectively but not *all-trans* lycopene at the 9′, 10′ double bond.[23] While we estimated a K_m of 3.5 μM for *all-trans* β-carotene based on the CMO2 expressed in SF9 cells, we could not calculate the kinetic constants of CMO2 for lycopene due to difficulty in controlling auto-isomerization, thus necessitating the use of mixed isomers of lycopene as the substrate for kinetic analysis. Since the lycopene substrate mixture contains only ~20% as *cis* isomers and considering the ferret CMO2 would not cleave *all-trans* lycopene, we speculate that the K_m for *cis*-lycopene is actually much lower than that of the lycopene isomer mixture. This indicates that *cis*-lycopene may act as a better substrate than *all-trans* β-carotene for the ferret CMO2. The mechanism whereby ferret CMO2 preferentially cleaves the 5-*cis* and 13-*cis*-isomers of lycopene into apo-10′-lycopenal but not *all-trans* lycopene is currently unknown. One possible explanation is that the chemical structure of *cis* isomers of lycopene could mimic the ring structure of the β-carotene molecule and fit into the substrate-enzyme binding pocket (Figure 3). Although this hypothesis warrants further investigation, the observation that supplementation of all-*trans* lycopene results in a significant increase in *cis*-lycopene tissue concentration in ferrets underlies the significance of this observation.[24–26]

REGULATION OF CAROTENE OXYGENASES

Unlike vitamin A, high-dose β-carotene supplementation does not result in hypervitaminosis A, indicating that cleavage of β-carotene to vitamin A is tightly regulated. A number of animal studies have demonstrated that CMO1 activity is affected by vitamin A status.[37,38] Other studies have indicated that expression of CMO1 may be regulated at the transcriptional level through feedback regulatory mechanisms via interactions between retinoic acid

and its nuclear receptors.[39,40] Recent molecular studies of the mouse and human CMO1 promoter demonstrated the presence of a peroxisome proliferator response element (PPRE).[41,42] PPARγ and RXRα agonists were shown to transactivate the CMO1 promoter-reporter when cotransfected with the corresponding nuclear receptor.[41] Analysis of the human CMO1 promoter identified an additional enhancer element. A myocyte enhancer factor-2 (MEF2) binding site was identified and when mutated reduced luciferase activity by ~30%.[42] The *in vivo* importance of the MEF2 binding site is not fully understood. Nonetheless, the regulation by PPAR and RXR indicates a regulatory link between carotenoid and lipid metabolism. Two recent reports have shed some light onto this relationship. In F344 rats supplemented with lycopene, CMO1 expression was significantly decreased in the adrenal gland and kidney.[43] Interestingly, fatty acid binding protein-3 (FABP-3), a PPARγ target gene, was downregulated in parallel with CMO1. While vitamin A production from β-carotene was abolished in CMO1-knockout (KO) mice, lipid metabolism was significantly altered.[21] There was a significant increase in several fatty acid metabolism genes, including CD36 and fatty acid binding protein-4 (FABP4) expression, both PPARγ target genes, in visceral adipose tissue. Additionally, there were significant increases in serum free fatty acids and total lipids resulting in hepatic steatosis. It has been suggested that cleavage products of β-carotene may fine-tune the crosstalk between the nuclear receptors that regulate lipid metabolism.[44–46] The relationship between carotenoid and lipid metabolism deserves further inquiry.

Regulation of CMO2 is less well understood. A recent analysis failed to identify a PPRE within the mouse CMO2 promoter.[43] Work in our laboratory also failed to identify any potential nuclear receptor response elements or any other enhancer sequences within the ferret CMO2 promoter (unpublished data). Recent *in vivo* studies have provided evidence of an effect of lycopene on CMO2 expression. After 9 weeks of lycopene supplementation, we observed an ~4-fold increase in CMO2 expression in the lungs of ferrets.[23] In F344 rats, however, there was no significant effect of lycopene on CMO2 expression in several tissues, including lungs.[43] β-carotene supplementation also had no significant effect on CMO2 expression. In ferrets supplemented with low- and high-dose β-carotene and exposed to cigarette smoke for 6 weeks, we observed no change in lung and liver CMO2 expression.[47] Similar findings were observed using CMO1-knockout mice. CMO1-KO mice supplemented with β-carotene accumulated significant amounts of β-carotene in various tissues.[21] However, there were no changes in CMO2 protein expression in any tissues analyzed. Clearly, more research is needed in order to gain a better understanding of the transcriptional regulatory mechanisms of CMO2.

LYCOPENE METABOLITES *IN VIVO*

While *in vitro* oxidation studies have yielded a large number of oxidative metabolites, *in vivo* studies have yielded a much smaller catalogue of lycopene metabolites.[48,49] Khachiket al.[50–52] first identified 5,6-dihydroxy-5, 6-dihydrolycopene in human serum. It was proposed that formation of this metabolite results from oxidation of lycopene forming 5,6 epoxide, which is then reduced to the 5,6-dihydroxy-5, 6-dihydrolycopene metabolite. The same group also identified epimeric 2,6-cyclolycopene-1, 5-diols in human milk and serum.[53] Based upon studies of lycopene oxidation with *m*-chloroperbenzoic acid,[54,55] it was proposed that lycopene is first oxidized at 1, 2 – and 5, 6 –positions to form lycopene 1,2-epoxide and lycopene 5,6-epoxide. Due to the instability of the epoxide rearrangement products, cyclization occurs and results in the corresponding diols. Interestingly, epimeric 2,6-cyclolycopene -1,5-diols are also found in low concentrations in tomato-based products.[55] Whether the presence of these metabolites results from consumption of tomato-based products or from *in vivo* oxidation or both is not fully understood.

The use of animal models has proven to be useful in the identification of *in vivo* lycopene metabolites. A study in preruminant cattle identified as 5, 6-dihydrolycopene and 5, 6-dihydro-5-*cis* lycopene in serum after 2 weeks of lycopene supplementation.[56] Using ^{14}C-labeled lycopene, Gajic et al.[57] detected both apo-8′-lycopenal and apo-12′-lycopenal in rat liver 24 h post dosing. In addition, a large quantity of very polar, unidentified short-chain compounds was detected. We have recently identified apo-10′-lycopenol in ferret lungs, which is the predicted cleavage product of CMO2, and certain unidentified compounds in the HPLC profiles of ferret lungs after lycopene supplementation for 9 weeks.[23] Since we did not detect apo-10′-lycopenal or apo-10′-lycopenoic acid in the lung tissues of lycopene-supplemented ferrets, it is likely that apo-10′-lycopenal is a short-lived intermediate compound and can be reduced to its alcohol form *in vivo*, and apo-10′-lycopenoic acid may be present at too low a concentration to be detected in our HPLC system. This was supported by our subsequent demonstrations that incubation of apo-10′-lycopenal with the post-nuclear fraction of hepatic tissue of ferrets resulted in both apo-10′-carotenol and apo-10′-lycopenoic acid, depending upon the presence of either NAD^+ or NADH. In the presence of NADH, apo-10′-lycopenal was converted to both the alcohol and acid forms (Figure 3). The latter could be due to the consumption of NADH for the reduction reaction, which makes NAD^+ available for the oxidation of

apo-10′-lycopenal. Nonetheless, the presence of specific metabolites has not been consistent across different animal models. Whether these differences are due to different methodological approaches, species used, or tissues analyzed should be ascertained.

BIOLOGICAL ACTIVITY OF LYCOPENE METABOLITES

The identification of lycopene metabolites *in vitro* and *in vivo* raises the question of whether lycopene metabolites, similar to other carotenoid metabolites that can possess either more or less activity than the parent compound or have entirely different functions,[58] may contribute, at least in part, to the biological functions ascribed to lycopene.

Antioxidant properties

Much of the biological activity ascribed to lycopene has been attributed to its antioxidant capabilities. Indeed, antioxidant properties of many carotenoids have been long believed to play critical roles in their anticarcinogenic actions.[59] Lycopene is a powerful *in vitro* antioxidant. Among naturally occurring carotenoids, lycopene has shown the strongest ability to scavenge free radicals[60] and chemically quench singlet oxygen,[61,62] being 2-fold and 10-fold more effective at quenching singlet oxygen than β-carotene and α-tocopherol, respectively.[62] Accordingly, several epidemiological studies have evaluated the role of lycopene as a potential *in vivo* antioxidant. Using tomatoes or tomato products, numerous studies have demonstrated decreased DNA damage,[63,64] decreased susceptibility to oxidative stress in lymphocytes,[65,66] and decreased LDL oxidation[67] or lipid peroxidation.[67,68] However, most *in vivo* studies have used tomato products, which also contain various micronutrients and phytochemicals, including other carotenoids, polyphenols, vitamin C, and vitamin E. Caution must be taken when attributing the beneficial effects of tomatoes and tomato products solely to lycopene. Data regarding the antioxidative effects of lycopene alone in biological systems are limited.

While evidence suggests that intact lycopene functions as an antioxidant, especially *in vitro*, there is little evidence to support an antioxidant role of lycopene metabolites. However, recent evidence suggests that lycopene metabolites may indeed possess putative antioxidant functions. We have recently provided evidence of a possible indirect antioxidant effect of the excentric cleavage product apo-10′lycopenoic acid in BEAS-2B cells. After 24 h of treatment with apo-10′-lycopenoic acid (3–10 μM), we observed a dose-dependent decrease in endogenous ROS production.[69] This decrease in ROS was comparable to control cells treated with *tert*-butylhydroquinone (tBHQ). We next determined if apo-10′-lycopenoic acid had any effect on H_2O_2-induced oxidative damage, as measured by lactate dehydrogenase release (LDH). Pre-treating BEAS-2B cells with apo-10′-lycopenoic acid (3–10 μM) for 24 h resulted in a dose-dependent inhibition of LDH release. These results were comparable to control cells pre-treated with tHBQ.[69] Taken together, our data suggests that lycopene metabolites in general, and apo-10′-lycopenoic acid in particular, may possess indirect antioxidant functions. More research is needed.

Gap-junction communication

Gap junctions are cell-to-cell channels that enable connected cells to exchange nutrients, waste products, and information. Each gap junction is derived from six connexin proteins from each adjacent cell for a total of 12 connexin proteins. The connexin family has >20 connexins that are expressed in mammals with both cell and developmental specificity of expression.[70] Although there are >20 connexins, connexin 43 (Cx43) is the most widely expressed connexin. More interestingly, Cx43 is the connexin most often induced by retinoids and carotenoids. Gap junction communication (GJC) has been implicated in the control of cell growth via adaptive responses, i.e., differentiation, proliferation, and apoptosis.[71] A large body of evidence now indicates loss of GJC is a hallmark of carcinogenesis.[72]

Targeting connexins as a possible strategy for chemoprevention has been suggested.[72] Retinoids and carotenoids have been shown to inhibit carcinogenesis. Moreover, retinoids and carotenoids increase GJC between normal and transformed cells.[73,74] Interestingly, it was demonstrated that both pro-vitamin A and non-provitamin A carotenoids inhibited carcinogen-induced neoplastic transformation[75] and upregulated Cx43 mRNA expression.[73,74] Furthermore, whereas treatment with retinoic acid increased Cx43 expression within 6 h, carotenoid treatment required approximately three times longer and produced the same response.[76,77] This lag in activity is often attributed to the formation of active metabolites.

Several lines of *in vitro* evidence indicate that carotenoid oxidative products/metabolites may be responsible for increased GJC, especially in the case of lycopene. After complete oxidation of lycopene with hydrogen peroxide and osmium tetroxide, Aust et al.[9] isolated an oxidative metabolite that effectively increased GJC. The compound, identified as 2,7,11-trimethyl-tetradecahexaene-1, 14-dial, induced GJC to a level comparable to retinoic acid. The oxidative metabolite lycopene-5,6-epoxide, which is found in tomatoes, was shown to increase Cx43 expression

in human keratinocytes.[52] Stahl et al.[78] demonstrated that the central cleavage product of lycopene, *acyclo*-retinoic acid (ACR), could increase GJC. However, an effect of ACR on GJC was only achieved at high concentrations, indicating that the contribution of ACR to the activity of lycopene on GJC appears to be minimal. More recently, we demonstrated cleavage of lycopene to apo-10′-lycopenal by ferret CMO2.[23] While the Cx43 promoter does not contain an RARE, it has been reported that RAR antagonists inhibited upregulation by retinoids and had no effect on the effect of carotenoids.[79] This is interesting due to the effect of both oxidative metabolites and enzymatic cleavage metabolites of lycopene on modulating GJC, which could provide two separate pathways of increasing GJC. Considering the bioconversion of lycopene into apo-10′-lycopenoids, whether apo-10-lycopenoids contribute to lycopene activity on GJC warrants further study.

Retinoid activity

Retinoids, the most important oxidative products of provitamin A carotenoids, play an essential role in several critical processes, including vision, reproduction, metabolism, differentiation, hematopoiesis, bone development, and pattern formation during embryogenesis.[80] Considerable evidence supports the role of retinoids in the management of several human chronic diseases, including cancer.[81] The mechanism by which retinoids elicit these responses is through their ability to regulate gene expression at specific target sites within the body. Beta-carotene and its excentric cleavage metabolites can serve as direct precursors for all-*trans* and 9-*cis*-retinoic acid,[30,82,83] which are ligands for both retinoic acid receptors (RAR) and retinoid X receptors (RXR). Retinoid receptors function as ligand-dependent transcription factors and regulate gene expression by binding as dimeric complexes to the retinoic acid response element (RARE) and the retinoid X response element (RXRE), which are located in the 5′ promoter region of responsive genes. RXR can not only form dimeric complexes with RAR, it can also dimerize with other members of the nuclear hormone receptor superfamily, such as thyroid hormone receptors, the vitamin D receptor (VDR), peroxisome proliferators-activated receptors (PPAR), and possibly other receptors with unknown ligands designated orphan receptors.

Upregulation of retinoid receptor expression and function by provitamin A carotenoids may play a role in mediating the growth inhibitory effects of retinoids in cancer cells.[84,85] However, it is unclear if non-provitamin A carotenoids and their metabolites may function in a similar fashion. Several reports have evaluated the ability of the CMO1 cleavage product, acyclo-retinoic acid (ACR), to transactivate the RARE. Using a RARE-reporter gene, Ben-Dor et al.[86] demonstrated that ACR transactivates the RARE-reporter gene through an interaction with RARα. However, the potency of activation was approximately 100-fold lower than retinoic acid. Binding affinity studies indicated that ACR bound RXRα with no appreciable affinity, whereas ACR bound RARα with an equilibrium dissociation constant in the range of 50–150 nM – two orders of magnitude lower than all-*trans* retinoic acid. Intact lycopene did not show any significant binding to either receptor. However, intact lycopene displayed a weak transactivation of the RARE-reporter gene.[86] Stahl et al.[78] demonstrated similar findings with the RAR-β2 promoter. Only when ACR was provided at concentrations 500-fold higher than retinoic acid was an effect on luciferase and β-galactosidase reporter activity observed.[78] There was no effect of intact lycopene on reporter transactivation at the concentrations used in this study. ACR was also found to have no significant effect on transactivation of RAR and RXR reporter systems in yet another study.[87] While an effect of ACR on retinoid signaling has not been substantiated *in vivo*, a synthetic acyclic retinoid, lacking one or two double bonds, has been shown to transactivate retinoid reporter systems with potential benefits in the treatment of hepatocellular carcinoma.[88–90]

Because of the similarity in chemical structures among apo-10′lycopenoic acid, *acyclo*-retinoic acid, and *all-trans* retinoic acid (Figure 3), we questioned whether apo-10′-lycopenoic acid is an activator of RARs. We hypothesized that the antiproliferative effects of the excentric cleavage product apo-10′-lycopenoic acid[91] may be mediated through induced expression of the RARβ receptor, which has been suggested to be a tumor suppressor gene playing a critical role in mediating the growth inhibitory effects of retinoids in various cancer cell lines.[92] Three cell lines – NHBE, a normal human bronchial epithelial cell line, BEAS-2B, an immortalized human bronchial epithelial cell line, and A549 cells, a non-small-cell lung cancer cell – representing different stages of lung carcinogenesis were incubated with increasing concentrations of apo-10′-lycopenoic acid (3–5 μmol/L).[91] After 48 h, we observed a dose-dependent increase in RARβ mRNA expression in both NHBE and BEAS-2B cell lines. The effect of apo-10′-lycopenoic acid was similar to that of all-*trans*-retinoic acid. However, there was only a slight increase in expression of RARβ in A549 cells. Compared to NHBE and BEAS-2B cells, A549 cells were less sensitive to apo-10′-lycopenoic acid and retinoic acid in terms of RARβ expression. We next sought to determine if increased RARβ mRNA expression was due to increased transactivation of the RARβ promoter region. A series of reporter constructs containing serially deleted fragments of the RARβ promoter were constructed. Treatment with apo-

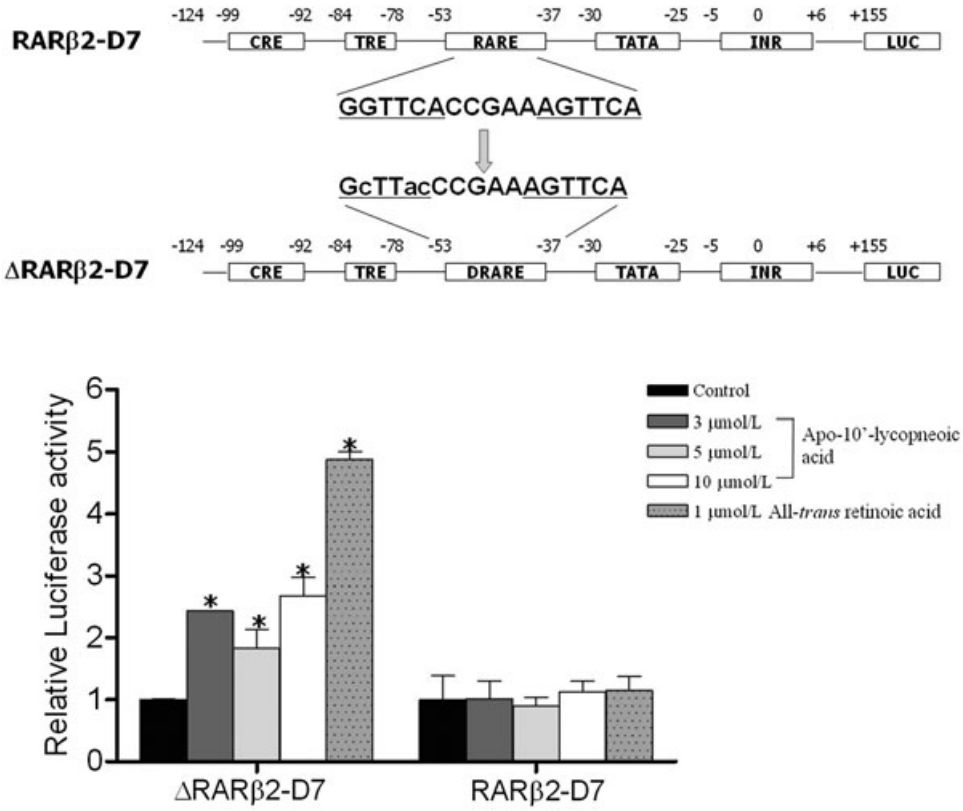

Figure 4 **The involvement of RARE on apo-10'-lycopenoic acid-transactivated RARβ expression.** Upper panel shows a diagram of the construction of the RARβ reporter vector with wild-type or mutated RARE. Lower panel, RARβ reporter vector was treated with apo-10′-lycopenoic acid or *all-trans* retinoic acid for 24 h. Luciferase activities were measured by dual-luciferase reporter system. Values are means ± SEM of three replicate assays.
*Statistically different, as compared with control in the same group, $P < 0.05$.
Adapted from Lian et al. (2007).[91]

10′-lycopeneoic acid increased luciferase activity in all constructs. However, the magnitude of the increase in luciferase was less than that induced by retinoic acid. The deletion of the promoter region between −1500 and −124 bp did not affect the transactivation activity of apo-10′-lycopenoic acid. To investigate the involvement of the RARE in the promoter, located between −53 and −37 bp, site-directed mutagenesis was utilized to abolish the RAR binding site (Figure 4). Both apo-10′-lycopenoic acid and retinoic acid significantly increased luciferase activity in the presence of the wild-type RARE. However, mutation of the RAR binding site completely abolished promoter activity induction by both retinoic acid and apo-10′-lycopenoic acid (Figure 4). These results suggest that the growth inhibitory actions of apo-10′-lycopenoic acid may be mediated through retinoid signaling.

Induction of phase II enzymes

The drug-metabolizing phase I and II enzymes respond to various compounds, including drugs, environmental compounds, pollutants, carcinogens, and dietary and endogenous compounds. Phase I enzymes, such as cytochrome P450, catalyze the addition of oxygen to carcinogens, thereby increasing the reactivity of carcinogens and the formation of DNA adducts, known as bioactivation.[93] In general, the action of phase II enzymes increases the hydrophilicity of carcinogens and enhances their detoxification and excretion.[94] Evidence in recent years has begun to accumulate indicating the beneficial effect of lycopene may be due, in part, to induction of phase II detoxification enzymes.[95] Induction of phase II enzymes is mediated through *cis*-regulatory DNA sequences located in the promoter or enhancer region, which are known as antioxidant response elements (ARE).[96] The major ARE transcription factor Nrf2 (nuclear factor E_2-related factor 2) is a primary factor involved in induction of antioxidant and detoxifying enzymes[97] and is essential for the induction of several phase II enzymes, including glutathione *S*-transferases, NAD(P)H:quinone oxidoreductase.[98]

Many carotenoids, including lycopene, have been shown to induce several phase I and II enzymes both

in vivo and *in vitro*.[43,99–101] Gradelet et al.[99] observed an induction in the phase II enzymes *p*-nitrophenol-UDP-glucuronosyltransferase and quinone oxidoreductase in rats fed various carotenoids. While canthaxanthin and astaxanthin induced phase II activity, lycopene and lutein had no effect after 15 days of feeding. In another study, Breinholt et al. demonstrated a dose-dependent induction of several phase I and II enzymes in female Wistar rats supplemented with lycopene at doses ranging from 0.001 to 0.1 g/kg body weight for 2 weeks.[100] Hepatic ethoxyresorufiin O-dealkylase (EROD) and benzyloxyresorufin O-dealkylase (BROD) increased approximately 2-fold and 50%, respectively, suggesting activation of cytochrome P450 enzyme (CYP) 1A (CYP1A) enzymes. In addition, several liver and red blood cell phase II enzyme activities, such as glutathione reductase, glutathione *S*-transferase, and quinone reductase, were significantly increased by lycopene feeding. The induction of phase II enzymes by lycopene has been reported in other animal studies.[43,102] However, it is unclear if induction of xenobiotic metabolizing enzymes is due to intact lycopene or lycopene metabolites. Ben-Dor et al. showed that lycopene induced phase II enzymes by activating Nrf2 transcription factor. More interestingly, an ethanolic extract of lycopene containing unidentified hydrophilic derivatives activated ARE-driven reporter gene at a similar potency to lycopene alone.[101] Although the identity of the lycopene oxidative derivatives is unknown, this study suggested that lycopene oxidative metabolites might be responsible for the induction of phase II enzymes through ARE-induced expression.

We have recently provided evidence that the lycopene CMO2 cleavage product, apo-10′-lycopenoic acid, induces phase II enzyme expression *in vitro*. Using BEAS-2B human bronchial epithelial cells, we demonstrated a dose- and time-dependent increase in nuclear Nrf2 protein accumulation with apo-10′-lycopenoic acid treatment.[69] In addition, apo-10′-lycopenoic acid significantly induced mRNA expression of several phase II enzymes, including heme-oxygenase-1 (HO-1), quinone oxidoreductase, glutathione S-transferase, glutathione reductase, glutamate-cysteine ligase (catalytic unit, GCLC; and modifier unit, GCLM), microsomal epoxide hydrolase 1, and UDP glucuronosyltransferase 1 family, polypeptide A6 compared to THF alone.[69] Additionally, all three apo-10′-lycopenoids were effective in activating the Nrf2-mediated induction of HO-1 (Figure 5). Although the exact mechanisms behind activation of the Nrf2-dependent HO-1 induction by these three lycopenoids remain unknown, the chemical properties of these compounds may contribute to the activation of Nrf2, which is controlled through multiple regulatory mechanisms, including Keap1-mediated ubiquitination and degradation, subcellular distribution, and phosphorylation. Notably, apo-10′-lycopenal showed the strongest induction of HO-1 as compared with apo-10′-lycopenoic acid and apo-10′-lycopenol. This may be due to the highly reactive aldehyde group in the compound, which is capable of Schiff base formation with the amino groups of protein and reactive with cellular macromolecules (e.g., directly modifying the reactive cysteine residues in Keap1 and interrupting Keap1-mediated Nrf2 ubiquitination and degradation). It is also possible that these lycopenoids affect upstream signaling pathways, such as mitogen-activated protein kinases, phosphoinositol 3-kinase, epidermal growth factor receptor, and protein kinase C, which all have been shown to play a role in the regulation of Nrf2-ARE in lung epithelial cells. Clearly, these hypotheses warrant further investigation.

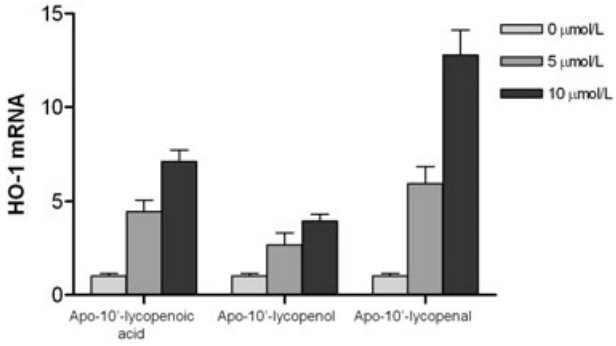

Figure 5 **Effect of apo-10'-lycopenoic acid, apo-10'-lycopenol, and apo-10'-lycopenal on hemo-oxygenase-1 gene expression.** Values are means ± SEM of three replicate assays.
Adapted from Lian et al. (2008).[69]

Interference with growth factors

It has been suggested that the insulin-like growth factors (IGF) signaling system may play a role in the biological action of lycopene.[103,104] IGFs (IGF-1 and IGF-2) are mitogens that play a central role in the regulation of cellular proliferation, differentiation, and apoptosis.[105] By binding to membrane IGF-1 receptors, IGFs activate intracellular phospatidylinositol 3′-kinase/Akt/protein kinase B and Ras/Raf/mitogen-activated protein kinase pathways, which regulate various biological processes such as cell cycle progression, survival, and transformation.[106] IGFs are sequestered in circulation by a family of binding proteins (IGFBP1–IGFBP6), which regulate the availability of IGFs to bind to IGF receptors.[106] Disruption of normal IGF signaling, leading to hyperproliferation and survival signal expression, has been implicated in the development of several tumor types.[107] Indeed, strong positive associations have been found between plasma IGF-I levels and prostate cancer risk,[108] breast

cancer risk,[109] and colorectal cancer risk.[110] Recent epidemiological studies provide supportive evidence that lycopene may have a chemopreventive effect against a broad range of epithelial cancers, particularly prostate, breast, colorectal, and lung cancer.[3,111–114] Sharoni et al. provided a potential mechanism whereby lycopene interfered with IGF-1-stimulated cell growth.[103,104,115] They showed that IGF-1-stimulated cell growth, as well as DNA binding activity of the AP-1 transcription factor, were reduced by physiological concentrations of lycopene in endometrial, mammary (MCF-7), and lung (NCI-H226) cancer cell lines. Lycopene has been shown to inhibit IGF-1-stimulated insulin receptor substrate 1 phosphorylation and cyclin D1 expression, block IGF-1-stimulated cell cycle progression,[103,116] and increase membrane-associated IGFBPs.[103,115] Consistent with previous *in vitro* findings, recent epidemiological studies demonstrated higher dietary intake of lycopene has been associated with lower circulating levels of IGF-1[117] and higher levels of IGFBPs.[118,119]

We have examined the effect of lycopene on prevention of IGF signaling in cigarette smoke-related lung carcinogenesis in the ferret model.[25] We found that plasma IGF-1 levels were not affected by cigarette smoke exposure or lycopene supplementation. However, IGFBP-3 levels were increased by lycopene supplementation and decreased by smoke exposure. Interestingly, lycopene increased plasma IGFBP-3 regardless of smoke exposure status. Increased plasma IGFBP-3 was associated with inhibition of cigarette smoke-induced lung squamous metaplasia, decreased proliferating cell nuclear antigen (PCNA), phosphorylated BAD levels, and cleaved caspase 3, suggesting inhibition of cell proliferation and induction of apoptosis.[25] These results, along with others, suggest that interference of IGF-1 signaling may be an important mechanism by which lycopene exerts its anticancer activity. However, whether intact lycopene or its metabolites are responsible for the observed effects on IGF-1 signaling remains unknown. We recently provided evidence that lycopene metabolites may be partly responsible. Treatment with apo-10′-lycopenoic acid (5–20 μM) resulted in a dose-dependent increase in IGFBP-3 mRNA levels in THLE-2 human liver cells. Similar concentrations of retinoic acid, lycopene, and ACR showed no significant effect on induction of IGFBP-3 mRNA levels (unpublished results). Research into this area is ongoing in our laboratory.

Cell proliferation and apoptosis

The growth inhibitory effect of lycopene was first demonstrated by Levy et al., who showed that lycopene is a stronger cell growth inhibitor than β-carotene.[104] This growth inhibitory effect was further observed in several cell lines, including breast cancer,[104,120] prostate cancer,[104] lung cancer,[104] human colon cancer cells,[121] and oral cavity cancer,[122] as well as normal prostate epithelial cells.[123] Cell proliferation is controlled by a series of cell cycle regulators, including cyclins, cyclin-dependent kinases (CDKs), and CDK inhibitors, which regulate cell cycle progression. Specifically, cyclin D regulates the transition from G_0 to early G_1 phase, while cyclin E regulates the transition of the cell from late G_1 phase to S phase; p21 and p27 CDK inhibitors bind and inhibit the activity of cyclin E/CDK2 complex, blocking cell cycle progression in G_1 phase. The growth inhibition of lycopene on MCF-7 breast cancer cells was associated with decreased G_1-S cell cycle progression, decreased cyclin D1 expression, and stabilization of p27 in the cyclin E-CDK complex.[116,120] Lycopene was recently associated with an inhibition of tumor invasion, cell proliferation, and angiogenesis in the lungs of nude mice injected with SK-Hep-1 human hepatoma cells.[124] In addition to cell proliferation inhibition, the growth inhibitory effect of lycopene may also be attributed to induction of apoptosis. Hwang et al. observed that 1 μM water-soluble lycopene inhibited the growth of LNCaP prostate cancer cells while 5 μM lycopene blocked cells in G2/M phase and induced apoptosis, suggesting high concentrations of lycopene can induce DNA damage.[125] In another study, physiological concentrations of lycopene (0.3–3 μM) did not affect proliferation of LNCaP cells but rather affected mitochondrial function and induced apoptosis.[126] Palozza et al.[127] reported that lycopene (0.5–2 μM) inhibited the growth of cigarette smoke condensate-exposed immortalized RAT-1 fibroblast cells by arresting cell cycle progression and inducing apoptosis.

Among identified lycopene metabolites, the central cleavage product, ACR, an analog of retinoic acid, is the best studied. It has been shown to inhibit cell proliferation[86,128,129] and induce apoptosis[130] in a variety of cell lines. Using lycopene, ACR, and retinoic acid, Ben-Dor et al. observed a decrease in cell growth and a decreased rate of cell cycle progression, especially G_1 to S transition, in MCF-7 mammary cancer cells.[86] Both ACR and retinoic acid inhibited cell growth with a similar potency (IC_{50} ~ 1–2 μM) to lycopene. Moreover, both ACR and retinoic acid decreased serum-stimulated cyclin D_1 protein expression, a similar finding also observed with intact lycopene.[120] In addition to ACR, the activity of other lycopene metabolites has been investigated. Zhang et al.[8] identified an oxidative product of lycopene, (E, E, E)-4-methyl-8-oxo-2,4,6-nonatrienal, that induced apoptosis in HL-60 cells. A dose-dependent reduction of cell viability with a concomitant increase in chromatin condensation and nuclear fragmentation, characteristic of apoptosis, were observed. Further analysis revealed an increased ratio of sub-G_1 cells and an increase in

caspase-8 and caspase-9 activity. These apoptotic changes were accompanied by a decrease in Bcl2 and Bcl-X_L protein expression but no changes in Bax expression. In spite of the evidence above, the physiologic role of these lycopene products remains unknown since none of these metabolites have been detected in biological systems.

Recently, we investigated the activity of the excentric cleavage product apo-10′-lycopenoic acid on cell proliferation in the following three cell lines: NHBE, a normal human bronchial epithelial cell line; BEAS-2B, an immortalized human bronchial epithelial cell line; and A549 cells, a non-small-cell lung cancer cell, which represent different stages of lung carcinogenesis.[91] We showed that apo-10′-lycopenoic acid treatment inhibited cell growth in all three cell lines, albeit with different sensitivity. Three micromoles apo-10′-lycopenoic acid significantly reduced cell numbers by 40 and 60% in NHBE and BEAS-2B, respectively, whereas cell numbers were reduced ~50% in A549 cells treated with 10 μM apo-10′-lycopenoic acid. The growth inhibitory effect was further examined in BEAS-2B and A549 cells using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Cell viability was decreased by ~20% in A549 cells while viability decreased ~80% in BEAS-2B cells treated with 0.5 μM apo-10′-lycopenoic acid. The growth inhibitory action of apo-10′-lycopenoic acid was largely due to decreased cell proliferation, as we did not observe any induction of apoptosis. The effects of apo-10′-lycopenoic acid on cell cycle distribution were analyzed by fluorescent-activated cell sorting analysis (FACS). Treatment with apo-10′-lycopenoic acid for 48 h significantly decreased A549 cells in S-phase from 31% in cells treated with THF alone to 24% and 21% in cells treated with 3 and 5 μM apo-10′-lycopenoic acid. Accordingly, there was a concomitant increase in the number of cells in G_1/G_0 phase. These results suggested the effect of apo-10′-lycopenoic acid on cell proliferation was due to effects on cell cycle regulators; thus, we investigated potential cell cycle regulators to identify potential targets of apo-10-lycopenoic acid. Treatment of A549 cells resulted in a dose-dependent decrease in mRNA and protein levels of cyclin E but not cyclin D. Analysis of p21 and p27 mRNA levels revealed no significant effects of apo-10′-lycopenoic acid on mRNA expression. However, there was a significant increase in p21 and p27 protein levels. Similar results were observed in BEAS-2B cells.[91] We also observed similar findings in human liver cells. Apo-10′lycopenoic acid dose-dependently inhibited cell growth and induced apoptosis in THLE-2 liver cells by stimulating the cyclin-dependent kinase inhibitor p21 and by reducing activation of Jun N-terminal kinase (JNK) and cyclin D1 gene expression.[131] In order to support our *in vitro* findings, an *in vivo* study was performed to evaluate the effect of apo-10′-lycopenoic acid on tumor development in the A/J mouse model of lung cancer. A/J mice were preloaded with control diet or diet containing 10, 40, or 120 mg/kg diet of apo-10′-lycopenoic acid for 2 weeks before lung tumors were induced by injection of 4-(*N*-methyl-*N* – nitrosamino)-1-(3-pyridal)-1-butanone (NNK). After 14 weeks on experimental diets, a significant decrease in tumor number (Figure 6) but not tumor incidence was observed in treated animals.[91] Interestingly, the plasma levels of apo-10′-lycopenoic acid, which demonstrated the protective effect against lung tumor formation in mice, was much lower than reported plasma lycopene concentrations in humans, suggesting that apo-10'-lycopenoic acid may, at least partially, mediate the chemopreventive activity of lycopene.

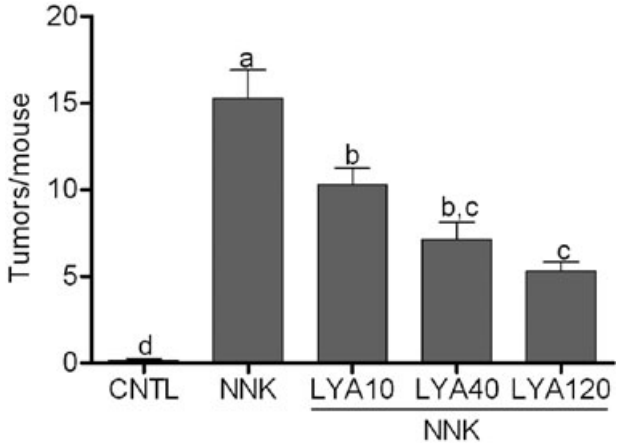

Figure 6 **Effect of apo-10'-lycopenoic acid supplementation on NNK-induced lung tumor development in A/J mice.** A/J mice were fed control diet or diet supplemented with apo-10′-lycopenoic acid for 16 weeks. Lung tumors were induced by injection of NNK at week 3 of supplementation. Tumor nodules on the surface of mouse lung tissues were counted and recorded as tumor multiplicity (tumors/mouse).
Abbreviations: *CNTL*, non-supplementation plus sham injection; *NNK*, non-supplementation plus NNK injection; *LYA10*, *LYA40*, *and LYA120*, 10, 40, and 120 mg/kg diet of apo-10′-lycopenoic acid supplementation plus NNK injection.
Values are presented as means ± SEM, $n = 12–14$. Groups that do not share a letter are significantly different, $P < 0.05$. Adapted from Lian et al. (2007).[91]

It should be pointed out that the potential use of lycopene metabolites, such as apo-10′-lycopenoic acid, as chemopreventive agents against cancers demands careful investigation. The *in vivo* metabolism of lycopene is complicated, and may be affected by a number of environmental factors, such as oxidative stress induced by cigarette smoking and alcohol consumption. For example, we have shown that high doses of β-carotene in an oxidative environment (such as the lungs of smokers)

may result in excess levels of polar metabolites, which can promote carcinogenesis, whereas lower doses of β-carotene have been shown to be protective.[132,133] In a very recent study, we observed that high-dose lycopene supplementation in the presence of alcohol ingestion increased hepatic inflammation and TNF-α expression.[134] While no apparent adverse effects, such as a decrease in body weight or tissue damage, were observed in our recent study of apo-10'-lycopenoic acid-supplemented, NNK-treated A/J mice,[91] an earlier study showed enhancement of benzo[a]pyrene-induced mutagenesis in mouse lung and colon tissues after lycopene supplementation.[135] These results suggest that lycopene or lycopene metabolites may, as β-carotene and its metabolites do, enhance carcinogenesis. Further investigation into the dose effects of lycopene, especially in response to smoke-exposure and/or alcohol ingestion, as well as a further understanding of the metabolism of apo-10′-lycopenoids on carcinogenesis is needed.

CONCLUSION

To gain a better understanding of the beneficial biological activities of lycopene upon cancer prevention, a greater knowledge of the metabolism of lycopene is needed. In particular, the identification of lycopene metabolites and oxidation products *in vivo*, the importance of tissue-specific lycopene cleavage by CMO1/CMO2, and the potential interaction between lycopene dose and smoking and alcohol ingestion remains a vital step towards a better understanding of lycopene actions. An important question that remains unanswered is whether the effect of lycopene on various cellular functions and signaling pathways is a result of the direct actions of intact lycopene or its derivatives. While evidence is presented in this review to support the latter, more research is clearly needed to identify and characterize additional lycopene metabolites and their biological activities, which will potentially provide invaluable insights into the mechanisms underlying the beneficial effects of lycopene to humans, especially in terms of cancer prevention.

Acknowledgments

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