Lycopenoids: Are Lycopene Metabolites Bioactive?

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Abstract

In vitro lycopene is the most potent antioxidant among carotenoids. While antioxidant function may be relevant to health, we hypothesize that metabolites of lycopene may be bioactive and responsible for the beneficial effects of tomato product consumption. We term these metabolites "lycopenoids," which we believe may be produced from carotenoid monooxygenase (CMO) II, paralleling the production of retinoids from β-carotene by CMO I. We present evidence suggesting that tomato carotenoid metabolites may be responsible for the reduced risk of prostate cancer seen in men consuming high levels of tomato products. Finally, we identify gaps in knowledge in this evolving area of carotenoid research.

Key words: lycopene; lycopenoids; phytoene; phytofluene; carotenoids; tomato; prostate cancer; carotenoid monooxygenase I; carotenoid monooxygenase II; beta-carotene

Intro

The red-pigmented carotenoid, lycopene, has garnered a great deal of interest as a bioactive compound that may help prevent chronic disease. A question for a number of years has been whether lycopene itself, or its metabolites or oxidation products, are responsible for the effects noted in various studies. In this paper we will focus on reviewing evidence to support the hypothesis that metabolites of lycopene, and other tomato carotenoids, are responsible for the bioactivity associated with higher intakes of tomato products. We coined a term for these lycopene metabolites, "**lycopenoids,"** because we believe these compounds are produced by an initial cleavage by carotenoid monooxygenase (CMO) II in a manner similar to how retinoids (retinal, retinol, and retinoic acid) are produced from the initial cleavage of β-carotene by CMO I (See Figure 1). In addition to lycopene, tomatoes contain significant amounts of the carotenoids phytoene and phytofluene [1]. We propose that "phytoenoids" and "phytofluenoids" may also be produced by an initial cleavage by CMO II.

Background

Lycopene is a potent antioxidant *in vitro*, but its role in health may be due to its bioactive metabolites. While there is interest in lycopene and cardiovascular disease, skin, and eye health, the majority of research has focused on prostate cancer. Many epidemiological studies have found that higher intakes of tomato and tomato product

consumption are associated with a reduced risk of prostate cancer [2, 3]. In addition, intake and serum levels of lycopene have also been inversely related to prostate cancer occurrence [2]. Therefore, many scientists have attributed the protective effect of tomatoes to lycopene because it is the carotenoid found in the highest concentration in tomato products. While tomatoes provide 85% of the lycopene in the diet, it is also found in watermelon, pink grapefruit, guava, and rosehip [4].

Despite epidemiological and small clinical trial evidence suggesting a possible protective effect of lycopene, the mechanism of action is still unclear [5-7]. Multiple studies have suggested that lycopene is bioactive *in vivo* and *in vitro* against prostate and other cancers [8-14]. For example, feeding lycopene altered expression levels of enzymes involved in androgen metabolism in both normal prostates and Dunning MatLyLu prostate tumors [15, 16]. Androgens are critical to the growth of prostate tumors, thus, these alterations could possibly explain the association between lycopene intake and reduced risk of prostate cancer.

For reasons that are not clear, lycopene preferentially accumulates in androgen-sensitive tissues such as the prostate [17]. When rats were fed diets containing tomato powder, the biodistribution of phytoene, phytofluene, and lycopene differed in various tissues. Interestingly, lycopene accumulated in higher amounts in the prostate even if tomato powder-fed animals received an oral dose of phytoene or phytofluene prior to study termination (unpublished data). Lycopene is the predominant carotenoid found in the prostate and its levels were significantly higher in malignant than in benign prostates [18]. Furthermore, in food lycopene is predominately found in the all-trans form, however, the prostate accumulated primarily cis-isomers of lycopene [18]. This can be explained by a study in ferrets that demonstrated that cis-isomers of lycopene are more bioavailable than the all-trans form [19].

There also appears to be differential tissue metabolism or accumulation of ^{14}C lycopene in rats prefed lycopene. Liver and the androgen-sensitive seminal vesicles, accumulated about 20% of radiolabeled lycopene dose as polar products. In contrast, the dorsolateral prostate, the region of the rat prostate most homologous to the peripheral region where men develop prostate cancer, accumulated up to 80% of the radiolabeled dose as polar products of lycopene [20]. We believe these polar products are lycopene metabolites and oxidation products that have yet to be identified. Further research determined that whether or not lycopene was prefed did not alter the amounts of polar products that accumulated in the prostate of rats after receiving a ^{14}C lycopene dose [21]. These studies together indicate that prostate tissue metabolizes or preferentially accumulates these polar lycopene products.

Candidate Cleavage Enzymes

The enzymes most likely responsible for the initial metabolism of lycopene are the two main mammalian carotenoid cleaving enzymes, CMO I and II. It is generally accepted that CMO I cleaves β-carotene centrally to form two molecules of retinal, while CMO II eccentrically cleaves β-carotene to form β-apo carotenals, which can then form retinoic acid after undergoing β-oxidation-like reactions (Figure 1). However, little is known about the *in vivo* action of CMO I & II on other carotenoids such as lycopene,

phytoene, and phytofluene. For lycopene, the products would theoretically be acycloretinal or apo-lycopenals for CMO I and II, respectively.

E. coli engineered to produce lycopene and express mouse CMO I exhibited color bleaching suggesting that CMO I cleaved lycopene [22]. However, when lycopene was provided at levels equivalent to β-carotene to purified recombinant mouse CMO I, no acycloretinal was detected. Lycopene levels had to be increased 2.5-3 fold higher than β-carotene to even detect this product. Similarly, recombinant human CMO I was found to have little or no activity towards lycopene and zeaxanthin, but did cleave the provitamin A carotenoids β-carotene and β-cryptoxanthin [23]. Therefore, these studies suggest that lycopene is a poor substrate for CMO I.

Due to its similarity to retinoic acid, several *in vitro* studies have examined the bioactivity of the potential CMO I lycopene cleavage product acycloretinoic acid. Acycloretinoic acid activated the retinoic acid receptor element in human mammary MCF-7 cells, however this effect was approximately 100-fold less potent than retinoic acid itself [24]. Like lycopene, acycloretinoic acid was able to stimulate gap junction communication in human fetal skin fibroblasts, but a 10-fold higher concentration was needed to be as effective as lycopene itself [25]. In further *in vitro* work, acycloretinoic acid reduced the viability of human prostate cancer cell lines and increased apoptosis, however, supraphysiological levels were required to generate these effects [26]. Based on low production and bioactivity, we hypothesize that central cleavage of lycopene by CMO I to form acycloretinal or acycloretinoic acid is not likely a candidate to explain the anticancer effects of lycopene.

Compared to CMO I, CMO II has been far less studied. *E. coli* engineered to produce lycopene and expressing mouse CMO II exhibited a color shift indicating that lycopene was cleaved. This was in contrast to *E. coli* engineered to produce β-carotene and express CMO II, which did not display a color shift [27]. We have recently completed a pilot study with CMO I knockout mice. In CMO I knockout mice and control mice fed lycopene, there were no differences in serum lycopene levels, however hepatic lycopene levels were approximately 5-fold lower in CMO I knockout mice (unpublished data). Previous work in another laboratory found that there was an approximate 4-fold increase in hepatic CMO-II mRNA expression levels in CMO I knockout mice compared to control (personal communication). Therefore, we believe the increase in hepatic CMO II levels in CMO I KO mice leads to increased cleavage of lycopene to lycopenoids, resulting in lower hepatic lycopene levels. Recently, the laboratory of Wang cloned the ferret CMO II gene and expressed it in *Spodoptera frugiperda 9* cells. When they exposed cells to mixtures of lycopene isomers they found evidence suggesting that cis-isomers of lycopene may be preferred substrates of CMO II compared to the parent all-trans lycopene or β-carotene [28].

In addition to CMO II, it is also possible that cytochrome P450 enzymes are involved in the cleavage of lycopene, phytoene, and phytofluene, but this is most likely to occur with high intakes of carotenoids. There appears to be a differential effect of carotenoids on inducing P450 enzymes. Ten μM of β-carotene or β-apo-carotenals has been found to induce the pregnane X receptor (PXR) reporter gene in HepG2 cells [29]. PXR induces the expression of cytochrome P450 enzymes such as CYP3A4, which is estimated to be responsible for approximately 60% of xenobiotic metabolism in humans [30]. However, unlike β-carotene, lycopene was found to have no effect on PXR levels

[29]**.** This finding does not mean that lycopene is not potentially metabolized by cytochrome P450 enzymes, but it does not appear to induce them to the same extent as β-carotene. The effects of lycopene on P450 activity *in vivo* are still in need of clarification. It has been reported that P450 enzyme activity was induced in female rats receiving lycopene by gavage for 2 weeks [31]. However, feeding tomato powder to male hamsters led to a decrease in P450 enzyme activity, while feeding lycopene to male rats had no effect on P450 activity [32, 33]. Whether the difference in these studies is due to the difference in sex of the animals, administration versus feeding, or other methodology issues has not been resolved.

In addition, there is *in vitro* evidence that lycopene is cleaved by soybean lipoxygenase [34, 35]. However, a recent molecular modeling study found that lycopene actually bound to the putative allosteric site of 5-lipoxygenase and not in the cleavage site. In contrast, the lycopene metabolites, 4-methyl-8-oxo-2,4,6-nonatrienal and 2,7,11-trimethyl-tetradecahexaene-1,14-dial, bound to the catalytic site of the enzyme [36]. There is little known about whether lycopene or carotenoids alter the expression or activity of 5-lipoxygenase.

Metabolic Products Identified

To our knowledge, the first *in vivo* lycopene metabolite reported was 5,6-dihydroxy-5,6-dihydrolycopene in human serum by Khachik and colleagues. The authors' hypothesized that this metabolite resulted from oxidation of lycopene to form lycopene 5,6-epoxide which then was metabolically reduced to 5,6-dihydroxy-5,6-dihydrolycopene [37-39]. This same lab also identified epimeric 2,6-cyclolycopene-1,5-diols in human serum and milk, which they hypothesized were produced in a similar fashion as 5,6-dihydroxy-5,6-dihydrolycopene [40]. In preruminant calf serum, Sicilia et al. reported the lycopene metabolites 5,6-dihyrolycopene, 5,6-dihydro-5-cis-lycopene, and tetrahydrolycopene of which the exact position of the hydrogenation could not be determined [41].

In the liver of F344 rats fed lycopene, we identified apo-8'-lycopenal and potentially apo-12'-lycopenal as metabolites of lycopene (See Figure 1). In addition, there are a number of other polar products that have yet to be identified [42]. Similar work has been done in the ferret lung [28]. However, only apo-10'-lycopenol was reported, a product not found in rat liver [42]. The cause of these metabolite differences in the last two studies, be it species, tissue, or methodology differences should be elucidated.

There have been a number of studies looking at formation of lycopene metabolites and oxidation products *in vitro*[34, 43-46]. We will only discuss two studies due to space constraints. Incubation of lycopene in toluene, aqueous Tween 40, or liposomal suspension under atmospheric oxygen at 37°C for 72 hours led to identification of 8 products 3,7,11-trimethyl-2,4,6,10-dodecapentaen-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 [43]. Two cleavage products and 4 oxidation products were identified from the incubation of deuterated lycopene with the postmitochondrial fraction of rat intestinal mucosa and soybean lipoxygenase. The cleavage products were 3-keto-apo-13-lycopenone and 3,4-dehydro-5,6-dihydro-15-apo-

lycopenal, while the oxidative metabolites were: 2-ene-5,8-lycopenal-furanoxide, lycopene-5, 6,5',6'-diepoxide, lycopene-5,8-furanoxide isomer, 3-keto-lycopene-5',8'-furanoxide [34]. Since some of the products produced in these *in vitro* systems have not been found *in vivo*, it remains to be sorted out whether they are relevant *in vivo*.

Antioxidant Function vs. Lycopenoid Bioactivity

Antioxidant function is the main mechanism through which lycopene has been thought to decrease chronic disease risk. For example, lycopene acting as an antioxidant can prevent DNA damage, which leads to mutations that cause cells to become cancerous. Lycopene is a potent *in vitro* antioxidant, being 2-fold and 10-fold more efficient at quenching singlet oxygen than β-carotene and α-tocopherol, respectively [47]. However, Freeman et al. found the human prostate concentration of α-tocopherol to be 162-fold higher than lycopene [48]. Therefore, even with reduced antioxidant potential, the much higher concentration of α-tocopherol in this tissue suggests that it is unlikely that lycopene itself plays a major role as an antioxidant. In *in vitro* experiments, not changing lycopene-containing media during the 3-day treatment period was significantly more effective in reducing proliferation of connexin 43 wild-type mouse embryonic fibroblasts, compared to changing the lycopene treatment and media daily (unpublished data). We believe that not changing the media allows for the build up of lycopene metabolites and oxidation products, thus leading to the more effective antiproliferative response. Taken together, these findings provide support to our hypothesis that lycopenoids are the compounds responsible for lycopene's antiproliferative activity**.**

In contrast to the findings with acycloretinoic acid, some lycopene metabolites and oxidation products have been found to be bioactive. Apo-10 lycopenoic acid was found to decrease the proliferation of non-small cell lung cancer cells *in vitro* (personal communication). An ethanolic extract containing lycopene metabolites and oxidation products was as effective as lycopene itself in transactivating antioxidant response element (ARE) genes [49]. Furthermore, the lycopene oxidation product, 2,7,11-trimethyl-tetradecahexaene-1,14-dial, enhanced gap junction communication in rat liver epithelial WB-F344 cells [45]. Lastly, both lycopene or phytofluene metabolites inhibited the growth of HL-60 human promyelocytic leukemia cells [50].

Are Tissue Levels of Lycopenoids High Enough To Produce Biological Effects?

The levels of lycopenoids in tissues appear to be equivalent or greater than the levels of retinoic acid. In rat liver, retinoic acid levels have been reported to be 11.3 pmol/g tissue, while we estimated the levels of apo-8' lycopenal to be ~600 pmol/g [42, 51]. In ferret lung, retinoic acid has been reported to be 17 pmol/g, while the levels reported of apo-10-lycopenol were 8 pmol/g [28, 52]. Due to their structural and polarity similarities to retinoic acid, we believe that lycopenoids may be agonist or antagonists for nuclear receptors such as RARs/RXRs/LXR/PXR/PPARs or activate response elements such as the ARE. For instance, we recently found that lycopene feeding caused a significant downregulation of PPARγ in rat kidney and adrenal and the PPARγ target gene fatty acid binding protein 3 (FABP3) [33].

Future Directions

There is a great deal we do not know about lycopene metabolism and the metabolic effects of lycopenoids. Identification, particularly *in vivo*, of metabolites and oxidation products is still an active area of investigation. In addition, further clarification is needed to determine which lycopenoids are produced enzymatically and nonenzymatically, as well as in what tissues they are produced and accumulate. If CMO II is indeed the key enzyme in the metabolism of lycopene, is this enzyme specific for lycopene, or does it have a broad specificity for a variety of carotenoids? For instance, it may be that CMO I cleaves provitamin A carotenoids, while CMO II is more active towards nonprovitamin A carotenoids. A further understanding is needed of the effects of levels and timing of a carotenoid dose on the production of metabolites and the resulting biological effects. For example, consumption of tomato powder, lycopene, or phytofluene for 4 days to 8-week old Fisher 344 rats led to a significant reduction in serum testosterone levels [53]. This is clearly an acute effect, as previously feeding tomato powder or lycopene for weeks or months did not affect serum testosterone levels [54 & unpublished data]. However, even acute decreases in androgens could potentially help to reduce the risk of developing prostate cancer. Furthermore, we know little about the differential effects of carotenoids lycopene, phytoene, phytofluene and β-carotene on metabolism and gene expression. Lastly an understanding of the potential synergistic or additive effect among tomato carotenoids may explain why whole food sources are more effective than single carotenoid supplements. For example, tomato powder consumption is more effective than lycopene alone in preventing the development of and progression of prostate cancer in rats [55 & unpublished data]. Overall, future research will continue to clarify the role of lycopene, tomato carotenoids, and their metabolites in producing the beneficial effects associated with tomato intake.

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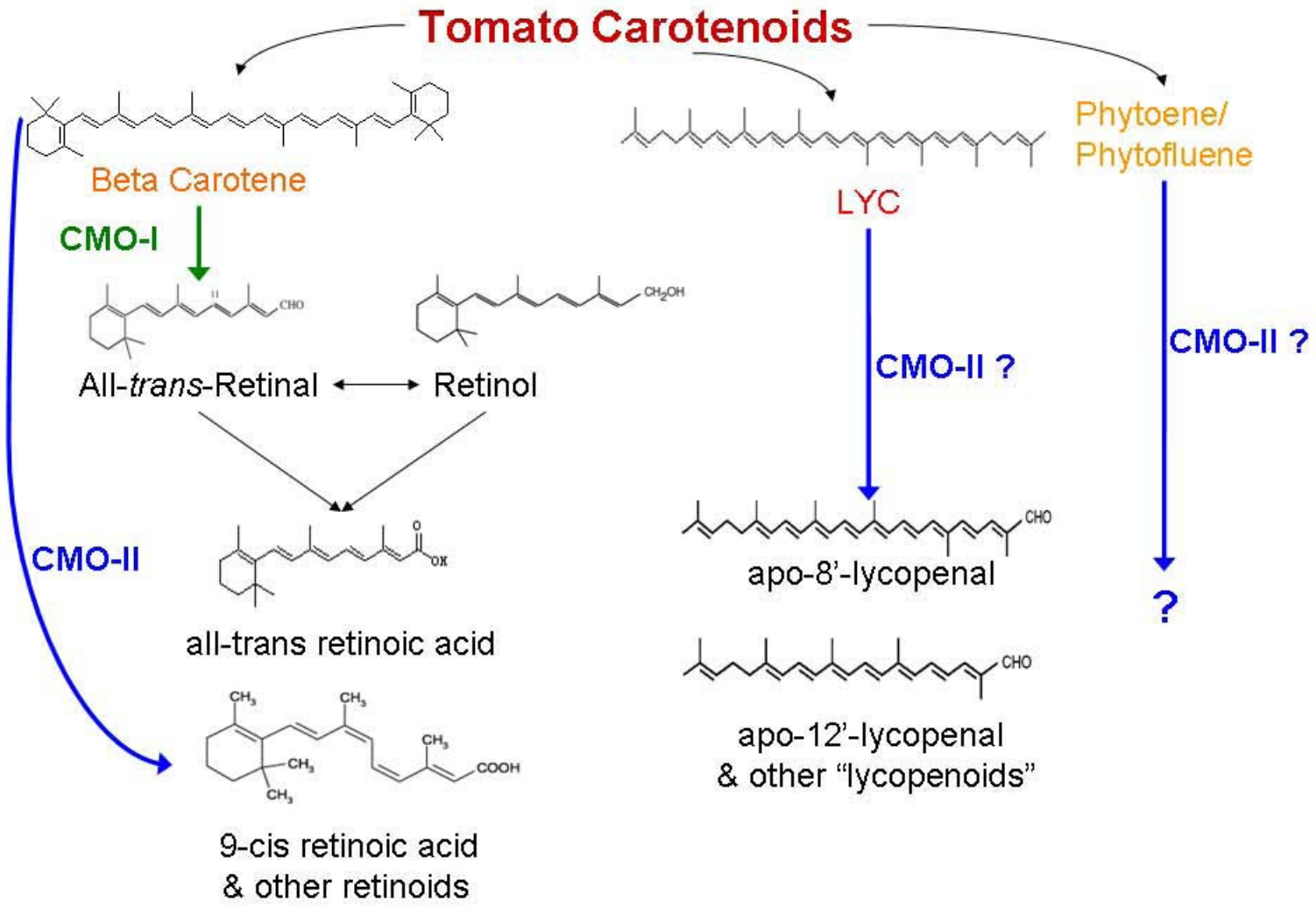
Figure 1 Legend

Proposed Metabolism of Tomato Carotenoids. The main metabolic pathway for β-carotene is central cleavage by CMO I to form retinal which can be reduced to retinol. Retinoic acid can be formed by the oxidation of retinal or retinol, or alternatively it is believed that CMO II eccentrically cleaves β-carotene to form β-apo carotenals. The β-apo carotenals can then undergo β-oxidation to eventually form retinoic acid. We believe lycopene cleavage by CMO II to lycopenoids parallels retinoid production from β-carotene by CMO I. Due to their similarity in structure, we believe that phytoene and phytofluene may also be cleaved like lycopene by CMO II to form phytoenoids and phytofluenoids.


Tomato Carotenoids
Beta Carotene
CMO-I
All-trans-Retinal
Retinol
CMO-II
all-trans retinoic acid
9-cis retinoic acid
& other retinoids
LYC
CMO-II ?
apo-8'-lycopenal
apo-12'-lycopenal
& other "lycopenoids"
Phytoene/
Phytofluene
CMO-II ?
?