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Review

Are lycopene metabolites metabolically active?*

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Lycopene is the most abundant carotenoid found in tomatoes and thus has been touted as the bioactive component for the reduced risk of chronic diseases such as prostate cancer. We and others hypothesize that lycopene metabolites are responsible for positively modulating biomarkers and risk factors for the prevention of chronic diseases. Lycopene metabolites circulate in serum and accumulate in tissues at concentrations equivalent to bioactive retinoids. Recent studies report that lycopene metabolites reduce the proliferation of cancer cells, induce apoptosis, enhance gap junction communication between cells, alter normal cell cycle progression, and modulate androgen signaling pathways. Here we review recent literature and provide new evidence to suggest that lycopene metabolites may be bioactive at physiological concentrations.

Key words: lycopene, carotene-monooxygenase, lycopenoids, testosterone

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INTRODUCTION

Epidemiological evidence suggests that consumption of lycopene, the red pigment of tomato products and a major carotenoid in human plasma and tissues, is inversely associated with the risk for a number of pathologies including most notably prostate cancer (Giovannucci *et al.,* 1995; Canene-Adams *et al.,* 2005). Based upon recent advances in understanding carotenoid metabolism, we hypothesize that lycopene metabolites may be responsible, at least in part, for this association.

In this review, we will show that lycopene metabolites circulate in serum and accumulate in tissues at physiologically-relevant concentrations in comparison to retinoids, establish that lycopene metabolites are biologically active, and introduce new findings that suggest these metabolites alter serum testosterone levels which are an important risk factor for prostate cancer.

LYCOPENE METABOLITES

Lycopene metabolites, also termed lycopenoids, are poly-isoprenoid compounds of less than 40 carbons in length derived from the parent compound, lycopene (Lindshield *et al.,* 2007). Previous studies identified and characterized lycopenoids and oxidation products generated *in vivo*; some of these metabolites demonstrate anticancer activity by inhibiting proliferation, inducing apoptosis, or enhancing cell to cell communication (King *et al.,* 1997; Zhang *et al.,* 2003; Ford *et al.,* 2011a).

A series of apo-lycopenals and short-chain carbonyl compounds were produced by *in vitro* autoxidation of lycopene including apo-14′-lycopenal, apo-12′-lycopenal, apo-10′-lycopenal and apo-6′-lycopenal (Kim *et al.,* 2001). Additional studies have identified other apo-lycopenals *in vitro* (Khachik *et al.,* 1998a; Khachik *et al.,* 1998b; Caris-Veyrat *et al.,* 2003). Although there are problems associated with the use of *in vitro* models for this type of work, the chemicals formed *in vitro* may be important representatives of carotenoid cleavage in the lungs of smokers, tumors in cancer patients, or other oxidatively stressed conditions (Ford *et al.,* 2011a).

Metabolites of lycopene have also been identified *in vivo* and interestingly, concentrations of lycopenoids are of comparable biological concentrations to retinoids produced from β-carotene (Table 1). Since all *trans* retinoic and 9-*cis* retinoic acids are important ligands for a variety of nuclear receptors, it is certainly plausible that some lycopenoids that have similar structure, polarity and molecular weights may be antagonists or agonists to some receptors.

LYCOPENE METABOLISM

Chain-shortened lycopene products may be produced by free radical oxidation, lipoxygenase activity, phase II detoxification enzymes, or carotenoid cleavage enzymes (see Mein *et al.,* 2008 for review). Enzymatic carotenoid metabolism is primarily catalyzed by two carotenoid monooxygenase enzymes, carotene-15,15′-monooxygenase (CMO-I) and carotene-9′10′-monooxygenase (CMO-II). CMO-I centrally cleaves carotenoids, such as β-carotene (Hessel *et al.,* 2007) whereas CMO-II primarily eccentrically metabolizes non-provitamin A carotenoids like lycopene (Hu *et al.,* 2006; Ford *et al.,* 2010). The expression of these carotenoid cleavage enzymes is differentially expressed in tissues (Table 2). The products of CMO-II are short chain aldehydes termed apo-lycopenals. As previously noted, apo-lycopenals have been identified *in vivo* at physiologically relevant concentrations (Table 1).

Recent studies from our lab and others have demonstrated that dietary lycopene alters the mRNA expression of CMO-II in animal models. We measured the relative mRNA expression of CMO-II by qRT-PCR in healthy prostate tissue and prostatic tumor from male Copenha-

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Abbreviations: AIN-93G, American Institute for Nutrition 1993 growth formula; CMO-I, carotene-15,15′-monooxygenase; CMO-II, carotene-9′,10′-monooxygenase; DHT, dihydrotestosterone; KO, knock-out; Nrf2, nuclear factor E2-related factor 2; qRT-PCR, quantitative real time polymerase chain reaction.

Table 1. Tissue and serum concentrations of lycopene and β-carotene metabolites.

Metabolite	Animal	Tissue	Concentration
Apo-6′-lycopenal	Humans	Plasma	0.076 ng/ml
Apo-8′-lycopenal	Rats	Liver	250 ng/g
Apo-8′-lycopenal	Humans	Plasma	0.142 ng/ml
Apo-10′-lycopenal	Humans	Plasma	0.076 ng/ml
Apo-12′-lycopenal	Rats	Liver	≥ 250 ng/g
Apo-12′-lycopenal	Humans	Plasma	0.137 ng/ml
Apo-10′-lycopenol	Ferrets	Lung	2-4 ng/g
Retinal	Rats	Intestine	8 μg/g
All-trans-Retinoic Acid	Humans	Plasma	3.5 ng/ml
All-trans-Retinoic Acid	Rats	Plasma	1.2 ng/ml
β-apo-8′-carotenal	Humans	Plasma	245 ng/ml

(De Leenheer *et al*., 1982; Gajic *et al*., 2006; Hu *et al*., 2006; Kopec *et al*., 2010)

gen rats in response to a 10% tomato powder diet or a control AIN-93G diet (Fig. 1). Quantification of CMO-II mRNA expression was described previously (Ford *et al.,* 2011b), the data was analyzed by student's *t*-test, and $p<0.05$ was considered statistically significant. The tomato powder diet significantly reduced the expression of CMO-II in healthy prostate tissue. However, in prostatic tumor tissue, the tomato powder diet induced mRNA expression of CMO-II relative to tissues of rats that were fed the control diet. In addition, we demonstrated that a lycopene-enriched diet significantly reduced the expression of CMO-II in renal tissue of F344 rats which we hypothesize was the result of feedback inhibition by lycopene or its metabolites (Zaripheh *et al.,* 2006). In a study from another laboratory, dietary lycopene increased the expression of CMO-II mRNA by 4-fold in the lungs of ferrets (Hu *et al.,* 2006). The apparent contradictions in these data sets suggest to us that these are tissue specific metabolic and/or oxidative environments that affect lycopene catabolism and/or the possible differential need for lycopene metabolites by specific tissues. We hypothesize that highly oxidative conditions such as lung and tumor tissue may produce or require greater concentrations of lycopene metabolites and therefore inducing CMO-II expression in these tissues. Overall, the effect of carotenoid consumption on the expression of carotenoid cleavage enzymes warrants further investigation.

Table 2. Relative tissue mRNA expression of CMO-II in mouse and human tissues.
CMO-II expression was analyzed by qRT-PCR using SYBR green fluorescence as described previously (Ford *et al.,* 2011b) and compared to published literature (Wyss 2004; Lindqvist *et al.,* 2005).

Tissue	Mouse (new data)	Mouse (literature)	Human (literature)
Liver	++	+++	+++
Duodenum	+++	+++	+++
Prostate	++	N/A	++
Testes	+*	++	++
Adrenals	N/A	++	N/A

(N/A) not available, (+) low, (++) moderate, (+++) high expression levels. (*) Published previously (Ford *et al.,* 2011b).

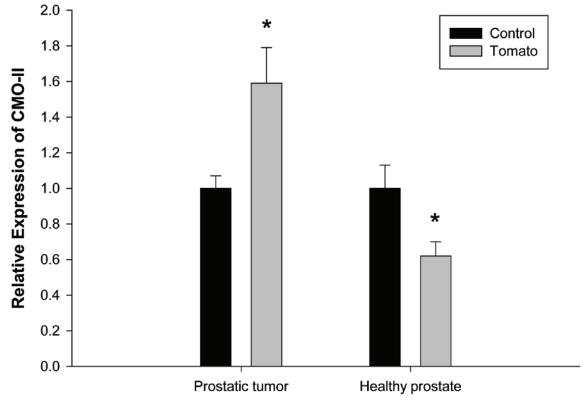

Figure 1. Relative CMO-II mRNA expression.
Prostate and prostatic tumor tissue express CMO-II but expression is differentially affected by consumption of a 10% tomato powder diet containing approximately 13 nmol lycopene per gram of diet. Quantitative RT-PCR analysis of the carotenoid monooxygenase II (CMO-II) was carried out in healthy rat prostatic tissue and prostatic tumor tissue from Dunning R3327-H prostate adenocarcinoma orthotopic transplant in male Copenhagen rats. mRNA expression is expressed relative to the ribosomal gene, 18S. Expression of CMO-II in each tissue was analyzed by student's *t*-test; (*) $p<0.05$ considered statistically significant.

BIOLOGICAL ACTIVITY OF APO-LYCOPENALS AND APO-LYCOPENOIC ACIDS

Recent strong scientific interest in lycopene metabolism has produced some early evidence to suggest that lycopenoids are biologically active. We recently reported that apo-10′-lycopenal and apo-12′-lycopenal treatment reduced DU145 prostate cancer cell proliferation in a dose-dependent manner in part through regulation of the normal cell cycle (Ford *et al.,* 2011a). A separate group demonstrated that apo-10′-lycopenoic acid reduced proliferation of lung cancer cells and tumorigenesis of an *in vivo* lung tumor mouse model through Nrf2-mediated induction of phase II detoxifying/antioxidant enzymes (Lian *et al.,* 2007). Future studies will further define the biological benefits of lycopenoids.

BIOLOGICAL ACTIVITY OF LYCOPENE OXIDATION PRODUCTS

Products produced from lycopene through a variety of oxidative procedures have been reported to modulate biological activity. It has been demonstrated that oxidation products of lycopene inhibit the growth of human leukemia cells *in vitro* (Nara *et al.,* 2001). Specifically, 6 μM of lycopene failed to alter leukemia cell proliferation while 6 μM of oxidative lycopene products (produced from incubation with toluene for 24 hours) dramatically inhibited cell growth by 97% relative to control after 120 hours in culture. In human promyelocytic leukemia cells, a different oxidative product, (*E,E,E*)-4-methyl-8-oxo-2,4-6-nonatrienal (5–15 μM), also reduced proliferation and induced the essential physiological mechanism to remove damaged DNA by apoptosis (Zhang *et al.,* 2003). In contrast, lycopene treatment alone did not affect apoptosis and had little effect on proliferation rates. Other oxidative products of lycopene have also been shown to inhibit prostate cancer cell growth through induction of apoptosis (Kotake-Nara *et al.,* 2002). In another study, a solution of undefined lycopene oxidative products stimu-

lated gap junction communication which is essential to reduce overgrowth of cells as typically found in cancerous tissues (Aust *et al.,* 2003). Another lab demonstrated that an ethanol extract of oxidized lycopene activated the electrophile/antioxidant response element which is known to induce phase II detoxification enzymes in human mammary cancer cells (Linnewiel *et al.,* 2009).

Early studies are starting to suggest that lycopene metabolites play a role in preventing or moderating certain cancer types. Therefore, identification of bioactive lycopene metabolites and further characterization of their *in vivo* function(s) is critical for cancer research.

PROSTATE CANCER, TESTOSTERONE AND LYCOPENE METABOLITES

Prostate cancer risk is positively associated with induced androgen signaling (Gann *et al.,* 1996; Shaneyfelt *et al.,* 2000). It was first reported in 1941 that the reduction of testosterone levels is a potent therapeutic agent for patients with advanced prostate cancer (Huggins *et al.,* 2002). Moreover, a causal relationship was suggested by a few case reports of prostate cancer patients who previously used androgens as anabolic agents or for medical treatment (Roberts *et al.,* 1986; Ebling *et al.,* 1997).

Recently, we established that testosterone levels in our mouse models are dependent upon an interaction of the expression of carotenoid cleavage enzymes and dietary levels of lycopene. Specifically, we reported that tomato powder or lycopene-containing diets reduced serum and testicular testosterone in CMO-I knock out (CMO-I KO) mice (Ford *et al.,* 2011b). It should be noted that the primary site of whole body testosterone production is from the testis tissue and we demonstrated the testis tissue of CMO-I KO mice have significantly elevated expression of CMO-II. Therefore, it is plausible that CMO-I KO mice have elevated production of lycopene metabolites in testis tissue which effectively reduced serum and testicular testosterone concentrations. Here, we further demonstrate in this mouse model that the expression of key testosterone metabolism genes or receptors is significantly reduced in response to a diet containing 248 nmol lycopene per gram of diet (Fig. 2). The mRNA expression of testicular 5-alpha reductase I, testicular 5-alpha reductase II, and prostatic androgen receptor was measured by qRT-PCR using the SYBR green dye as previously described (Ford *et al.,* 2010). The conversion of testosterone to its more potent androgen dihydrotestosterone, DHT, is catalyzed by the 5-alpha reductase enzyme. Either testosterone or DHT may bind the androgen receptor thus inducing androgen responsive genes. The significant reduction in the expression of these genes by a lycopene-containing diet suggests a reduction in androgen signaling in the testes and prostate. Therefore, it is conceivable that the enhanced production of lycopenoids in the testis of CMO-I KO mice reduced androgen signaling and therefore may potentially reduce prostate cancer risk.

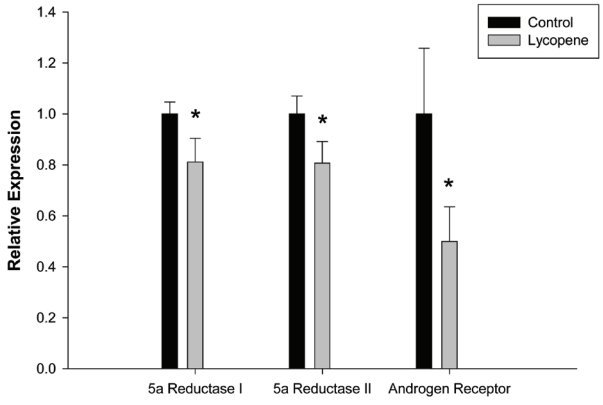

Figure 2. Relative mRNA expression of steroid pathway genes. Testicular 5 alpha reductase I, testicular 5 alpha reductase II, and prostatic androgen receptor are differentially expressed in CMO-I knock-out mouse tissues in response to a lycopene-containing diet (248 nmol/g diet). CMO-I knock-out mice have significantly elevated expression of testicular CMO-II (Ford *et al.,* 2011b). mRNA expression is expressed relative to the ribosomal gene, 18S. mRNA expression of target genes in each tissue was analyzed by student's t-test; (*) $p<0.05$ considered statistically significant.

CONCLUSIONS

Lycopene metabolites have been identified *in vivo* in similar concentrations to the biologically active retinoids. Recent reports suggest that lycopenoids reduce proliferation of cancer cells, induce apoptosis, regulate flow through the cell cycle, induce nuclear transcription factors, enhance cell to cell communication, and reduce androgen signaling. The consumption of lycopene containing foods and the tissue specific expression of carotenoid cleavage enzymes determines tissue lycopenoid concentrations. New reports suggest that these lycopenoids are biologically active and may reduce the risk for chronic diseases. We provide data that suggest that lycopeniods influence androgen metabolism in rodent models.

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