

# Promises and Perils of Lycopene/Tomato Supplementation and Cancer Prevention

## Lycopene Effects on Rat Normal Prostate and Prostate Tumor Tissue<sup>1</sup>

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### EXPANDED ABSTRACT

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Epidemiological evidence links tomato consumption with reduced risk of prostate cancer (1–3). Prostate cancer remains a severe health problem throughout the Western world, and the success in treating advanced prostate cancer remains poor. Therefore, the importance of prevention through dietary measures is growing. Lycopene, a non-provitamin A carotenoid and the red pigment of tomatoes, is considered the major active compound in tomatoes. The health function of lycopene has been mainly linked to its potent antioxidant effects (i.e., singlet-oxygen-quenching properties) resulting in protection against oxidative DNA damage in vitro and in vivo [reviewed in (4)]. Lycopene also inhibits cell proliferation, at least in part due to its interference with insulin-like growth factor-1 (IGF-1)<sup>5</sup> signaling (4).

Vitamin E, the major lipid-soluble antioxidant in cell membranes, is also linked to a lower prostate cancer risk (5,6). Vitamin E scavenges lipid peroxyl radicals, which are the chain-carrying species propagating lipid peroxidation. Although a variety of effects of lycopene and vitamin E have been shown, the molecular mode of action underlying the reduced risk for prostate cancer is not well understood.

To obtain insight into the in vivo mechanisms by which

lycopene and/or vitamin E contribute to prostate cancer risk reduction, we (7) used the MatLyLu Dunning prostate cancer model. Thirty male Copenhagen rats were randomly assigned to 5 groups. The 2 control groups were nonsupplemented controls and placebo-supplemented controls. Treatment groups were fed diets containing 200  $\mu\text{g}$  lycopene/g diet (redivivo<sup>TM</sup> 5% TG, DSM), 540  $\mu\text{g}$  vitamin E/g diet (Rovimix E-50 SD, DSM), or both. Placebo-fed controls received a diet containing the same volume of placebo beadlets for both the lycopene and the vitamin E formulation. The lycopene-supplemented diet also contained vitamin E placebo beadlets, and the vitamin E-supplemented diet also contained lycopene placebo beadlets. After 4 wk of supplementation, tumors were induced by injection of  $10^5$  MatLyLu prostate tumor cells into the ventral prostate lobe, and supplementation continued for 18 d of tumor growth. Lycopene and vitamin E uptake were assessed by HPLC. The effects of the nutrients in tumor tissue were evaluated by in vivo MRI after 14 d of tumor growth and by GeneChip analysis at trial termination after 18 d of tumor growth. Key gene regulations were confirmed by TaqMan<sup>TM</sup> RT-PCR.

After 4 wk of supplementation with vitamin E or with vitamin E and lycopene, plasma vitamin E levels rose to 47.98 and 46.44  $\mu\text{mol/L}$ , respectively. In the lycopene-supplemented groups, lycopene plasma levels were 1.02 and 0.92  $\mu\text{mol/L}$  after 4 wk of supplementation. These vitamin E and lycopene plasma levels correspond to high physiological levels measured in humans.

Within 18 d of tumor growth, vitamin E and lycopene accumulated in the tumor tissue. Vitamin E levels were 75.46 and 47.60  $\mu\text{mol/L}$  in the vitamin E- and the vitamin E and lycopene-treated groups, respectively. Lycopene levels were 0.38 and 0.42  $\mu\text{mol/L}$  in the 2 lycopene-fed groups.

After 14 d of tumor growth, tumors were examined in vivo by MRI. Both vitamin E and lycopene single treatment substantially increased the necrotic area of the tumors, to 36.4 and 36.0%, respectively, compared to 20.0 and 23.3% in the 2

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<sup>5</sup> Abbreviations used: 5 $\alpha$ -DHT, 5- $\alpha$ -dihydrotestosterone; 17 $\beta$ -HSD, 17 $\beta$ -hydroxysteroid-dehydrogenases; DHEA, dehydroepiandrosterone; ER $\beta$ , estrogen receptor  $\beta$ ; IGF-1, insulin like growth factor 1; NF- $\kappa$ B, nuclear factor- $\kappa$ B.

control groups. The combination of vitamin E and lycopene nonsignificantly increased the necrosis rate to 27.5%.

Affymetrix GeneChip technology was employed to investigate the influence of lycopene and/or vitamin E on gene expression in prostate tumors. Gene regulation was analyzed by comparing the expression level of genes in the treated groups with the expression levels of the same genes in the placebo group. Gene regulation data are given as fold changes relative to the expression in tumors of the placebo group, which was set to 1.

The hallmark of both the lycopene and the vitamin E effect was suppression of genes involved in steroid metabolism and signaling. Lycopene reduced steroid  $5\alpha$ -reductase 1 expression in the lycopene group (0.36-fold) and in the cotreated group (0.48-fold).  $5\alpha$ -Reductase is responsible for the conversion of androstenedione and testosterone into the most potent natural androgen,  $5\alpha$ -dihydrotestosterone ( $5\alpha$ -DHT). Consistent with downregulation of  $5\alpha$ -reductase, a set of androgen target genes (cystatin-related proteins 1 and 2; prostatic spermine-binding protein; prostatic steroid-binding proteins C1, C2, and C3 chain; probasin) was consistently downregulated in both lycopene-treated groups, with downregulation up to 0.02-fold. Although vitamin E did not alter the expression of steroid  $5\alpha$ -reductase 1, the expression of the same set of androgen target genes was downregulated as in the lycopene-treated groups. Moreover, vitamin E alone or together with lycopene reduced aromatase expression (0.57- and 0.65-fold). Lycopene and vitamin E cotreatment downregulated androgen target genes in an additive manner. In addition to the effects on steroid metabolism and signaling, lycopene alone inhibited local IL-6 expression. Furthermore, the prostate-specific expression of IGF-I was substantially downregulated by lycopene both alone and in combination with vitamin E.

#### Summary of lycopene effects in the MatLyLu Dunning prostate tumor model

Lycopene supplementation increased necrosis rates in prostate tumors. This effect was associated with a downregulation of local androgen signaling, as well as IGF-I and IL-6 expression. Because all of these mechanisms are known to be important in prostate cancer development, their inhibition by lycopene is a plausible explanation for the link between high lycopene consumption and reduced prostate cancer risk.

#### Effect of lycopene in healthy rats

For true primary prostate cancer prevention, these mechanisms must be influenced by lycopene that has been acquired in young normal prostate tissue. Moreover, due to the frequent consumption of processed tomato-based products, like pizza, spaghetti bolognese, and ketchup, children consume considerable amounts of lycopene independent of an intention to prevent prostate cancer. For young adolescents (6–16 y) in the United States, a mean lycopene serum concentration of 0.45  $\mu\text{mol/L}$  was reported, with maximum levels reaching 1.7  $\mu\text{mol/L}$  (8). Hence, it is important to know the influence of lycopene on normal prostate tissue. To investigate the effect of lycopene on normal prostate tissue, we (9) conducted a similar study in healthy young male rats. Forty-two male Copenhagen rats were randomly assigned to supplementation groups. The placebo group ( $n = 8$ ) received a basal diet, containing 40  $\mu\text{g}$  vitamin E/g diet (originating from the placebo formulation), a reduced vitamin A content (4000 IU/kg), and no phytosterols. Lycopene-supplemented rats were fed a basal diet enriched with 200  $\mu\text{g}$  lycopene/g diet (redivivo 5% TG, DSM). Starting

at d 0 of supplementation, every 2 wk, a group of 6 to 8 lycopene-fed rats was killed. After 8 wk of supplementation, 8 lycopene-fed rats and the 8 placebo-fed rats were killed. For each rat, 1 lobe per prostate lobe pair (anterior, dorsal, lateral, ventral) was used to measure lycopene accumulation by HPLC. In rats supplemented for 8 wk, gene expression was assessed individually in the other lobe of the dorsal and lateral prostate lobe pair, using 1 chip per lobe and rat. Gene regulations in key pathways were confirmed by TaqMan RT-PCR.

During 8 wk of supplementation, the total prostate lycopene content increased gradually and reached a mean total lycopene concentration of  $0.46 \pm 0.09$  nmol/g, with all-*trans* lycopene representing the major isoform (~60%). Lycopene uptake was lobe-specific. The highest lycopene concentrations were achieved in the lateral lobe ( $0.78 \pm 0.07$  nmol/g). The isomer distribution was comparable for all prostate lobes. Prostate lobe weight was not influenced by lycopene uptake, indicating that lycopene did not interfere with normal prostate growth.

GeneChip analysis was used to identify genes regulated by lycopene in normal prostate tissue. Gene expression profiling was performed in the dorsal and lateral prostate lobes, which are considered the rat equivalent of the human peripheral zone, the zone from which human prostate cancer predominantly arises. Lycopene had an overall mild, but significant, effect on gene expression. The most important effects with respect to primary prostate cancer prevention were observed in local steroid metabolism and signaling, in local IGF-I expression, and in expression of inflammatory markers. Hence, lycopene influenced the same mechanisms in normal rat prostate as in rat prostate tumors.

The expression profiles of 3 major enzymes of androgen metabolism [ $5\alpha$ -reductase 2,  $17\beta$ -hydroxysteroid-dehydrogenases ( $17\beta$ -HSD), Cyp7B1] suggest that lycopene supplementation reduced the activation of androgens in normal prostate tissue.  $5\alpha$ -Reductase 2 was mildly, but consistently, downregulated by lycopene in both lobes [0.66-fold change in the dorsal lobe (d); 0.81-fold in the lateral lobe (l)], indicating reduced androgen activation. In line with this, androgen targets were also downregulated by lycopene (prostatic steroid binding proteins C1 and C3, cystatin-related protein 2, and seminal vesicle secretion protein IV).  $17\beta$ -HSD type IV is responsible for dehydroepiandrosterone (DHEA) generation, whereas Cyp7B1 regulates  $3\beta$ Adiol catabolism. Both DHEA and  $3\beta$ Adiol are ligands for the estrogen receptor  $\beta$  (ER $\beta$ ). Upregulation of  $17\beta$ -HSD type IV [1.55-fold (d)] as well as downregulation of Cyp7B1 [0.66-fold (d); 0.74-fold (l)] by lycopene suggests increased formation of both ER $\beta$  ligands, presumably at the expense of  $5\alpha$ -DHT. Selective ER $\beta$  ligands are crucially involved in controlling proliferation of prostate epithelium and prostate size and are hence employed as agents against prostate cancer.

Moreover, lycopene reduced IGF-I expression in normal prostate tissue [0.43-fold (d); 0.60-fold (l)]. The IGF-I axis is regarded as a major pathway in prostate cancer development (10). Downregulation of IGF-I expression in prostate tissue indicates that lycopene interferes with the local IGF-I signaling in prostate tissue.

Inflammatory cytokines, like IL-1 $\beta$  and the CXC chemokines MIP-2 and LIX, were downregulated by lycopene, suggesting an anti-inflammatory effect of lycopene. Furthermore, transcripts encoding immunoglobulins and immunoglobulin Fc receptor were less abundant in prostate tissue after lycopene supplementation, also supporting an anti-inflammatory effect of lycopene. These results are in line with the downregulation of IL-6 by lycopene found in rat prostate tumors, because the above-mentioned cytokines regulate IL-6 transcription via the

nuclear factor- $\kappa$ B (NF- $\kappa$ B) pathway. Members of the NF- $\kappa$ B pathway were either not included on the GeneChip or not regulated. Chronic prostatitis is likely linked with increased prostate cancer risk. Hence, an anti-inflammatory action of lycopene may also contribute to prostate cancer prevention.

#### ***Summary of lycopene effects in normal rat prostate tissue***

Lycopene accumulated in all 4 prostate lobes, with the lateral lobe achieving the highest lycopene levels. Lycopene did not interfere with normal prostate growth. Lycopene mildly, but significantly, regulated gene expression. Lycopene reduced local prostatic androgen signaling, IGF-I expression, and inflammatory signals.

#### ***Conclusion for lycopene effects in normal and tumorous rat prostate tissue***

Lycopene regulated the same mechanisms in normal prostate tissue as in prostate tumor tissue, and all of these mechanisms are crucially involved in prostate cancer development. The degree of regulation was different for the 2 models. The anti-androgen effect was milder, and the anti-inflammatory effect of lycopene was more pronounced in normal prostate tissue compared to prostate tumor tissue. The consistency of pathway regulation by lycopene in both normal prostate and prostate tumor tissue suggests that these mechanisms are major targets of lycopene. The mechanisms identified can explain how dietary lycopene may reduce prostate cancer risk.

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