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Lycopene and Vitamin E interfere with autocrine/paracrine loops in the Dunning prostate cancer model

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ABSTRACT

Epidemiological studies have consistently associated high intakes of lycopene or vitamin E with a reduced prostate cancer risk. Both compounds were tested in the MatLyLu Dunning prostate cancer model to gain insight into the in vivo action of lycopene and vitamin E. Supplementation for 4 weeks with 200 ppm lycopene, 540 ppm vitamin E, or both led to plasma levels comparable with those in humans. Both compounds also accumulated in tumor tissue. Macroscopic evaluation of the tumors by magnetic resonance imaging showed a significant increase in necrotic area in the vitamin E and the lycopene treatment groups. Microarray analysis of tumor tissues revealed that both compounds regulated local gene expression. Vitamin E reduced androgen signaling without affecting androgen metabolism. Lycopene interfered with local testosterone activation by down-regulating 5-α-reductase and consequently reduced steroid target genes expression (cystatin-related protein 1 and 2, prostatic spermine binding protein, prostatic steroid binding protein C1, C2 and C3 chain, probasin). In addition, lycopene down-regulated prostatic IGF-I and IL-6 expression. Based on these findings, we suggest that lycopene and vitamin E contribute to the reduction of prostate cancer by interfering with internal autocrine or paracrine loops of sex steroid hormone and growth factor activation/synthesis and signaling in the prostate.

Key words: carotenoid • steroid • nutrigenomics • magnetic resonance imaging

Prostate cancer represents a severe health problem, especially in the Western world. In 2000, 415,568 of the 542,990 prostate cancer cases diagnosed worldwide were diagnosed in more developed countries, 211,950 of them in North America (1). For 2003, 220, 900 cases of prostate cancer (2) are expected to be diagnosed in the United States.

During recent years, considerable efforts have been made to find regimens for cancer chemoprevention. Pharmacological drugs like flutamide, DFMO, bicalutamide (casodex), celecoxib, sulindac sulfone (exisulind), GTx-006, and finasteride, as well as natural compounds

such as selenium, soy/genistein, vitamin D, vitamin E and lycopene, are currently being tested in clinical chemoprevention studies for their potential to prevent prostate cancer (3, 4). Furthermore, a variety of additional natural compounds and food ingredients like omega-3 fatty acids and EGCG are considered to have chemopreventive potential for prostate cancer (5) but have not yet been studied clinically.

According to epidemiological studies, the intake of tomato sauce, tomatoes, or pizza is significantly associated with lower prostate cancer risk (6), an effect thought to be mediated by lycopene, the main carotenoid in tomatoes. The health function of lycopene has been linked mainly to its potent antioxidant, singlet oxygen-quenching properties, which result in protection of oxidative DNA damage in vitro and in vivo [reviewed in (7)]. Furthermore, both lycopene and vitamin E can trap peroxynitrite, an important biological oxidant. Lycopene also inhibits cell proliferation by interference with IGF-I signaling (7).

Vitamin E functions as the major lipid-soluble antioxidant in cell membranes. α-Tocopherol scavenges lipid peroxyl radicals, which are the chain-carrying species propagating lipid peroxidation (8). In several studies, vitamin E was effective in reducing the growth of prostate tumors in vitro (9) and in vivo (10). A study of 2974 subjects with a 17-year follow-up indicated that low α-tocopherol levels were associated with higher prostate cancer risk (11). In the α-Tocopherol β-Carotene (ATBC) Study, prostate cancer incidence was reduced in the vitamin E supplemented group when compared with the control group (12).

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 less understood.

To gain insight into the in vivo mechanisms by which lycopene and/or vitamin E contribute to prostate cancer risk reduction, we used the MatLyLu Dunning prostate cancer model. Animals were pre-supplemented with diets containing lycopene or vitamin E, or a combination of both, for four weeks until tumor induction. Thereafter, supplementation was continued for an additional 18 days of tumor growth. Lycopene and vitamin E accumulation in plasma and tumor tissue was followed by HPLC analysis, and the activities of lycopene and vitamin E in tumor tissue were evaluated by in vivo MR imaging, as well as by GeneChip® analysis.

MATERIALS AND METHODS

Animals

30 male Copenhagen rats (8–10 weeks of age, 180–200 g; Charles River, Sulzfeld, Germany) were randomly assigned to five groups according to their supplementation. The control group received a basal diet consisting of Kliba diet #2019 (Provimi Kliba AG, Kaiseraugst, Switzerland) with added 6% (w/w) coconut fat. This basal diet contained less than 5 ppm vitamin E and a reduced vitamin A content (4000 iU/kg) and was devoid of phytosterols. The exact composition of the five experimental diets is given in Table 1.

All animals were pre-supplemented for four weeks. Subsequently, 1×10^5 MatLyLu Dunning prostate cancer cells (13) were injected into the ventral prostate, and supplementation was

continued until trial termination 18 days post-injection. At 14 days after tumor cell injection, orthotopic tumors were examined by using magnetic resonance (MR)-imaging. Plasma samples were taken before and after four weeks of supplementation, as well as at the end of the experiment. Tumors were weighed at sacrifice. All procedures during the animal trial were consistent with the German Animal Welfare Act.

MRI

MRI was performed at 1.5T (Magnetom Vision, Siemens, Erlangen, Germany). All images were acquired with a heavily T1-weighted thin section spin-echo sequence with a total acquisition time of 6.82 min. Images were obtained before and after intravenous injection of a conventional, low-molecular-weight gadolinium-containing contrast medium (Gd-DTPA) at a dosage of 0.3 mm Gd/kg. Necrosis rates were determined from the cross-sectional view of the tumor and was calculated as the percentage of necrotic area within the largest cross-sectional view. Differences in necrotic areas were assessed by unpaired *t*-test.

Lycopene analysis

Tissue samples were homogenized in acetone plus magnesium sulfate. The acetone extract was diluted with n-hexane and applied to an open magnesium sulfate column. Lycopene was eluted with n-hexane, dried, and dissolved in n-hexane/acetone (99:1 v/v). For the analysis of plasma samples, proteins were removed by ethanol precipitation. Lycopene was extracted by using n-hexane, which was dried and re-dissolved in n-hexane/acetone (99:1 v/v).

Analysis of lycopene isomers was performed by HPLC chromatography using three Nucleosil 300-5 columns (250×4.6 mm; Macherey-Nagel, Oensingen, Switzerland) coupled to a stationary phase of 750 × 4.6 mm. As mobile phase n-hexane/n-ethyldiisopropylamine (998.5:1.5 v/v) was used. For the detection of all-trans lycopene, as well as cis-isomers, the absorption was measured at 472 nm.

Vitamin E analysis

The tissue was saponified in a methanolic potassium hydroxide solution. The solution was diluted in 35% ethanol and extracted with hexane/toluol. α-Tocopherol was quantified by isocratic HPLC analysis by using a Lichrosorb^{TM} Si 60.5 μm, 20 × 4 mm precolumn coupled to a Lichrosorb^{TM} Si 60.5 μm, 125×4 mm column (Stagroma, Reinach, Switzerland) as stationary phase, and 3% 1.4-dioxane in n-hexane as mobile phase. For detection, fluorescent emission was measured at 330 nm after excitation at 295 nm.

RNA isolation

RNA isolation was performed by phenol-chloroform extraction (TRIzol Reagent, Invitrogen, Basel, Switzerland). Phenol-chloroform-purified RNA was re-purified with RNeasy mini spin columns (Qiagen, Basel, Switzerland) and an on-column DNase digest (RNase-Free DNase Set, Qiagen), according to the manufacturer's description. The integrity of the isolated RNA was tested on an agarose gel.

Affymetrix GeneChip® hybridization

RNA (10 µg) was subjected to double-stranded (ds)-cDNA synthesis with T7-d$(T)_{24}$ primers and the Superscript™ II choice system (Invitrogen). The ds-cDNA was used as template in an in vitro transcription reaction (T7 MegaScript kit, Ambion, Austin, TX) to generate the hybridization probes. The cRNA was labeled by incorporation of biotin-11-CTP and biotin-16-UTP (Roche Molecular Systems, Penzberg, Germany). cRNA (10 µg) was fragmented by incubation in 40 mM Tris-acetate, pH 8.1; 100 mM KOAc; and 30 mM MgOAc for 35 min at 95°C.

Affymetrix Rat Genome U34A microarrays were hybridized as described in the Gene Chip Expression Analysis Technical Manual (Affymetrix, Oxford, UK). Each tumor was represented by one chip. Briefly, fragmented cRNA was hybridized overnight at a stringency of 1× MES, 1M $[Na^+]$, and 0.01% Tween-20 at 45°C. The arrays were washed at a maximal stringency of 1× MES, 0.1 M $[Na^+]$, and 0.01% Tween-20 at 50°C (Fluidics program EukGE-WS2). Hybridization signals were detected by streptavidin-phycoerythrine staining (Molecular Probes, Leiden, Netherlands), which was amplified by incubation with anti-streptavidin antibody (Vector Labs, Orton Southgate, Great Britain) and a second staining with streptavidin-phycoerythrine. Subsequently, the microarrays were subjected to laser scanning (Hewlett Packard, Palo Alto, CA), and the hybridization signals were analyzed with the GeneChip® Analysis Suite MAS 5.0 (Affymetrix).

GeneChip® data analysis

The gene chip data analysis was performed by using RACE-A (Roche Affymetrix Chip Experiment-Analysis), a Roche proprietary software package for differential expression analysis. The workflow supported by RACE-A comprises the following steps: (i) selection and quality assessment of probe arrays; (ii) reading probe set intensities reported by Affymetrix chip analysis software; (iii) assignment of chip replicates to experimental conditions; (iv) comparisons of experimental conditions; (v) filtering of comparison results based on a variety of attributes calculated by RACE-A; and (vi) output to Excel® table format.

During step (iii), probe arrays were normalized against the mean signal intensity of each chip. Outlier genes, as defined by a Nalimov outlier test at 95% confidence level (14), were removed in step (iii). Changes of expression levels are reported as "fold induction" relative to the placebo-treated control.

For each gene analyzed, changes in gene expression in each comparison were considered significant when the expression level represented by the mean^{AvgDiff}-value of the gene in one or both groups compared was greater than 10 (switched on or off, or modulated above expression minimum), and the fold induction was above 1.5 or below 0.66.

TaqMan™ real time RT-PCR analysis

cDNA was synthesized by using random hexamer oligonucleotides and Superscript II (Invitrogen). Synthesis reactions were performed at 42°C, followed by enzyme inactivation at 70°C and RNase H (Invitrogen) digestion.

Real-Time-PCR was performed with a TaqMan ABI PRISM 7700 Sequence Detector System (PE Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. The housekeeping gene 18 S RNA served as calibrator gene. All primer and probe sets were tested for their amplification as recommended by Applied Biosystems.

For analysis, data were transferred to Excel©, where "fold inductions" were calculated as described in User Bulletin #2 (Applied Biosystems). To maintain consistent data quality, the inclusion criteria for the statistical analyses were defined by the detection of signals of minimal template concentrations as determined by a standard curve. Induction of gene expression within a treatment group was calculated as the geometric mean +/– standard error of the single fold induction values. Statistical analysis was performed with the Statview® software package. Non-parametric statistical tests, which are distribution-independent and thus more conservative, were applied to determine the significance levels, as recommended by Müller et al. (15).

RESULTS

General health status

There was no difference in the development of body weight between the groups during the first four weeks. Following tumor cell injection, rats were increasingly less active and stopped gaining weight, as their food consumption dropped due to continuous tumor growth.

Lycopene and vitamin E uptake

All plasma samples, as well as prostate tumor tissue samples, were analyzed for their lycopene and vitamin E content to evaluate the uptake of lycopene and vitamin E (Table 2).

After 28 days of pre-supplementation, plasma vitamin E concentrations of 21.67 μM and 26.38 μM in the vehicle and in the lycopene group were comparable with physiological levels of 25 μM in un-supplemented humans (16). At the same time, the vitamin E and the co-treated animals showed elevated serum vitamin E levels of 47.98 μM and 46.44 μM, which were in the same range as seen in humans after three years of supplementation in the ATBC study (17).

Lycopene supplementation for 28 days led to plasma concentrations of 1.02 and 0.92 μM. These concentrations correspond to the average plasma concentrations of 0.95 and 1.29 μM found in people who live in the Mediterranean region (18).

At late stages of tumor growth, the feed intake dropped, resulting in decreasing plasma levels of lycopene and vitamin E in all supplementation groups. This was especially evident for lycopene due to its short half-life in blood. However, lycopene levels in tumor tissue analyzed at Day 46 reached 0.38 and 0.42 μM, respectively, in the two lycopene-treated groups. At the same time, vitamin E increased two- to threefold in tumor tissues of supplemented animals and reached an average of 61.53 μM in the two vitamin E treated groups.

In vivo examination of MatLyLu tumor tissue by MR-imaging

On Day 14 after tumor cell injection, the tumors were examined in vivo by MR-imaging. As a result, both lycopene or vitamin E increased the percentage of necrotic area in the tumors (Table 3). Tumor volumes 14 days after tumor cell injection and tumor weights after 18 days of tumor growth (Day 46) were not significantly different between study groups (Table 3).

Vitamin E treatment increased the mean necrotic areas to 36.37% (P=0.0062), and lycopene increased it to 35.97% (P=0.0223). This finding compares with 19.98% necrosis in untreated and 23.27% in vehicle-treated animals. In the lycopene/vitamin E co-treated group, the percentage of necrotic area was non-significantly increased to 28.47%.

GeneChip® analysis of MatLyLu prostate tumor tissue

To establish the mechanisms by which lycopene and vitamin E had increased tumor necrosis rates, we analyzed tumor tissue for changes in gene regulation elicited by these compounds. The expression level of each gene in the three treatment groups was compared with the expression level in the vehicle group.

Vitamin E treatment alone up-regulated 90 genes and down-regulated 119 genes. In the lycopene-treated group, 101 genes, and in the lycopene/vitamin E co-treated group, 71 genes were up-regulated, while 261 and 260 genes, respectively, were repressed. This dominant suppression points to a general inhibitory effect of lycopene on tumor tissue gene expression.

Lycopene interferes with autocrine loops in tumor tissue

Most changes in gene expression by lycopene treatment were moderate. Only 25% of all genes affected by lycopene showed fold inductions ≥2 or ≤0.5.

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-α-reductase 1 expression in the lycopene [fold induction (fold) 0.36] and in the co-treated group (fold 0.48). This finding is of relevance because testosterone is converted to the more potent 5αDHT by 5-α-reductase in normal prostate development (19), as well as in prostate cancer development (20). Consequently, a set of androgen target genes (cystatin related protein 1 and 2, prostatic spermine binding protein, prostatic steroid-binding protein C1, C2 and C3 chain, probasin) was consistently down-regulated in both lycopene-treated groups with fold up to 0.04 (prostatic steroid-binding protein C1 chain) in the lycopene group, and up to 0.02 (prostatic steroid-binding protein C1 chain) in the co-treated group (Table 4). This was confirmed by TaqMan™ real-time RT-PCR analysis with three androgen target genes (Fig. 1).

Additionally, lycopene treatment significantly reduced local expression of IL-6, an autocrine growth factor in prostate cancer (21). Importantly, insulin and IGF-I were also significantly reduced by lycopene on the mRNA level. Epidemiological data indicate that IGF-I signaling is a factor involved in prostate carcinogenesis (22). Two reactive oxygen species (ROS) generating enzymes, inducible nitric oxide synthase (iNOS) and p22-phox, a subunit of the NADPH oxidase, were down-regulated by lycopene. Arylamine n-acetyltransferase 2 (GenBank accession

no U01348) was also down-regulated by lycopene. In the sequence of 171 basepairs represented by the Affymetrix probe set for NAT2, the sequences specific for n-acetyltransferase type 1 or type 2 differed by 3 basepairs. Thus, the probe sets will detect both NAT1 and NAT2. Recently, Hein et al. suggested that a low n-acetyltransferase 2 activity in the liver and a high n-acetyltransferase 1 activity in the prostate contribute to the prostate cancer risk due to heterocyclic amines (23).

Vitamin E affects androgen target genes

Vitamin E supplementation of rats led to changes in expression of 209 genes in the prostate tumor tissue. Similar to lycopene treatment, the major effect was observed on steroid signaling (Table 4), resulting in the decreased expression of a set of androgen target genes. As for the lycopene-treated group, this finding was confirmed by TaqMan™ real-time RT-PCR (Fig. 1).

In contrast to lycopene supplementation, vitamin E treatment did not influence steroid 5-α-reductase expression in the prostate tumors. Vitamin E, however, significantly reduced aromatase expression, suggesting reduced estrogen synthesis. Also, in contrast to lycopene treatment, neither IGF-I nor the ROS generating iNOS or NADPH oxidase were affected by vitamin E.

Effects in the lycopene/vitamin E co-treated group are characterized by the inhibitory action of lycopene

Lycopene/vitamin E co-treatment affected 331 genes, of which 71 genes were induced (Table 4). The effect of co-treatment is therefore mainly dominated by lycopene's inhibitory features, with the strongest effect seen in steroid metabolism and signaling. As in the vitamin E group, the estrogen-metabolizing enzymes aromatase and 17β-estradiol inactivating estradiol 17β-dehydrogenase 4 (24) were significantly down-regulated. Furthermore, androgen activation was reduced, similar to the findings for the lycopene group. In line with the inhibitory effect on steroid metabolism, the same set of androgen target genes was affected as in the lycopene group. In comparison with the groups treated with vitamin E and lycopene alone, the inhibitory activities on androgen target gene expression seemed to be additive in the co-treated group. This additive effect can be explained by lycopene acting on the autocrine/paracrine 5αDHT synthesis and by vitamin E acting downstream of androgen metabolism by inhibiting transduction via the androgen receptor (25).

In addition to steroid metabolism and signaling, the expression of Jun-D, C-Jun, and Jun dimerization protein 2 (JDP2) was significantly reduced by co-treatment, while the single treatment led only to an insignificant reduction. Jun-D, C-Jun, and JDP2 all participate in JNK signaling, which is known to be induced by oxidative stress and is involved in the regulation of apoptosis in response to oxidative stress (26). The stronger decrease in JNK signaling by lycopene/vitamin E co-treatment can be explained by a cooperative reduction in oxidative stress due to the combination of the two antioxidants.

The reduction of IGF-I, insulin, iNOS, and arylamine n-acetyltransferase 2 (GenBank accession no U01348) were comparable with the changes seen in the lycopene group, which indicates that these changes in gene expression were induced by lycopene.

Separation of lycopene and vitamin E effects: Lycopene causes 5-α-reductase and IGF-I reduction in MatLyLu tumor tissue

It has to be noted that the lycopene formulation, and accordingly the placebo, contained low amounts of vitamin E as stabilizer (Table 1). Therefore, the lycopene effect could not be separated from that of vitamin E, and changes in gene expression might have been the result of a joint lycopene/low vitamin E action. To distinguish the lycopene effect from the vitamin E effect, the data were re-grouped in correlation to the actual vitamin E concentration in the tumors. The re-organized groups were analyzed for the lycopene activity in the presence of very low (<15 mg/l), low (15–25 mg/l) or high (≥25 mg/l) vitamin E levels (Table 5). Within each of the three defined vitamin E concentration ranges, we compared the gene expression of the lycopene-free samples with the gene expression of those samples containing detectable amounts of lycopene.

The statistical analysis of samples containing <15 mg vitamin E/L ("very low E") was not possible, as there were only two tissue samples containing lycopene. In the two resulting comparisons, "low E" (15–25 mg vitamin E/L) and "high E" (>25mg vitamin E/L), the changes in gene expression induced by lycopene addition were calculated, and the genes were filtered according to former analysis.

This analysis confirmed the findings described above. The down-regulation of 5-α-reductase I (fold 0.33) as well as the reduction of androgen target gene expression and IGF-I expression were more pronounced in the presence of a high vitamin E concentration (Table 4). The reduction in IL-6 expression was stronger in the presence of a moderate vitamin E concentration. However, the analysis revealed that both in presence of a low, and in presence of a high vitamin E value lycopene was responsible for the reduction in androgen target gene expression in prostate tumors.

DISCUSSION

In our study, we demonstrate that lycopene and vitamin E increase tumor necrosis in the Dunning prostate cancer model. By gene expression analysis of tumors we provide two major mechanisms by which this effect is likely mediated: (I) reduction in tissue internal androgen activation and thereby a reduction in steroid signaling, and (II) reduction in local IGF-I and IL-6 expression, which interferes with the endocrine loop of local synthesis and signaling.

The orthotopic Dunning tumor model used in this study represents the most widely studied animal model for prostate cancer (27). In this model, prostate cancer cells are injected into the rat ventral prostate and grow in their natural environment. Among the characterized Dunning prostate cancer cell lines, the MatLyLu cell line used in our study represents the most aggressive subline showing the shortest doubling time and being highly metastatic (28). The informative doses are reported to be 10^4 MatLyLu cells subcutaneously injected to test immune response (29)

and 2×10^5 cells intravenously injected for skeletal metastasis induction (30). In our experiment, we used 10^5 cells injected into the ventral prostate lobe as the dose reported to be informative for prostate cancer induction (31).

Pre-supplementation with lycopene and vitamin E for 4 weeks until tumor cell injection led to physiologically relevant plasma concentrations of both compounds. Within 18 days of tumor growth, lycopene and vitamin E were accumulated in the tumor tissue.

At 14 days after tumor cell injection, tumors were evaluated macroscopically by in vivo MR-imaging, a method frequently used to evaluate the therapeutic efficacy in tumor models (32). We found no difference in tumor volume between the different treatment groups. However, we did find a significant increase in necrotic area of tumors from animals supplemented with lycopene or vitamin E. Such an increased necrosis rate represents a remarkable effect in this aggressive model, especially for nutritional intervention. In several cancer models, it has been shown that treatment can increase tumor internal cell death without affecting the tumor volume. For example, RIF-1 tumors treated with cyclophosphamide resulted in an estimated tumor cell kill of 67% with no changes in tumor volume (33). Moreover, the treatment of rat intracranial 9L gliomas with BCNU led to an estimated 90% cell kill within a week, whereas tumor volume doubled over time (34). However, although this effect would suggest therapeutic activity, caution should be taken to promote lycopene for therapy, unless further research has proven the efficacy as strong enough for therapeutic use.

We further investigated the effect of treatment on gene expression level by GeneChip® analysis. The expression data were filtered with low stringency regarding the minimum expression intensity on the mRNA level and changes in expression. This was done under the assumption that nutrition-based cancer prevention is mediated by chronic exposure to moderately active ingredients. The second reason for the low stringency was the short time frame of 18 days between tumor cell injection and sacrifice for accumulation and action within the tumor tissue.

Unexpectedly, we found steroid hormone synthesis and signaling being most strongly affected in MatLyLu tumors. In 1986, this cell line was reported to be androgen insensitive (28). This statement was based on the finding that authors saw no difference in androgen receptor content of cytosolic fractions between tumor tissue samples of uncastrated tumor-bearing rats and tumor-bearing rats, which were castrated 24 h earlier. Furthermore, in cells grown in vitro, authors could not detect androgen receptor or estrogen receptor expression (28). The GeneChip® data and TaqMan™ analysis from the current study showed the presence of androgen receptor mRNA, although at a low level (data not shown). Estrogen receptor α was undetectable, but estrogen receptor β (ERβ) mRNA was clearly present (data not shown). This discrepancy concerning steroid receptor expression can be explained by sensitivity differences in the methods used and by different growth conditions.

In our hands, changes in androgen target gene expression in the MatLyLu tumor clearly indicate a response to androgen signaling. A massive down-regulation was seen in all three treatment groups. Steroid 5-α-reductase 1 expression was reduced in both groups receiving lycopene. Consequently, a set of androgen target genes was consistently and profoundly down-regulated.

Comparisons based on the vitamin E and lycopene concentrations in tumor tissue showed that the reduction of both steroid 5-α-reductase and androgen target genes was mediated by lycopene.

In the vitamin E-treated group, the reduced androgen target gene expression is in agreement with studies demonstrating that vitamin E inhibits the androgen receptor (25). In addition, vitamin E affected estrogen metabolism by down-regulating aromatase expression. Weihua and co-workers have recently shown that the major endogenous estrogen in the prostate is not 17β-estradiol, but 5α-androstane-3β,17β-diol (3βAdiol; 35). The latter is synthesized from the 5-α-reductase product 5αDHT by 3β-hydroxysteroid dehydrogenase activity. Thus, down-regulation of 5-α-reductase in combination with aromatase reduction, as seen in the lycopene/vitamin E co-treated group, affects the local synthesis of both estrogenic steroids in the prostate. A complete block of both androgen and estrogen signaling leads to atrophic luminal epithelial cells with no secretory activity (36). Thus, it is conceivable that lycopene's beneficial impact on prostate cancer, especially in combination with vitamin E, is at least in part due to a local reduction of the autocrine/paracrine loop of steroid synthesis and signaling within the prostate.

Additionally, the changes in gene expression in the co-treated group indicated an interaction between lycopene and vitamin E on oxidative stress reduction. The down-regulation of JDP2, C-Jun, and Jun-D, indicative of reduced JNK signaling, was stronger in the co-treated group than in the individually treated groups and was paralleled by weaker necrosis rate as determined by MR-imaging. Oxidative stress is known to activate JNK and p38 pathways (26). In prostate cancer cells, JNK signaling was recently shown to contribute to apoptosis induction (37). Both lycopene and vitamin E are known to have antioxidative properties. Therefore, the concerted action of lycopene and vitamin E on JNK signaling indicates a stronger reduction in oxidative stress in the co-treated group and might explain a weaker necrosis rate. In healthy tissue, reduction of oxidative stress is desired in terms of prevention as radicals/reactive oxygen species are involved in the process of initiation and promotion of neoplastic diseases (38, 39).

Ample evidence from preclinical studies (9), epidemiological observations (11, 40), and controlled trials (12, 17) indicates that vitamin E prevents the development or progression of prostate cancer. Compelling data supporting the use of vitamin E in this setting comes from secondary analyses of the ATBC study, a large, randomized, double-blind, placebo-controlled trial (41). Based primarily on these and other research results, a new Phase III, randomized, double-blind, placebo-controlled intervention trial, the SELECT study, was recently launched, to test the efficacy of vitamin E and selenium, alone and in combination, in the prevention of prostate cancer (42). Consistent with the evidence of vitamin E as effective preventive agent in the development of prostate cancer, our findings suggest that vitamin E acts via suppression of estrogen metabolism and androgen signaling in prostate.

Epidemiologically, the intake of lycopene in the form of tomato-based products is associated with a reduced prostate cancer risk (6, 43). In addition, lycopene also prevented the progression of existing tumors. In a nested case-control study (44), embedded in the Physicians' Health Study, and in a case-control study in Americans of different ethnic origin (45), serum lycopene was inversely associated with prostate cancer risk, particularly for the late stages, the aggressive forms of the disease. Our finding that lycopene affects the very rapid growing and highly metastatic MatLyLu tumor agrees with these epidemiological data.

Another important finding was the reduction of IGF-I expression by lycopene. Although epidemiological data draw an inconclusive picture about the role of systemic IGF-I levels in prostate cancer, IGF-I is regarded as a risk factor for prostate cancer (22). Over-expression of IGF-I in prostate basal epithelial cells leads to spontaneous hyperplasia (46), and in a transgenic adenocarcinoma of mouse prostate (TRAMP) model, the prostate cancer progression is associated with increased IGF-I expression (47). Insulin receptors, which have been characterized on prostate epithelial cells (48), and IGF-I receptor both converge in their signaling by activating insulin receptor substrate 1 (49, 50), and induce proliferation of primary prostate epithelial cells (51). Therefore, down-regulation of local IGF-I production in prostate tumors by lycopene might interfere with the autocrine or paracrine action of IGF-I on prostate tumor progression.

The expression of IL-6 and its receptor in benign and in neoplastic prostate tissue is known to build paracrine and autocrine loops (52), which contribute to prostate cancer growth in vivo (53). In our study, lycopene interfered with the autocrine loop of local IL-6 expression and signaling. Reduced expression of the proinflammatory cytokine IL-6 expression is beneficial, as a history of prostatitis is epidemiologically associated with an elevated prostate cancer risk (54). Furthermore, focal prostatic atrophy lesions (also termed “proliferative inflammatory atrophy”) are often associated with chronic inflammation, and they are discussed as being an intermediate stage, which may progress to prostatic intraepithelial neoplasia and/or adenocarcinoma (55, 56).

In summary, we have shown that lycopene and vitamin E are accumulated and act locally in prostate tumor tissue. We have successfully identified signaling events in prostate tumor tissue affected by nutritional intervention. Assuming that lycopene and vitamin E interact with the same signaling pathways in healthy prostate tissue and throughout prostate cancer development, we consider our results a plausible mechanistic explanation for the epidemiologically observed risk reduction for prostate cancer by lycopene and vitamin E. To prove our concept, further studies are needed to confirm that lycopene and vitamin E perform their activities via the identified mechanisms in healthy prostate tissue.

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REFERENCES

1. Ferlay, J., Bray, F., Pisani, P., and Parkin, D. M. GLOBOCAN 2000: Cancer Incidence, Mortality and Prevalence Worldwide, Version 1.0, IARC CancerBase No. 5. Lyon, IARCPress, 2001. http://www-dep.iarc.fr/globocan/globocan.html

2. American Cancer Society (2003) Cancer Facts & Figures 2003, http://www.cancer.org/downloads/STT/CAFF2003PWSecured.pdf

3. Parnes, H. L., House, M. G., Kagan, J., Kausal, D. J., and Lieberman, R. (2004) Prostate cancer chemoprevention agent development: the National Cancer Institute, Division of Cancer Prevention Portfolio. *J. Urol.* **171,** S68–74; discussion S75

4. Lieberman, R. (2002) Chemoprevention of prostate cancer: current status and future directions. *Cancer Metastasis Rev.* **21,** 297–309

5. Kucuk, O. (2002) Chemoprevention of prostate cancer. *Cancer Metastasis Rev.* **21,** 111–124

6. Giovannucci, E., Ascherio, A., Rimm, E. B., Stampfer, M. J., Colditz, G. A., and Willett, W. C. (1995) Intake of carotenoids and retinol in relation to risk of prostate cancer. *J. Natl. Cancer Inst.* **87,** 1767–1776

7. Goralczyk, R., and Siler, U. (2004) The Role of Lycopene in Health and Disease. In *Phytochemicals in Health and Disease* (Bao, Y., and Fenwick, R., eds) pp. 285–309, Marcel Dekker, Inc, New York

8. Wang, X., and Quinn, P. J. (1999) Vitamin E and its function in membranes. *Prog. Lipid Res.* **38,** 309–336

9. Israel, K., Sanders, B. G., and Kline, K. (1995) RRR-alpha-tocopheryl succinate inhibits the proliferation of human prostatic tumor cells with defective cell cycle/differentiation pathways. *Nutr. Cancer* **24,** 161–169

10. Fleshner, N., Fair, W. R., Huryk, R., and Heston, W. D. (1999) Vitamin E inhibits the high-fat diet promoted growth of established human prostate LNCaP tumors in nude mice. *J. Urol.* **161,** 1651–1654

11. Eichholzer, M., Stahelin, H. B., Gey, K. F., Ludin, E., and Bernasconi, F. (1996) Prediction of male cancer mortality by plasma levels of interacting vitamins: 17-year follow-up of the prospective Basel study. *Int. J. Cancer* **66,** 145–150

12. Albanes, D., Heinonen, O. P., Huttunen, J. K., Taylor, P. R., Virtamo, J., Edwards, B. K., Haapakoski, J., Rautalahti, M., Hartman, A. M., Palmgren, J., et al. (1995) Effects of alpha-tocopherol and beta-carotene supplements on cancer incidence in the Alpha-Tocopherol Beta-Carotene Cancer Prevention Study. *Am. J. Clin. Nutr.* **62,** 1427S–1430S

13. Isaacs, J. T., Yu, G. W., and Coffey, D. S. (1981) The characterization of a newly identified, highly metastatic variety of Dunning R 3327 rat prostatic adenocarcinoma system: the MAT LyLu tumor. *Invest. Urol.* **19,** 20–23

14. Kaiser, R., and Gottschalk, G. (1972) *Elementare Tests zur Beurteilung von Messdaten*, Bibliographisches Institut, Mannheim/Wien/Zuerich

15. Muller, P. Y., Janovjak, H., Miserez, A. R., and Dobbie, Z. (2002) Processing of gene expression data generated by quantitative real-time RT-PCR. *Biotechniques* **32,** 1372–1379

16. Dixon, L. B., Winkleby, M. A., and Radimer, K. L. (2001) Dietary intakes and serum nutrients differ between adults from food-insufficient and food-sufficient families: Third National Health and Nutrition Examination Survey, 1988-1994. *J. Nutr.* **131,** 1232–1246

17. (1994) The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. The Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group. *N. Engl. J. Med.* **330,** 1029–1035

18. Van Kappel, A. (2000) Carotenoids in blood: Biochemical markers of fruit and vegetables consumption and relation to the risk of breast cancer, Library Bu Santé, Code 321051/2000/162, Theses University Claude Bernard, Lyon 1

19. Bonkhoff, H., and Remberger, K. (1996) Differentiation pathways and histogenetic aspects of normal and abnormal prostatic growth: a stem cell model. *Prostate* **28,** 98–106

20. Debes, J. D., and Tindall, D. J. (2002) The role of androgens and the androgen receptor in prostate cancer. *Cancer Lett.* **187,** 1–7

21. Giri, D., Ozen, M., and Ittmann, M. (2001) Interleukin-6 is an autocrine growth factor in human prostate cancer. *Am. J. Pathol.* **159,** 2159–2165

22. Pollak, M. (2001) Insulin-like growth factors and prostate cancer. *Epidemiol. Rev.* **23,** 59–66

23. Hein, D. W., Leff, M. A., Ishibe, N., Sinha, R., Frazier, H. A., Doll, M. A., Xiao, G. H., Weinrich, M. C., and Caporaso, N. E. (2002) Association of prostate cancer with rapid N-acetyltransferase 1 (NAT1*10) in combination with slow N-acetyltransferase 2 acetylator genotypes in a pilot case-control study. *Environ. Mol. Mutagen.* **40,** 161–167

24. Labrie, F., Luu-The, V., Labrie, C., and Simard, J. (2001) DHEA and its transformation into androgens and estrogens in peripheral target tissues: intracrinology. *Front. Neuroendocrinol.* **22,** 185–212

25. Zhang, Y., Ni, J., Messing, E. M., Chang, E., Yang, C. R., and Yeh, S. (2002) Vitamin E succinate inhibits the function of androgen receptor and the expression of prostate-specific antigen in prostate cancer cells. *Proc. Natl. Acad. Sci. USA* **99,** 7408–7413

26. Martindale, J. L., and Holbrook, N. J. (2002) Cellular response to oxidative stress: signaling for suicide and survival. *J. Cell. Physiol.* **192,** 1–15

27. Bostwick, D. G., Ramnani, D., and Qian, J. (2000) Prostatic intraepithelial neoplasia: animal models 2000. *Prostate* **43,** 286–294

28. Isaacs, J. T., Isaacs, W. B., Feitz, W. F., and Scheres, J. (1986) Establishment and characterization of seven Dunning rat prostatic cancer cell lines and their use in developing methods for predicting metastatic abilities of prostatic cancers. *Prostate* **9,** 261–281

29. Friedman, E. J., Orth, C. R., Brewton, K. A., Ponniah, S., and Alexander, R. B. (1997) Cryosurgical ablation of the normal ventral prostate plus adjuvant does not protect Copenhagen rats from Dunning prostatic adenocarcinoma challenge. *J. Urol.* **158,** 1585–1588

30. Geldof, A. A., van den Tillaar, P. L., Newling, D. W., and Teule, G. J. (1997) Radionuclide therapy for prostate cancer lumbar metastasis prolongs symptom-free survival in a rat model. *Urology* **49,** 795–801

31. Lein, M., Jung, K., Ortel, B., Stephan, C., Rothaug, W., Juchem, R., Johannsen, M., Deger, S., Schnorr, D., Loening, S., et al. (2002) The new synthetic matrix metalloproteinase inhibitor (Roche 28-2653) reduces tumor growth and prolongs survival in a prostate cancer standard rat model. *Oncogene* **21,** 2089–2096

32. Chenevert, T. L., Stegman, L. D., Taylor, J. M., Robertson, P. L., Greenberg, H. S., Rehemtulla, A., and Ross, B. D. (2000) Diffusion magnetic resonance imaging: an early surrogate marker of therapeutic efficacy in brain tumors. *J. Natl. Cancer Inst.* **92,** 2029–2036

33. Zhao, M., Pipe, J. G., Bonnett, J., and Evelhoch, J. L. (1996) Early detection of treatment response by diffusion-weighted 1H-NMR spectroscopy in a murine tumour in vivo. *Br. J. Cancer* **73,** 61–64

34. Chenevert, T. L., McKeever, P. E., and Ross, B. D. (1997) Monitoring early response of experimental brain tumors to therapy using diffusion magnetic resonance imaging. *Clin. Cancer Res.* **3,** 1457–1466

35. Weihua, Z., Makela, S., Andersson, L. C., Salmi, S., Saji, S., Webster, J. I., Jensen, E. V., Nilsson, S., Warner, M., and Gustafsson, J. A. (2001) A role for estrogen receptor beta in the regulation of growth of the ventral prostate. *Proc. Natl. Acad. Sci. USA* **98,** 6330–6335

36. Huynh, H., Alpert, L., Alaoui-Jamali, M. A., Ng, C. Y., and Chan, T. W. (2001) Co-administration of finasteride and the pure anti-oestrogen ICI 182,780 act synergistically in modulating the IGF system in rat prostate. *J. Endocrinol.* **171,** 109–118

37. Shimada, K., Nakamura, M., Ishida, E., Kishi, M., and Konishi, N. (2003) Roles of p38- and c-jun NH2-terminal kinase-mediated pathways in 2-methoxyestradiol-induced p53 induction and apoptosis. *Carcinogenesis* **24,** 1067–1075

38. Hussain, S. P., Hofseth, L. J., and Harris, C. C. (2003) Radical causes of cancer. *Nat. Rev. Cancer* **3,** 276–285

39. Gromadzinska, J., and Wasowicz, W. (2000) The role of reactive oxygen species in the development of malignancies. *Int. J. Occup. Med. Environ. Health* **13,** 233–245

40. Hsing, A. W., Comstock, G. W., Abbey, H., and Polk, B. F. (1990) Serologic precursors of cancer. Retinol, carotenoids, and tocopherol and risk of prostate cancer. *J. Natl. Cancer Inst.* **82,** 941–946

41. Heinonen, O. P., Albanes, D., Virtamo, J., Taylor, P. R., Huttunen, J. K., Hartman, A. M., Haapakoski, J., Malila, N., Rautalahti, M., Ripatti, S., et al. (1998) Prostate cancer and supplementation with alpha-tocopherol and beta-carotene: incidence and mortality in a controlled trial. *J. Natl. Cancer Inst.* **90,** 440–446

42. Klein, E. A., Thompson, I. M., Lippman, S. M., Goodman, P. J., Albanes, D., Taylor, P. R., and Coltman, C. (2003) SELECT: the selenium and vitamin E cancer prevention trial. *Urol. Oncol.* **21,** 59–65

43. Giovannucci, E. (2002) A review of epidemiologic studies of tomatoes, lycopene, and prostate cancer. *Exp. Biol. Med. (Maywood)* **227,** 852–859

44. Gann, P. H., Ma, J., Giovannucci, E., Willett, W., Sacks, F. M., Hennekens, C. H., and Stampfer, M. J. (1999) Lower prostate cancer risk in men with elevated plasma lycopene levels: results of a prospective analysis. *Cancer Res.* **59,** 1225–1230

45. Vogt, T. M., Mayne, S. T., Graubard, B. I., Swanson, C. A., Sowell, A. L., Schoenberg, J. B., Swanson, G. M., Greenberg, R. S., Hoover, R. N., Hayes, R. B., et al. (2002) Serum lycopene, other serum carotenoids, and risk of prostate cancer in US Blacks and Whites. *Am. J. Epidemiol.* **155,** 1023–1032

46. DiGiovanni, J., Kiguchi, K., Frijhoff, A., Wilker, E., Bol, D. K., Beltran, L., Moats, S., Ramirez, A., Jorcano, J., and Conti, C. (2000) Deregulated expression of insulin-like growth factor 1 in prostate epithelium leads to neoplasia in transgenic mice. *Proc. Natl. Acad. Sci. USA* **97,** 3455–3460

47. Kaplan, P. J., Mohan, S., Cohen, P., Foster, B. A., and Greenberg, N. M. (1999) The insulin-like growth factor axis and prostate cancer: lessons from the transgenic adenocarcinoma of mouse prostate (TRAMP) model. *Cancer Res.* **59,** 2203–2209

48. Carmena, M. J., Fernandez-Moreno, M. D., and Prieto, J. C. (1986) Characterization of insulin receptors in isolated epithelial cells of rat ventral prostate: effect of fasting. *Cell Biochem. Funct.* **4,** 19–24

49. White, M. F. (1998) The IRS-signaling system: a network of docking proteins that mediate insulin and cytokine action. *Recent Prog. Horm. Res.* **53,** 119–138

50. Furstenberger, G., and Senn, H. J. (2002) Insulin-like growth factors and cancer. *Lancet Oncol.* **3,** 298–302

51. Cohen, P., Peehl, D. M., Lamson, G., and Rosenfeld, R. G. (1991) Insulin-like growth factors (IGFs), IGF receptors, and IGF-binding proteins in primary cultures of prostate epithelial cells. *J. Clin. Endocrinol. Metab.* **73,** 401–407

52. Hobisch, A., Rogatsch, H., Hittmair, A., Fuchs, D., Bartsch, G., Jr., Klocker, H., Bartsch, G., and Culig, Z. (2000) Immunohistochemical localization of interleukin-6 and its receptor in benign, premalignant and malignant prostate tissue. *J. Pathol.* **191,** 239–244

53. Smith, P. C., and Keller, E. T. (2001) Anti-interleukin-6 monoclonal antibody induces regression of human prostate cancer xenografts in nude mice. *Prostate* **48,** 47–53

54. Dennis, L. K., Lynch, C. F., and Torner, J. C. (2002) Epidemiologic association between prostatitis and prostate cancer. *Urology* **60,** 78–83

55. De Marzo, A. M., Marchi, V. L., Epstein, J. I., and Nelson, W. G. (1999) Proliferative inflammatory atrophy of the prostate: implications for prostatic carcinogenesis. *Am. J. Pathol.* **155,** 1985–1992

56. Putzi, M. J., and De Marzo, A. M. (2000) Morphologic transitions between proliferative inflammatory atrophy and high-grade prostatic intraepithelial neoplasia. *Urology* **56,** 828–832

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Table 1

Diet composition for the five supplementation groups

Supplementation	Basal diet[a] supplements: Powder cellulose[b]	Lycopene beadlet[c]	Lycopene beadlet placebo[d]	Vitamin E beadlet[e]	Vitamin E beadlet placebo[f]	final Vitamin E content	final Lycopene content
Control	5 g/kg					<5 ppm	0
Vehicle			4 g/kg		1 g/kg	40 ppm	0
Vitamin E			4 g/kg	1 g/kg		540 ppm	0
Lycopene		4 g/kg			1 g/kg	40 ppm	200 ppm
Lyc + Vit E		4 g/kg		1 g/kg		540 ppm	200 ppm

[a] Basal diet: Kliba #2019 (Provimi Kliba AG, Kaiseraugst, Switzerland) + 6% (w/w) coconut fat.

[b] Sanacel 300 (Schneider+Co. AG, Winterthur, Switzerland) to compensate the volume of the supplements.

[c] Lycopene 5% TG (DSM Nutritional Products, Basel, Switzerland).

[d] Lycopene 5% TG placebo formulation (DSM Nutritional Products, Basel, Switzerland).

[e] Rovimix® E-50 SD (DSM Nutritional Products, Basel, Switzerland).

[f] Rovimix® E-50 SD placebo formulation (DSM Nutritional Products, Basel, Switzerland).

Table 2

Lycopene and vitamin E concentrations in plasma and prostate tumor tissue during the supplementation period

	Lycopene					Vitamin E			
	Plasma			Prostate		Plasma			Prostate
Diet	Day 0 µM	Day 28 µM	Day 46 µM	Day 46 µM[a]	% 5cis	Day 0 µM	Day 28 µM	Day 46 µM	Day 46 µM[a]
Control	ND	ND	ND	ND		NM	9.29 (+/– 0)	7.74 (+/– 1.34)	23.99 (+/– 3.79)
Vehicle	ND	ND	ND	ND		NM	21.67 (+/– 3.55)	15.48 (+/– 1.34)	55.34 (+/– 7.69)
Vitamin E	ND	ND	ND	ND		NM	47.98 (+/–7.09)	20.12 (+/–1.34)	75.46 (+/–9.71)
Lycopene	ND	1.02 (+/–0.30)	0.004 (+/–0.01)	0.38 (+/–0.11)	38.9 (+/–1.58)	23.22 (+/–2.32)	26.31 (+/–1.34)	12.38 (+/–1.34)	44.89 (+/–3.75)
Lyc + Vit E	ND	0.92 (+/–0.22)	0.051 (+/–0.068)	0.42 (+/–0.18)	39.0 (+/–1.64)	23.99 (+/–1.34)	46.44 (+/–6.97)	21.67 (+/–3.55)	47.60 (+/–34.77)

Average concentration (+/–stdev)

ND not detected

NM not measured

[a]1µmol/kg tissue corresponds to 1µM

Table 3

Percentage of necrotic area in a cross-sectional view of prostate tumor tissue determined by in vivo MR-imaging, and tumor weight

	% Necrotic area			Tumor weight (g)	
Treatment	Mean	Stdev	P	Mean	Stdev
Control	19.98	4.66		11.46	1.98
Vehicle	23.27	10.47		11.60	2.68
Control/Vehicle	21.25	8.20	1	11.52	2.25
Vitamin E	36.37	11.92	0.0062	12.08	1.47
Lycopene	35.97	16.53	0.0223	11.35	1.73
Lyc / Vit E	28.47	10.82	0.1448	11.69	1.73

Table 4

GeneChip® analysis of MatLyLu prostate tumor tissue: changes in gene expression

		Fold induction				
	Grouping by:	Supplementation[a]			Prostate tissue concentrations[b]	
	Reference condition:	Vehicle group			Lycopene free prostate samples	
AFFY_ID	DESCR	Lyc	Vit E	Lyc/VitE	Low E	High E
J05035_g_at	Steroid 5-alpha-reductase 1 (ec 1.3.99.5)	0.88	0.67	0.48	1.04	0.50
J05035_at	steroid 5-alpha-reductase 1 (ec 1.3.99.5)	0.41	1.03	0.64	0.39	0.33
S81448_s_at	steroid 5-alpha-reductase 1 (ec 1.3.99.5)	0.36	1.01	0.85	1.33	0.52
M33986mRNA_at	cytochrome p450 19 (aromatase) (ec 1.14.14.1)	0.70	0.57	0.65	0.90	0.33
rc_AI101743_s_at	estradiol 17 beta-dehydrogenase 4 (ec 1.1.1.62)	0.73	0.98	0.63	0.74	0.51
U56853_s_at	rattus norvegicus 21-hydroxylase mrna, complete cds	0.32	0.78	0.48	0.52	0.52
U89280_at	oxidative 17beta hydroxysteroid dehydrogenase type 6	0.73	0.71	0.65	0.95	0.52
Z13993_f_at	cystatin related protein 1 precursor (crp-1)	0.32	0.37	0.13	0.84	0.34
M58169_f_at	cystatin related protein 2 precursor	0.27	0.09	0.03	0.27	0.10
M58169_I_at	cystatin related protein 2 precursor	0.23	0.13	0.08	0.27	0.11
rc_AI639100_at	crp2 gene for cystatin related protein 2	0.39	0.39	0.24	0.44	0.58
J02675_at	prostatic spermine-binding protein precursor (sbp)	0.13	0.23	0.04	0.13	0.11
rc_AI639036_f_at	best hit: rat prostatic spermine-binding protein (sbp)	0.74	0.43	0.47	0.79	0.83
J00774cds_s_at	prostatic steroid-binding protein c1 chain precursor	0.07	0.05	0.02	0.07	0.13
V01255_at	prostatic steroid-binding protein c1 chain precursor	0.04	0.05	0.02	0.04	0.17
J00776cds_s_at	prostatic steroid-binding protein c2 chain precursor	0.28	0.09	0.04	0.28	0.08
X05034_at	prostatic steroid-binding protein c2 chain precursor	0.23	0.20	0.05	0.24	0.09
J00777_at	prostatic steroid-binding protein c3 chain precursor	0.47	0.18	0.09	0.25	0.19
J00772_s_at	prostatic steroid-binding protein c3 chain precursor	0.25	0.10	0.04	0.25	0.07
M27156_g_at	probasin precursor (pb) (m-40)	0.61	0.30	0.21	0.89	0.39
M27156_at	probasin precursor (pb) (m-40)	0.50	0.27	0.14	0.78	0.39
M26744_at	interleukin-6 precursor (il-6)	0.58	1.05	0.79	0.62	0.83
M15481_at	insulin-like growth factor ia precursor (igf-ia)	0.63	0.80	0.56	0.73	0.26
D00698_s_at	insulin-like growth factor ia precursor (igf-ia)	0.50	0.90	0.49	0.52	0.61
M15481_g_at	insulin-like growth factor ia precursor (igf-ia)	0.74	1.02	0.74	0.77	0.58
AF050159_at	rattus norvegicus insulin receptor substrate 2 (irs-2)	0.92	1.03	0.53	1.10	0.50
M31837_at	insulin-like growth factor binding protein 3 precursor	1.16	1.04	1.46	1.11	1.50
rc_AI014020_f_at	insulin 2 precursor	0.61	0.63	0.37	0.58	0.53
M25584_at	insulin 1 precursor	0.64	0.76	1.01	0.93	1.18
E00001cds_f_at	dna coding for rat pro-insulin	0.37	1.09	0.60	0.62	0.68
D26307cds_at	transcription factor jun-d	0.75	0.95	0.64		
rc_AA944014_at	rat c-jun oncogene mrna for transcription factor ap-1	0.81	0.93	0.61		
U53449_at	rattus norvegicus jun dimerization protein 2 (jdp-2)	0.93	0.92	0.55		
AF006619_s_at	nitric oxide synthase, inducible (ec 1.14.13.39) (inos)	0.67	0.73	1.08		

D44591_s_at	nitric oxide synthase, inducible (ec 1.14.13.39) (inos)	0.81	1.21	0.60
rc_AA817764_at	best hit: rat mrna for endothelial p22-phox	0.53	0.95	0.78
rc_AA892382_at	putative n-acetyltransferase camello 1	1.70	1.22	1.27
rc_AI639293_at	gene for choline acetyltransferase, exon 1 (non coding)	1.46	1.83	1.03
D86373_s_at	sterol o-acyltransferase 1	0.70	0.91	0.66
U01348_g_at	arylamine n-acetyltransferase 2 (nat-2) (at-2)	0.46	0.82	0.47

[a]Samples were grouped and analyzed according to supplementation.

[b]The samples were grouped according to lycopene and vitamin E concentrations in tumor tissue (15-25mg/l or ≥25mg/l vitamin E) resulting in sample groups with comparable vitamin E contents, but differing in lycopene. Calculated changes in gene expression represent the effect of lycopene addition in the presence of different vitamin E concentrations.

Table 5

Lycopene and vitamin E prostate tumor tissue concentrations in re-grouped samples (Thresholds: 15 mg vitamin E/l, 25mg vitamin E/l and 0 μg lycopene/l)

New groups	Vit E (mg/l)	Lyc (μg/l)	Comparison
Very low E	10.33 (+/–1.63)	ND	
			(Very low E)
Lyc very low E	2.50 (+/–3.54)	277.00 (+/–100.41)	
Low E	22.00 (+/–2.16)	ND	
			Low E
Lyc low E	21.50 (+/–2.07)	186.00 (+/–83.02)	(see Table 4)
High E	31.25 (+/–4.23)	ND	
			High E
Lyc high E	32.33 (+/–4.51)	248.67 (+/–37.87)	(see Table 4)

average concentration (+/–stdev); ND not detected

Fig. 1

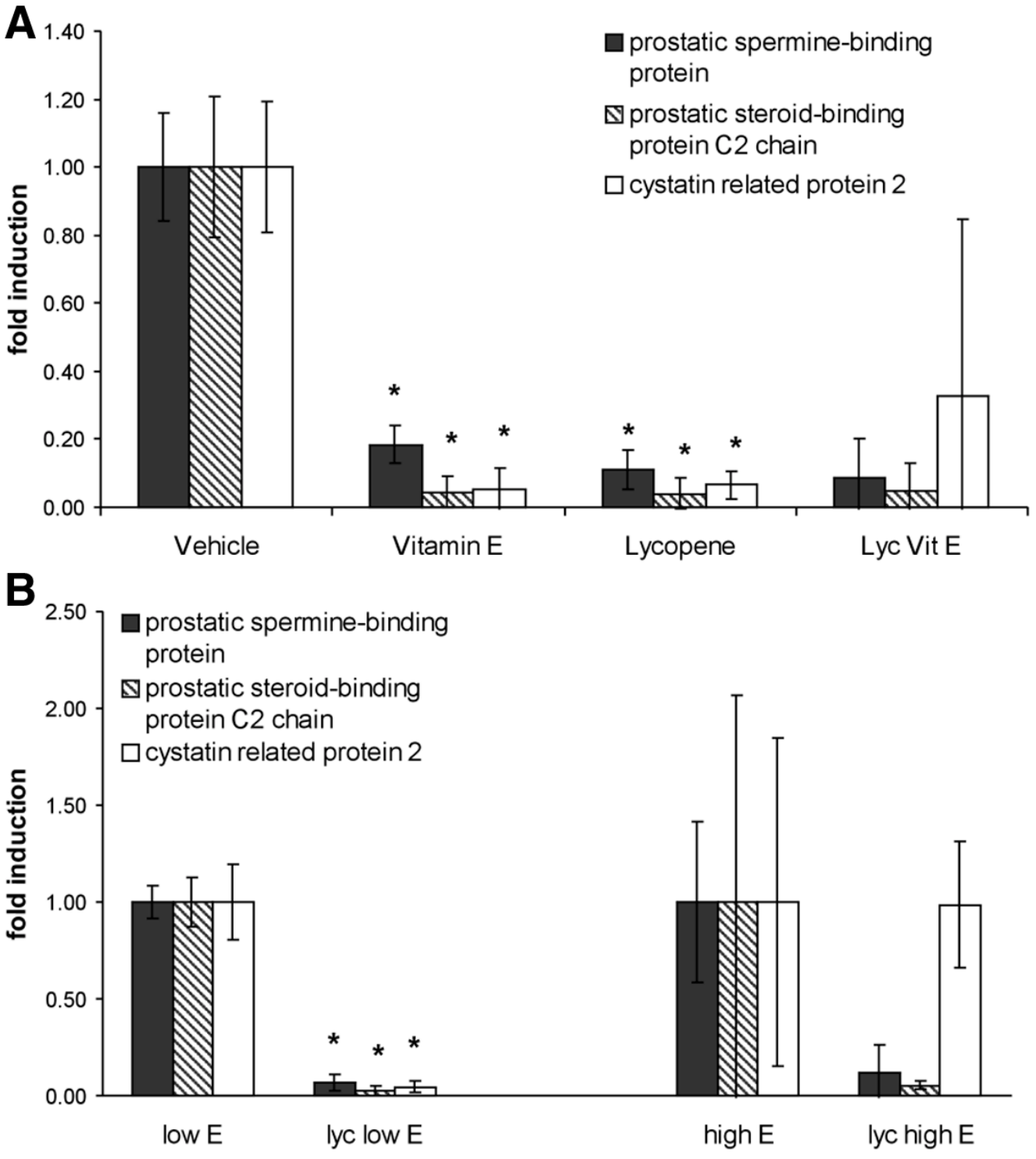

Figure 1. Confirmation of androgen target gene expression by real-time quantitative RT-PCR. TaqMan™ real-time RT-PCR of selected candidate genes was used to confirm the results obtained with the GeneChip® arrays. ***A*)** Treatment-induced changes of gene expression are given as fold induction relative to the expression in the vehicle-treated group (+/– standard error). ***B*)** Samples were re-grouped according to the tissue lycopene and vitamin E concentration (see **Table 5**), and the changes in gene expression were re-calculated according to the new grouping. Please note that the expressions within the "low E" and in the "high E" groups were individually set as 100% for the comparison with "lyc low E" or "lyc high E", respectively. (* = $P<0.05$; Mann-Whitney).