

Phase II Randomized Clinical Trial of Lycopene Supplementation before Radical Prostatectomy¹

Omer Kucuk,² Fazlul H. Sarkar, Wael Sakr, Zora Djuric, Michael N. Pollak, Fred Khachik, Yi-Wei Li, Mousumi Banerjee, David Grignon, John S. Bertram, John D. Crissman, Edson J. Pontes, and David P. Wood, Jr.

Division of Hematology and Oncology [O. K., Z. D.], Departments of Pathology [F. H. S., W. S., Y.-W. L., D. G., J. D. C.], Urology [E. J. P., D. P. W.], and Biostatistics [M. B.], Wayne State University, and Barbara Ann Karmanos Cancer Institute, Detroit, MI, 48201; Department of Medicine, McGill University and Jewish General Hospital, Montreal, Quebec H3T 1E2, Canada [M. N. P.]; Joint Institute for Applied Nutrition, Department of Chemistry and Biochemistry, University of Maryland, College Park, MD, 20742 [F. K.]; and Cancer Research Center of Hawaii, University of Hawaii, Honolulu, HI 96813 [J. S. B.].

Abstract

An inverse association has been observed between dietary intake of lycopene and the risk of prostate cancer. We investigated the effects of lycopene supplementation in patients with prostate cancer. Twenty-six men with newly diagnosed, clinically localized (14 T₁ and 12 T₂) prostate cancer were randomly assigned to receive 15 mg of lycopene ($n = 15$) twice daily or no supplementation ($n = 11$) for 3 weeks before radical prostatectomy. Biomarkers of differentiation and apoptosis were assessed by Western blot analysis on benign and malignant parts of the prostate gland. Prostatectomy specimens were entirely embedded, step-sectioned, and evaluated for pathological stage, Gleason score, volume of cancer, and extent of high-grade prostatic intraepithelial neoplasia. Plasma levels of lycopene, insulin-like growth factor-1 (IGF-1), IGF binding protein-3, and prostate-specific antigen were measured at baseline and after 3 weeks of supplementation or observation. Eleven (73%) subjects in the intervention group and two (18%) subjects in the control group had no involvement of surgical margins and/or extra-prostatic tissues with cancer ($P = 0.02$). Twelve (84%) subjects in the lycopene group and five (45%) subjects in the control group had tumors <4 ml in size ($P = 0.22$). Diffuse involvement of the prostate by high-grade prostatic intraepithelial neoplasia was present in 10 (67%) subjects in the intervention group and in 11 (100%) subjects in the control group ($P = 0.05$). Plasma

prostate-specific antigen levels decreased by 18% in the intervention group, whereas they increased by 14% in the control group ($P = 0.25$). Expression of connexin 43 in cancerous prostate tissue was 0.63 ± 0.19 absorbance in the lycopene group compared with 0.25 ± 0.08 in the control group ($P = 0.13$). Expression of bcl-2 and bax did not differ significantly between the two study groups. IGF-1 levels decreased in both groups ($P = 0.0002$ and $P = 0.0003$, respectively). The results suggest that lycopene supplementation may decrease the growth of prostate cancer. However, no firm conclusions can be drawn at this time because of the small sample size.

Introduction

Epidemiological studies have shown an inverse association between dietary intake of tomatoes, tomato products, lycopene, and PCa³ risk (1–4). Lycopene, the major carotenoid in tomatoes, has been postulated to be the protective compound against PCa (1–5). Possible mechanisms of action for lycopene include the following: (a) inhibition of growth and induction of differentiation in cancer cells by modulating the expression of cell cycle regulatory proteins (6–10); (b) modulation of the IGF-1/IGFBP-3 system (10–19); (c) up-regulation of tumor suppressor protein Cx43 and increased gap junctional intercellular communication (20–32); (d) modulation of redox signaling (33); (e) prevention of oxidative DNA damage (34, 35); and (f) modulation of carcinogen metabolizing enzymes (36).

Lycopene is a potent antioxidant and quencher of singlet oxygen (37, 38) and a predominant carotenoid in the plasma and tissues (39–42). Clinton *et al.* (43) have studied the concentrations of various carotenoids in human prostate glands obtained by radical prostatectomy from patients with PCa. Lycopene and β -carotene were the predominant carotenoids, with means \pm SE of 0.80 ± 0.08 and 0.54 ± 0.09 nmol/g, respectively. The presence of lycopene in the prostate at concentrations that are biologically active in laboratory studies supports the hypothesis that lycopene may have direct effects within the prostate and contribute to the reduced PCa risk associated with the consumption of tomato-based products. Intake of encapsulated lycopene can increase the serum levels of lycopene within days (44). Rao *et al.* (44) provided 19 healthy human subjects with tomato juice, spaghetti sauce, and tomato oleoresin capsules (Lyc-O-Mato) and observed a 2-fold increase in serum lycopene level and diminished amounts of serum thiobarbituric acid-reactive substances, a biomarker of oxidative stress. The same group observed significantly lower

Received 1/18/01; revised 4/27/01; accepted 5/24/01.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¹ The study was supported by a Virtual Discovery Grant from the Barbara Ann Karmanos Cancer Institute, Detroit, MI. Results were presented in part at the 90th annual meeting of the AACR in Philadelphia, PA, in April, 1999.

² To whom requests for reprints should be addressed, at Division of Hematology and Oncology, 3990 John R, 5 Hudson, Detroit, MI, 48201. Phone: (313) 745-2748; Fax: (313) 993-0559; E-mail: kucuko@karmanos.org.

³ The abbreviations used are: PCa, prostate cancer; IGF-1, insulin-like growth factor-1; IGFBP-3, insulin-like growth factor binding protein-3; HGPIN, high-grade prostatic intraepithelial neoplasia; Cx43, connexin 43; PSA, prostate-specific antigen; HPLC, high-performance liquid chromatography; THF, tetrahydrofuran; NCI, National Cancer Institute.

serum and prostate tissue lycopene levels (44%; $P = 0.04$ and 78%; $P = 0.05$, respectively) in patients with PCa than in their controls in a case-control study (45).

Evolving evidence suggests that carotenoids may modulate processes related to mutagenesis, carcinogenesis, cell differentiation, and proliferation independently of their role as antioxidants or precursors of vitamin A (8, 20–32, 46–48). One action of lycopene, as well as many other carotenoids, is to increase gap-junctional intercellular communication by increasing the expression of a widely expressed gap junctional gene, *Cx43* (21–23). This action correlates strongly with the ability of these carotenoids to suppress neoplastic transformation in model cell culture systems (23), an action which is shared by retinoids (26). This action of carotenoids has been proposed to have mechanistic significance by enabling the transfer of growth-regulatory signals between normal growth-inhibited cells and preneoplastic cells. Indeed, when neoplastic cells were forced into junctional communication with quiescent normal cells, the neoplastic cells became growth-arrested in direct proportion to their extent of junctional communication (29). Consistent with the hypothesis of growth control via junctional communication, first proposed by Loewenstein (30), have been multiple reports that connexin expression and/or junctional communication is severely impaired in most solid tumors (reviewed in Ref. 49). Progressive decreases with disease severity in the expression of *Cx43* have been reported in the human prostate (50), and there is evidence in prostatic carcinoma cell lines that some of this loss of junctional communication may result from defects in the assembly of *Cx43* protein into gap junctions (51). When functional communication was restored in a human prostatic cell line, cells had more normal differentiation, reduced proliferation, and suppressed tumorigenicity (52).

IGFs have mitogenic and antiapoptotic effects on normal and transformed prostate epithelial cells (19, 53, 54). Whereas most circulating IGF-1 originates in the liver, IGF bioactivity in tissues is related to both circulating levels of IGF and IGFBP as well as to local production of IGFs, IGFbps, and IGFBP proteases (55). Whereas IGF-1 is an important mitogen for prostate cells, IGFbps have opposing actions, in part by binding IGF-1, but also by direct inhibitory effects on target cells. As mitogens and antiapoptotic agents, IGFs may be important in carcinogenesis, possibly by increasing the risk of cellular transformation by enhancing cell turnover. In recent epidemiological studies, relatively high IGF-1 and low IGFBP-3 plasma levels have been independently associated with greater risk of PCa (11, 14, 16–18).

Despite the inverse association between lycopene intake and PCa observed in epidemiological studies, no clinical intervention studies have previously reported the effect of lycopene supplements in men with PCa. We conducted a randomized clinical trial to investigate the effect of lycopene supplementation on cancerous and benign prostate tissues and on serum levels of PSA, IGF-1, and IGFBP-3 of patients with PCa. We hypothesized that lycopene supplementation would decrease growth and induce apoptosis in premalignant and malignant prostate cells by up-regulating *Cx43*, down-regulating IGF-1 and decreasing the ratio of *bcl-2/bax* in patients with localized PCa.

Materials and Methods

Study Design. The study design was a randomized, two-arm intervention study. Patients with a diagnosis of PCa who were scheduled to undergo radical prostatectomy were randomly assigned to either lycopene supplementation or no intervention

for 3 weeks before surgery. At baseline, patients gave a blood sample and completed a food frequency questionnaire. Another blood sample was obtained for biomarker studies after 3 weeks immediately before surgery. Then the patients had radical prostatectomy, and the prostate specimens were step-sectioned, entirely embedded, and evaluated for pathological stage, Gleason score, the volume of PCa as well as the extent of HGPIN in the gland. The specimens were examined for biomarkers of cell differentiation and apoptosis. Comparisons were made between intervention and control groups. Biomarkers of differentiation (*Cx43*) and apoptosis (*bcl-2* and *bax*) were assessed by Western blotting in benign and malignant tissue samples obtained from radical prostatectomy specimens. Plasma lycopene levels were measured at baseline and after 3 weeks of intervention and tissue lycopene levels were measured in benign tissue samples obtained from prostatectomy specimens.

Subjects. Patients with clinically localized PCa who agreed to have radical prostatectomy and signed a written informed consent form were entered on the study. To be eligible, the subjects had to have a pathological diagnosis of adenocarcinoma of the prostate, which was clinically localized to the prostate gland as determined by digital rectal examination, computerized tomographic scan of pelvis, and normal liver function tests. The patients were excluded if they had any prior therapy for PCa, such as hormonal therapy, chemotherapy, and radiation therapy, or if they took any nutritional and herbal supplements other than a single multivitamin tablet once daily.

Ineligible Subjects. Nine patients (four on the intervention arm and two on the control arm) were excluded from the analysis, because they were found to be ineligible after randomization on the study. The reasons for ineligibility included incorrect diagnosis (one patient), refusal to participate in the study after randomization (one patient), refusal of surgery (one patient), and prior hormone therapy (six patients). Therefore, data analysis was restricted to 26 eligible patients who were randomly assigned to the lycopene arm ($n = 15$) or the control arm ($n = 11$) of the study.

Study Procedures. Subjects were registered on the study if they agreed: (a) to take lycopene supplementation twice a day; (b) not to take any other micronutrient supplements during the study; (c) to complete a food frequency questionnaire at baseline; and (d) to sign an informed consent form. Patients were then randomly assigned by to lycopene supplement or no intervention (control) groups according to a randomization schedule provided to the hospital pharmacy by the study biostatistician. Patients randomized to the control group were instructed to continue their usual diet but also received the NCI's 5-A-Day pamphlet, and patients randomized to the intervention group were asked to take 15 mg lycopene twice daily with meals. Lycopene capsules were stored at the hospital pharmacy and dispensed by the study manager. No stratification was done at the time of randomization. Blood samples were obtained at baseline and immediately before surgery for plasma biomarker studies and PSA levels. Subjects also completed a food frequency questionnaire at the time of study entry.

Baseline Clinical Evaluation. Before study entry, subjects had history and physical examination, digital rectal examination of the prostate gland, complete blood count, chemistry profile, serum PSA, and computerized tomographic scan of the pelvis.

Study Intervention. Subjects randomized to the intervention arm were given a 4-week supply of 15 mg lycopene capsules (Lyc-O-Mato; LycoRed Natural Products Industries, Beer-Sheva, Israel). Extra supplies of lycopene capsules were given

because of possible changes in the date of surgery. Subjects randomized to the control arm were asked to continue their regular diet and were given the NCI's recommendations to increase daily fruit and vegetable intake to five servings a day. Subjects randomized to the intervention arm were asked to take one lycopene capsule twice daily with meals, preferably at breakfast and dinner.

Lycopene Capsules. The lycopene soft gel capsules contained 15 mg of lycopene, 2.5 mg phytoene/phytofluene, and some minor carotenoids suspended in natural tomato matrix and encapsulated in gelatin. There were no added chemicals or micronutrients. Lyc-O-Mato is produced from specially bred and cultivated lycopene-rich tomato varieties developed in Israel by the late Prof. Rafael Frankel (Volcani Center, Tel Aviv, Israel). These tomatoes contain approximately three times greater amounts of lycopene than regular tomatoes. LycORed's hybrid tomatoes were developed through conventional cross-breeding techniques without using genetic engineering methods. In addition to lycopene, small quantities of other bioactive molecules are also found in the Lyc-O-Mato capsules (see "Results").

Study Compliance. Compliance was assessed by performing remaining pill counts at the end of the intervention when the patients were admitted to the hospital for surgery. Seven subjects were noncompliant to varying degrees. One subject received 77 capsules in 4 weeks because of delayed surgery; one subject received 39 capsules (93% compliance) in 3 weeks; five subjects received 23–33 capsules in 13–30 days with compliance rates varying from 55% to 79%.

Toxicity Monitoring. Subjects were asked to report any possible adverse effects or unusual symptoms they noticed during the study period. At the time of surgery, each had a complete physical examination and complete laboratory work-up, including a complete blood count and chemistry profile.

Tissue Procurement. All subjects had radical prostatectomy with *en bloc* removal of the entire prostate gland, seminal vesicals, and surrounding soft tissues. The radical prostatectomy specimens were delivered fresh to the tissue laboratory immediately after excision. Fresh tissue samples were obtained with the exact anatomical source of each sample clearly indicated on a specimen diagram for subsequent microscopic confirmation. In cases where gross identification of the tumor was difficult to establish, a frozen-section slide of the suspected area was generated and stained with H&E to guide the acquisition protocols. When possible, 1 g of tissue from benign areas of the gland was kept frozen for lycopene analysis. Appropriate tissue samples were taken for Western blotting and histological examination. Tissue samples were obtained from benign and malignant parts of the glands and were stored at -70°C for biomarker studies. The specimens were then entirely embedded in paraffin, step-sectioned, and microscopically examined by the study pathologists, who were blinded to the randomization status of the patients.

Blood samples and tissue samples were collected using aseptic technique and placed on ice, protected from light, and immediately transferred to the laboratory for processing and aliquoting before storing at -70°C . Blood samples were obtained at registration and after intervention immediately before surgery.

Study End Points. The study end points included biomarkers of cell growth and differentiation, bcl-2, bax, and Cx43, as assessed by Western analysis, and plasma levels of IGF-1 and IGFBP-3. We have also evaluated clinical end points, such as the grade, volume, and extension of tumor to and/or beyond the

resection margins, and the extent of prostatic involvement with HGPIN (*i.e.*, focal, multifocal, or diffuse) and the plasma levels of PSA.

Histological Examination. Prostatectomy specimens and tissue samples were handled similarly by the tissue core laboratory according to predetermined instructions. Pathology slides were examined by two experts in prostate pathology, Dr. Wael Sakr and Dr. David Grignon. All results were entered in the institutional PCa database. At the time of histological assessment of grade and volume of tumor, multifocality of HGPIN and surgical margins of the tumor, the study pathologists had no knowledge of the randomization status of the patients.

Western Blotting. Markers studied by Western blotting included Cx43, bcl-2, and bax. Detection of gene expression was performed by routine Western blotting procedures as described previously (56). Briefly, identical amounts of protein (20 μg) were separated on 12–16% polyacrylamide gels and transferred onto nitrocellulose membranes. Target protein was detected using enhanced chemiluminescence (Amersham) Western detection system with primary antibodies to respective proteins. Antibodies to Bax, bcl-2, and β -actin proteins were obtained from Trevigen (Gaithersburg, MD), Dako (Carpinteria, CA), and Sigma Chemical Co. (St. Louis, MO), respectively. Cx43 was detected with a rabbit polyclonal antibody to the 19 COOH-terminal residues of Cx43. This antibody had previously been shown to detect Cx43 in human samples by immunohistochemistry and to recognize a M_r 43,000 band on Western blotting (57).

Analysis of Carotenoids by HPLC. Carotenoids in serum and prostate tissues were extracted and analyzed by HPLC by the method of Khachik *et al.* (58–60). Inasmuch as detailed description of the analytical methods used have been published elsewhere, these methods are only briefly described here.

Reference Samples of Carotenoids, Tocopherols, and Retinol. Carotenoid standards were either synthesized or isolated from natural sources according to published procedures (61–63). Vitamin A, γ -tocopherol, and α -tocopherol were obtained from Sigma Chemical Co. Synthetic samples of 9-*cis*(Z)-lycopene, 13-*cis*(Z)-lycopene, 5-*cis*(Z)-lycopene, 9-*cis*(Z)- β -carotene, and 13-*cis*(Z)- β -carotene were donated by Hoffmann-La Roche (Basel, Switzerland). 5,5'-di-*cis*(Z)-Lycopene was synthesized according to the method of Hengartner *et al.* (64). 2,6-Cyclolycopene-1,5-diols (lycopene metabolites) were synthesized according to a published procedure (65).

Identification of Carotenoids (Fig. 1). Carotenoids were identified by comparison of their retention times and HPLC/UV-visible spectra obtained by a photodiode array detector with those of authentic standards.

Extraction Procedure. All extractions were performed under yellow fluorescent light. Serum and prostate tissues were immediately extracted after thawing. Serum samples were extracted according to the method of Khachik *et al.* (59). Prostate tissues (~ 0.3 – 0.7 g) were homogenized with 20 ml of THF (containing 0.1% butylated hydroxytoluene) by sonication in an ice-bath for 30 min. The solvent was decanted off into a 100 ml round-bottomed flask. The residue was ground by a pestle and the extraction was repeated twice as above. The combined THF extract was concentrated on a rotary evaporator at 40°C . The residue was microfiltered into a graduated 5-ml centrifuge vial using dichloromethane, and the solvent was evaporated under nitrogen. Reversed-phase HPLC injection solvents (~ 0.2 ml) were added, and the sample was centrifuged before HPLC

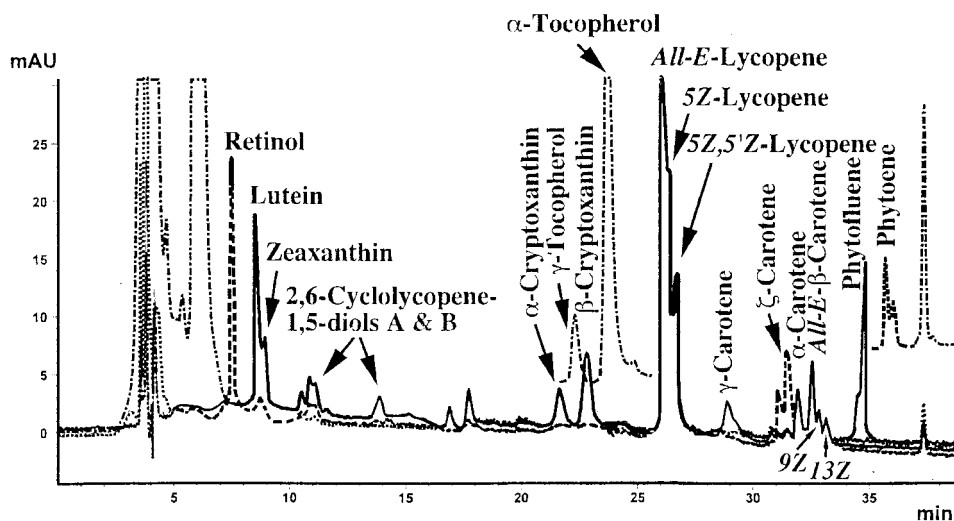


Fig. 1. Typical carotenoid HPLC profile of benign prostate tissues from human subjects supplemented daily with lycopene for 3 weeks before surgery.

analysis. Contents of the lycopene capsules were extracted into THF before HPLC analysis.

Verification of Accuracy. To monitor the accuracy and reproducibility of the HPLC analysis of carotenoids, a solution containing known concentrations of lycopene, lutein, α -cryptoxanthin, β -cryptoxanthin, α -carotene, β -carotene, retinol, γ -tocopherol, and α -tocopherol was routinely analyzed. The reproducibility of the HPLC analysis for carotenoids, vitamin A, and tocopherols was shown to be >95%.

Plasma IGF-1 and IGFBP-3 Levels. Plasma IGF-I and IGFBP-3 were assayed by ELISA as previously reported (11).

Statistical Analysis. χ^2 tests were used to compare the control versus intervention groups in terms of distribution of HGPIN (focal versus multifocal/diffuse), Gleason score (≤ 6 versus > 6), tumor volume (≤ 4 ml versus > 4 ml) and surgical stage (margin positivity/extraprostatic extension present versus absent). Fisher's exact test was used to determine statistical significance when small numbers were encountered in the contingency tables (*i.e.*, the expected value of any cell is < 5).

Tissue levels of lycopene and expressions of Cx43, bcl-2, and bax (in tumor as well as benign tissue) were compared between the intervention and control groups using a two-sample (independent) *t* test. We also compared pre- and postintervention plasma PSA levels based on a paired *t* test.

Results

Baseline Characteristics of Study Subjects (Table 1). There were no significant differences between the intervention and control groups with regard to age (median age 61 for both groups) or racial distribution [two African Americans (13%) were in the intervention group; three African Americans (27%) were in the control group; the remainder of the subjects were Caucasian Americans]. With regard to other potential confounding factors, baseline clinical stage, Gleason score, and PSA values were not different in a statistically significant fashion between the two study groups, although there appeared to be more patients with T₁ disease and lower a Gleason score in the intervention group.

Modulation of Clinical and Pathological End Points (Table 2). Plasma PSA levels decreased by 18% in the intervention group, whereas levels increased by 14% in the control group over the study period ($P = 0.22$). In the intervention group, 11

of 15 patients (73%) had involvement of surgical margins and/or extra-prostatic tissues with cancer compared with 2 of 11 patients (18%) in the control group ($P = 0.02$). Twelve of 15 patients (80%) in the lycopene group had tumors that measured 4 ml or less compared with 5 of 11 (45%) in the control group ($P = 0.22$). Multifocal and/or diffuse involvement by HGPIN was observed in 10 of 15 subjects (67%) in the lycopene group compared with all 11 subjects (100%) in the control group ($P = 0.05$). The apoptotic index determined by immunohistochemistry on tissue sections of both study arms was exceedingly low (data not shown), precluding the establishment of a differential value between the control and the intervention arms with respect to this parameter.

Modulation of Biomarkers in Cancerous Areas of the Prostate (Table 3). There were sufficient malignant tissues available for analysis of biomarkers on four subjects in the lycopene group and four subjects in the control group. The expression of gap junctional protein, Cx43, in the malignant part of the prostate glands, was $0.63 \pm 0.19 A_{280 \text{ nm}}$ units in the lycopene group compared with the $0.25 \pm 0.08 A_{280 \text{ nm}}$ units in the control group ($P = 0.13$). The expression of two important cell cycle regulatory proteins, bcl-2 and bax, were not significantly different between the two groups, although the level of bax seemed to be higher in the lycopene group (1.05 ± 0.29) compared with the control group (0.68 ± 0.18).

Modulation of Biomarkers in Benign Areas of the Prostate (Table 3). There were sufficient tissue samples from benign parts of the gland available for biomarker analysis in eight subjects in the intervention group and in six subjects in the control group. The expression of Cx43 was 0.64 ± 0.12 in the lycopene group compared with 0.51 ± 0.10 in the control group. The expression of bcl-2 was 0.63 ± 0.04 in the intervention group and 0.58 ± 0.04 in the control group ($P = 0.31$), and the expression of bax was 0.62 ± 0.10 in the intervention group and 0.79 ± 0.11 in the control group ($P = 0.28$). There were no statistically significant differences between the two groups in any of the biomarkers.

Modulation of Plasma IGF-1 and IGFBP-3 Levels (Table 4). Plasma samples were available from 13 subjects in the intervention group and from 10 subjects in the control group. Plasma levels of IGF-1 decreased by 29% (from 233 ± 21 ng/ml to 169 ± 23 ng/ml) in the lycopene group ($P = 0.0002$)

Table 1 Baseline clinical characteristics of the study subjects (n = 26)

Characteristic	Intervention (n = 15)	Control (n = 11)	P ^a
Age			
Median (range)	61 (51–71)	61 (53–70)	
Mean ± SE ^b	62.3 ± 1.9	62.0 ± 1.8	0.92
Race			
African American	2	3	
Caucasian American	13	8	0.62
Baseline clinical stage ^c			
T _{1C}	10	4	
T _{2A}	2	4	
T _{2B}	3	3	0.13 ^a
Baseline Gleason score			
≤6	11	5	
>6	4	6	0.23
Baseline plasma PSA (ng/ml)	6.89 ± 0.81 ^b	6.74 ± 0.88	0.89

^a P is for comparing the distribution of T₁ versus T₂ in the two groups.

^b Mean + SE.

^c American Urologic Association (AUA) Staging System: T_{1C}, tumor on needle biopsy of nonpalpable mass; T_{2A}, tumor involving one lobe; T_{2B}, tumor involving both lobes.

and by 30% (from 199 ± 20 ng/ml to 140 ± 16 ng/ml; *P* = 0.0003) in the control group. Interestingly, a similar degree of reduction in was observed in both groups. IGFBP-3 levels also decreased in both intervention (25%) and control (21%) groups during the study period. The decreases in the plasma IGFBP-3 levels in the intervention (from 5230 ng/ml to 3924 ng/ml) and control groups (5200 ng/ml to 4070 ng/ml) were statistically significant (*P* = 0.0002 and *P* = 0.0001, respectively).

Tissue and Plasma Lycopene Levels. Prostatic tissue lycopene levels were 47% higher in the intervention group (0.53 ± 0.03 ng/gm of prostate tissue) compared with the control group (0.36 ± 0.06), which was a significant difference (*P* = 0.02). Unfortunately, there were sufficient amounts of prostatic tissue available for lycopene analysis in only five subjects from the intervention group and three subjects from the control group. On the other hand, plasma levels of lycopene levels did not change significantly during the study period in either group of patients (Table 2). In the intervention group, plasma lycopene levels increased in 5 of 11 patients, whereas only 1 of 6 subjects in the control group had an increase (Fisher's exact test; *P* = 0.33). The level of postintervention plasma lycopene was 23.5 μg/dl in the intervention group and 17.5 μg/dl in the control group (*P* = 0.15). However, a significant difference in plasma levels was not found, probably because of great variability between subjects.

Dietary Intake of Tomato Products. There was no difference between the two groups with regard to dietary intake of tomatoes, tomato products, or other major foods at baseline (data not shown). However, there was no dietary reassessment during and at the end of intervention. Therefore, we cannot determine whether there were any changes in the intake of tomato products or other foods during the intervention period.

Carotenoid Content of Lycopene Capsules. Detailed carotenoid analysis of the capsules was conducted by HPLC. In addition to all-*E*(*trans*)-lycopene (13.50 mg), 5-*Z*(*cis*)-lycopene (1.05 mg), and 5*Z*,5'*Z*-lycopene (0.45 mg), the capsules contained small quantities of the typical tomato carotenoids, such as γ-carotene (0.09 mg), ζ-carotene (0.24 mg), β-carotene (0.16 mg), phytofluene (1.03 mg), and phytoene (1.16 mg).

Toxicity of Lycopene. No adverse effects were reported by patients or their physicians during or after lycopene supple-

Table 2 Clinical and pathologic findings in the two study groups

	Intervention group (n = 15)	Control group (n = 11)	P
Plasma PSA level (mean ± SE, ng/ml)			
Preintervention	6.89 ± 0.81	6.74 ± 0.88	
Postintervention	5.64 ± 0.87	7.65 ± 1.78	0.25 ^a
	(<i>P</i> = 0.22) ^b		
Plasma lycopene level (mean ± SE, ng/ml)			
Preintervention	28.3 ± 6.9	28.6 ± 7.1	
Postintervention	23.5 ± 2.4	17.5 ± 3.1	0.15 ^c
	(n = 11)	(n = 6)	
Extent of HGPIN (n)			
Focal	5	0	
Multifocal/diffuse	10	11	0.05
Postsurgical Gleason score			
≤6	7	4	
>6	8	7	0.70
Prostatic tumor volume (cm ³)			
≤4	12	5	
>4	3	6	0.22
Surgical tumor stage (n)			
Confined to prostate	11	2	
Not confined to prostate ^d	4	9	0.02

^a P is for comparing the change from pre- to postintervention PSA in the two groups.

^b P is for comparing the preintervention and postintervention PSA in the lycopene group.

^c P value is for comparing the postintervention mean values between the two groups.

^d Resection margins are positive, and/or extraprostatic invasion is present.

mentation. No abnormalities were observed in blood counts or chemistries.

Discussion

We report here the results of a small, randomized clinical trial, showing that lycopene supplementation may increase prostate tissue levels of lycopene, modulate biomarkers of growth and differentiation, and decrease clinical parameters of disease aggressiveness. To our knowledge, this is the first report of a clinical intervention trial with a lycopene supplement investigating the modulation of biological and clinical end points in prostatectomy specimens from patients with clinically localized PCa. The results suggest that oral intake of 15 mg of lycopene twice daily for 3 weeks may be sufficient to modulate clinical markers of disease. The microscopic extension of PCa to surgical margins and/or to extraprostatic tissues appeared to have decreased as a result of lycopene supplementation. Patients in the lycopene group had a decrease in the plasma PSA level, which is a parameter of PCa burden. The implication of these results is that lycopene may have an antitumor effect and thus may even be useful as an adjunct to standard treatments of PCa, such as surgery, radiation therapy, hormones, and chemotherapy. In addition, lycopene supplementation appears to have reduced the diffuse involvement of the prostate gland with HGPIN, which is widely accepted as a precursor of PCa (66). This finding suggests that lycopene may have a role in the prevention of PCa.

Our study did not find a significant increase in plasma lycopene levels in the intervention group. However, 5 of 11 intervention subjects, but only 1 of 6 control subjects, had an increase in plasma lycopene levels after the intervention period. On the other hand, the level of lycopene was significantly higher in prostate tissue obtained from the intervention group compared with the control group (*P* = 0.02). Previous studies

Table 3 Biomarkers in prostatectomy specimens

Biomarker	Lycopene group (n = 4) (Mean ± SE)	Control group (n = 4) (Mean ± SE)	P
Tumor tissue			
Connexin 43 (absorbance ^a)	0.63 ± 0.19	0.25 ± 0.08	0.13
bcl-2 (absorbance)	0.54 ± 0.01	0.51 ± 0.06	0.59
bax (absorbance)	1.05 ± 0.29	0.68 ± 0.18	0.33
bax/bcl-2	1.9 ± 0.5	1.5 ± 0.4	0.54
Benign tissue			
Connexin 43 (absorbance ^a)	0.64 ± 0.12	0.51 ± 0.10	0.44
bcl-2 (absorbance)	0.63 ± 0.04	0.58 ± 0.04	0.31
bax (absorbance)	0.62 ± 0.10	0.79 ± 0.11	0.28

^aAbsorbance corrected for expression of β -actin.

have shown good bioavailability from the lycopene capsules, which were used in this study (44, 67). We have analyzed the carotenoid content of the capsules taken by the intervention group in our study. Each capsule contained 15 mg of lycopene (13.50 mg all-E(*trans*)-, 1.05 mg 5-Z(*cis*)-, and 0.45 mg 5Z,5'-Z-lycopene), 1.16 mg phytoene, 1.03 mg phytofluene, and smaller amounts of β -, γ -, and ζ -carotene. All of the carotenoids in study capsules were detected in the prostate tissues of our subjects (Fig. 1). It is possible that some of the minor components of the capsules other than lycopene may have played a role in the modulation of biological and clinical end points reported here.

We observed a significant clinical effect of lycopene on the tumor despite the short duration of the intervention. Decreased involvement of surgical margins and extraprostatic tissues with tumor and reduced amount of diffuse pattern HGPIN in the gland were seen in the subjects who received lycopene. We had no difficulty evaluating the margins and extraprostatic tissues for tumor involvement, because there was no histological or immunohistochemical (data not shown) evidence of increased apoptosis in the tissues examined. However, the small size of the study precludes any conclusions regarding the biological relevance of surgical tumor stage as a pathological marker to judge the effects of lycopene. Although there were no statistically significant changes in biochemical markers, we observed changes in the serum PSA level and tumor tissue Cx43 level in the expected direction. Furthermore, a significant decrease in the extent of HGPIN, a precancerous lesion of the prostate gland, was also observed.

HGPIN is considered a precursor lesion for PCa, and the presence of this lesion is highly predictive of development of PCa (66). HGPIN has been proposed as an intermediate end point in chemoprevention studies (66). Therefore, we have evaluated the degree of involvement of the prostate gland by HGPIN and classified the lesions as focal, multifocal, or diffuse. Diffuse and/or multifocal involvement of the prostate with HGPIN was less common among patients randomized to the lycopene arm compared with the control subjects on the study. This finding suggests that lycopene may prevent the development of PCa by decreasing HGPIN. Furthermore, the modulation of HGPIN by lycopene supports the hypothesis that HGPIN is a suitable intermediate end point for PCa chemoprevention trials.

A possible mechanism for the observed decrease in tumor parameters is up-regulation of Cx43. Decreased expression of *connexins*, including Cx43, has been widely reported in human tumors in comparison to normal tissue (49), and *connexins* are regarded by many as putative tumor suppressor genes (68, 69).

Table 4 Preintervention and postintervention plasma levels of IGF-1 and IGFBP-3

	Lycopene group (n = 13) (Mean ± SE)	Control group (n = 10) (Mean ± SE)	P ^a
Plasma IGF-1 level			
Preintervention	232.7 ± 20.6	199.4 ± 19.8	0.27
Postintervention	169.2 ± 23.3	139.9 ± 16.0	0.34
Percentage of change	28.8 ± 5.5	29.9 ± 5.3	0.88
P ^b	0.0002	0.0003	
Plasma IGFBP-3 level			
Preintervention	5230 ± 244	5200 ± 403	0.94
Postintervention	3924 ± 298	4070 ± 263	0.73
Percentage of change	24.9 ± 4.7	20.6 ± 3.1	0.49
P ^b	0.0002	0.0001	

^aP is for comparison between intervention and control groups.

^bP is for comparison between pre- and posttreatment values.

Increased expression of Cx43 in tumor tissue in the intervention group, although not reaching statistical significance perhaps because of the small number of subjects, may have mechanistic importance to the observed differences in pathology between these two groups. Increased expression of Cx43 and resulting increases in gap junctional communication have previously been shown to occur after treatment of human and murine cells in culture with diverse carotenoids, including lycopene (22). Up-regulated junctional communication has in turn been linked to decreased proliferation in normal and preneoplastic cells (70). Furthermore, recent studies have shown that in human cells, genetically engineered to be inducible for Cx43 expression, gene induction leads to decreases in their neoplastic potential as measured by changes in anchorage-independent growth and by growth as tumors in the nude mouse (71). Previously, others have reported that Cx43 expression is progressively decreased in the prostate with increased disease severity, implying that Cx43 expression is negatively selected during tumor progression (50). Similar reductions have been seen in tumor *versus* normal prostate cells (72). The concept of negative selection during disease progression received support from studies demonstrating that the forced expression of Cx43 in a human prostatic cell line results in the decreased neoplastic potential of these cells (52). Thus the changes in Cx43 expression reported here (Table 3) would be expected to result in decreased cell proliferation. Clearly, these studies need to be repeated in a larger group of patients.

Another possible mechanism for the observed decrease in tumor parameters is decreased IGF-1 level or increased IGFBP-3 level. Decreased IGF-1 level would also be associated with decreased cell proliferation, but comparable reductions in IGF-1 levels were seen in both intervention and control groups. The puzzling change in IGF-1 level of control subjects could be the result of possible changes in diet and lifestyle factors of the control subjects. It is possible that the control subjects decided to eat a healthier diet on their own because they were not given lycopene capsules, and because they were told to increase their fruit and vegetable intake according to the NCI's 5-A-Day guidelines. Unfortunately no data were collected on the diet of subjects during and after the study period. Clearly, the observed "down-staging" of the tumor cannot be attributable simply to changes in proliferation but may be a result of cell differentiation and apoptosis. However, there was no difference between the two groups with respect to the expression of bax and bcl-2, probably because of the small study population. Because both groups had similar changes in IGF-1 and IGFBP-3 levels, other

pathways possibly modulated by lycopene could be responsible for the observed changes in tumor stage and HGPIN.

We cannot rule out the possibility that the observed results may be a consequence of a larger number of patients with favorable characteristics in the intervention group compared with the control group. Because of the small numbers of patients in each group and the lack of stratification for baseline clinical parameters at study entry, the observed differences between the intervention and control groups with regard to clinical extent and aggressiveness of disease may be a result of an imbalance in baseline clinical parameters. However, there were no statistically significant differences between the two groups with respect to baseline clinical characteristics of disease, and the trend for decreased PSA levels in the lycopene group and increased levels in the control group do not support this conclusion.

The results suggest that lycopene supplementation may decrease the growth of PCa, perhaps because of up-regulation of Cx43. However, because of the small sample size, a definitive conclusion cannot be reached regarding preventive and/or therapeutic effects of lycopene on PCa. Clearly, the results deserve confirmation by larger clinical trials. If confirmed, lycopene could be a useful agent in PCa prevention and/or treatment. The efficacy as well as the appropriate dose and duration of lycopene supplementation remains to be determined for men at high risk of developing PCa.

Acknowledgments

We thank the patients for participating in the study and Sara Heath and Martha Heath for providing assistance in patient recruitment and data collection.

References

- Giovannucci, E., and Clinton, S. K. Tomatoes, lycopene, and prostate cancer. *Proc. Soc. Exp. Biol. Med.*, *218*: 129–139, 1998.
- Gann, P. H., Ma, J., Giovannucci, E., Willett, W., Sacks, F. M., Hennekens, C. H., and Stampfer, M. J. Lower prostate cancer risk in men with elevated plasma lycopene levels: results of a prospective analysis. *Cancer Res.*, *59*: 1225–1230, 1999.
- Giovannucci, E. Tomatoes, tomato-based products, lycopene, and cancer: review of the epidemiologic literature. *J. Natl. Cancer Inst.*, *91*: 317–331, 1999.
- Giovannucci, E., Ascherio, A., Rimm, E. B., Stampfer, M. J., Colditz, G. A., and Willett, W. C. Intake of carotenoids and retinol in relation to risk of prostate cancer. *J. Natl. Cancer Inst.*, *87*: 1767–1776, 1995.
- Kelloff, G. J., Lieberman, R., Steele, V. E., Boone, C. W., Lubet, R. A., Kopelovitch, L., Malone, W. A., Crowell, J. A., and Sigman, C. C. Chemoprevention of prostate cancer: concepts and strategies. *Euro. Urol.*, *35*: 342–350, 1999.
- Bertram, J. S. Carotenoids and gene regulation. *Nutr. Rev.*, *57*: 182–191, 1999.
- Amir, H., Karas, M., Giat, J., Danilenko, M., Levy, R., Yermiah, T., Levy, J., and Sharoni, Y. Lycopene and 1,25-dihydroxyvitamin D3 cooperate in the inhibition of cell cycle progression and induction of differentiation in HL-60 leukemic cells. *Nutr. Cancer*, *33*: 105–112, 1999.
- Levy, J., Bosin, E., Feldman, B., Giat, Y., Miinster, A., Danilenko, M., and Sharoni, Y. Lycopene is a more potent inhibitor of human cancer cell proliferation than either α -carotene or β -carotene. *Nutr. Cancer*, *24*: 257–266, 1995.
- Park, C. K., Ishimi, Y., Ohmura, M., Yamaguchi, M., and Ikegami, S. Vitamin A and carotenoids stimulate differentiation of mouse osteoblastic cells. *J. Nutr. Sci. Vitaminol.*, *43*: 281–296, 1997.
- Karas, M., Amir, H., Fishman, D., Danilenko, M., Segal, S., Nahum, A., Koifmann, A., Giat, Y., Levy, J., and Sharoni, Y. Lycopene interferes with cell cycle progression and insulin-like growth factor I signaling in mammary cancer cells. *Nutr. Cancer*, *36*: 101–111, 2000.
- Chan, J. M., Stampfer, M. J., Giovannucci, E., Gann, P. H., Ma, J., Wilkinson, P., Hennekens, C. H., and Pollak, M. Plasma insulin-like growth factor-I and prostate cancer risk: a prospective study. *Science (Wash. DC)*, *279*: 563–566, 1998.
- Nickerson, T., Pollak, M., and Huynh, H. Castration-induced apoptosis in the rat ventral prostate is associated with increased expression of genes encoding insulin-like growth factor binding proteins 2, 3, 4 and 5. *Endocrinology*, *139*: 807–810, 1998.
- Miyake, H., Pollak, M., and Gleave, M. E. Castration-induced up-regulation of insulin-like growth factor binding protein-5 potentiates insulin-like growth factor-I activity and accelerates progression to androgen independence in prostate cancer models. *Cancer Res.*, *60*: 3058–3064, 2000.
- Giovannucci, E. Insulin-like growth factor-I and binding protein-3 and risk of cancer. *Horm. Res.*, *51*(Suppl. 3): 34–41, 1999.
- Rajah, R., Khare, A., Lee, P. D., and Cohen, P. Insulin-like growth factor-binding protein-3 is partially responsible for high-serum-induced apoptosis in PC-3 prostate cancer cells. *J. Endocrinol.*, *163*: 487–494, 1999.
- Mantzoros, C. S., Tzonou, A., Signorello, L. B., Stampfer, M., Trichopoulos, D., and Adami, H. O. Insulin-like growth factor-1 in relation to prostate cancer and benign prostatic hyperplasia. *Br. J. Cancer*, *76*: 1115–1118, 1997.
- Wolk, A., Mantzoros, C. S., Andersson, S. O., Bergstrom, R., Signorello, L. B., Lagiou, P., Adami, H. O., and Trichopoulos, D. Insulin-like growth factor-1 and prostate cancer risk: a population-based, case-control study. *J. Natl. Cancer Inst.*, *90*: 911–915, 1998.
- Pollak, M., Beamer, W., and Zhang, J. C. Insulin-like growth factors and prostate cancer. *Cancer Metastasis Rev.*, *17*: 383–390, 1998–1999.
- Rajah, R., Valentinis, B., and Cohen, P. Insulin-like growth factor (IGF)-binding protein-3 induces apoptosis and mediates the effects of transforming growth factor- β -1 on programmed cell death through a p53- and IGF-independent mechanism. *J. Biol. Chem.*, *272*: 12181–12188, 1997.
- Matsushima-Nishiwaki, R., Shidoji, Y., Nishiwaki, S., Yamada, T., Moriwaki, H., and Muto, Y. Suppression by carotenoids of microcystin-induced morphological changes in mouse hepatocytes. *Lipids*, *30*: 1029–1034, 1995.
- Zhang, L.-X., Cooney, R. V., and Bertram, J. S. Carotenoids enhance gap junctional communication and inhibit lipid peroxidation in C3H/10T1/2 cells: relationship to their cancer chemopreventive action. *Carcinogenesis (Lond.)*, *12*: 2109–2114, 1991.
- Bertram, J. S., Pung, A., Churley, M., Kappock, T. J., IV, Wilkins, L. R., and Cooney, R. V. Diverse carotenoids protect against chemically induced neoplastic transformation. *Carcinogenesis (Lond.)*, *12*: 671–678, 1991.
- Zhang, L.-X., Cooney, R. V., and Bertram, J. S. Carotenoids up-regulate *connexin43* gene expression independent of their provitamin A or antioxidant properties. *Cancer Res.*, *52*: 5707–5712, 1992.
- Hotz-Wagenblatt, A., and Shalloway, D. Gap junctional communication and neoplastic transformation. *Crit. Rev. Oncog.*, *4*: 541–558, 1993.
- Bertram, J. S., and Bortkiewicz, H. Dietary carotenoids inhibit neoplastic transformation and modulate gene expression in mouse and human cells. *Am. J. Clin. Nutr.*, *62* (Suppl. 6): 1327s–1336s, 1995.
- Hossain, M. Z., Wilkens, L. R., Mehta, P. P., Loewenstein, W., and Bertram, J. S. Enhancement of gap junctional communication by retinoids correlates with their ability to inhibit neoplastic transformation. *Carcinogenesis (Lond.)*, *10*: 1743–1748, 1989.
- Mehta, P. P., Bertram, J. S., and Loewenstein, W. R. The actions of retinoids on cellular growth correlate with their actions on gap junctional communication. *J. Cell Biol.*, *108*: 1053–1065, 1989.
- Beyer, E. C., Paul, D. L., and Goodenough, D. A. Connexin43: a protein from rat heart homologous to a gap junction protein from liver. *J. Cell Biol.*, *105*: 2621–2629, 1987.
- Mehta, P. P., Bertram, J. S., and Loewenstein, W. R. Growth inhibition of transformed cells correlates with their junctional communication with normal cells. *Cell*, *44*: 187–196, 1986.
- Loewenstein, W. R. Junctional intercellular communication and the control of growth. *Biochem. Biophys. Acta*, *560*: 1–65, 1979.
- Yamasaki, H. Gap junctional intercellular communication and carcinogenesis. *Carcinogenesis (Lond.)*, *11*: 1051–1058, 1990.
- Chen, S.-C., Pelletier, D. B., Peng, A., and Boynton, A. L. Connexin43 reverses the phenotype of transformed cells and alters their expression of cyclin/cyclin-dependent kinases. *Cell Growth Diff.*, *6*: 681–690, 1995.
- Gius, D., Botero, A., Shah, S., and Curry, H. A. Intracellular oxidation/reduction status in the regulation of transcription factors NF- κ B and AP-1. *Toxicol. Lett.*, *106*: 93–106, 1999.
- Riso, P., Pinder, A., Santangelo, A., and Porrini, M. Does tomato consumption effectively increase the resistance of lymphocyte DNA to oxidative damage? *Am. J. Clin. Nutr.*, *69*: 712–718, 1999.
- Rao, A. V., Fleshner, N., and Agarwal, S. Serum and tissue lycopene and biomarkers of oxidation in prostate cancer patients: a case-control study. *Nutr. Cancer*, *33*: 159–164, 1999.
- Jewell, C., and O'Brien, N. M. Effect of dietary supplementation with carotenoids on xenobiotic metabolizing enzymes in the liver, lung, kidney and small intestine of the rat. *Br. J. Nutr.*, *81*: 235–242, 1999.

37. Conn, P. F., Schlach, W., and Truscott, T. G. The singlet oxygen and carotenoid interaction [published erratum appears in *J. Photochem. Photobiol. B*, 17: 89, 1993]. *J. Photochem. Photobiol. B*, 11: 41–47, 1991.
38. DiMascio, P., Kaiser, S., and Sies, H. Lycopene as the most efficient biological carotenoid singlet oxygen quencher. *Arch. Biochem. Biophys.*, 274: 1–7, 1989.
39. Kaplan, L. A., Lau, J. M., and Stein, E. A. Carotenoid composition, concentrations, and relationships in various human organs. *Clin. Physiol. Biochem.*, 8: 1–10, 1990.
40. Parker, R. S. Carotenoid and tocopherol composition of human adipose tissue. *Am. J. Clin. Nutr.*, 47: 33–36, 1988.
41. Raica, N., Scott, J., Lowry, L., and Sauberlich, H. E. Vitamin A concentration in human tissues collected from five areas in the United States. *Am. J. Clin. Nutr.*, 25: 291–296, 1972.
42. Schmitz, H. H., Poor, C. L., Wellman, R. B., and Erdman J. W., Jr. Concentrations of selected carotenoids and vitamin A in human liver, kidney and lung tissue. *J. Nutr.*, 121: 1613–1621, 1991.
43. Clinton, S. K., Emenhiser, C., Schwartz, S. J., Bostwick, D. G., Williams, A. W., Moore, B. J., and Erdman, J. W., Jr. *Cis-trans* lycopene isomers, carotenoids and retinol in human prostate. *Cancer Epidemiol. Biomarkers Prev.*, 5: 823–833, 1996.
44. Rao, A. V., and Agarwal, S. Bioavailability and *in vivo* antioxidant properties of lycopene from tomato products and their possible role in the prevention of cancer. *Nutr. Cancer*, 31: 199–203, 1998.
45. Rao, A. V., Fleshner, N., and Agarwal, S. Serum and tissue lycopene and biomarkers of oxidation in prostate cancer patients: a case-control study. *Nutr. Cancer*, 33: 159–164, 1999.
46. He, Y., and Campbell, T. C. Effects of carotenoids on aflatoxin B1-induced mutagenesis in *S. typhimurium* TA 100 and TA 98. *Nutr. Cancer*, 13: 243–253, 1990.
47. Bertram, J. S. Cancer prevention by carotenoids. In: L. M. Canfield, N. I. Krinsky, and J. A. Olson (eds.), *Carotenoids in Health*, Vol. 691, pp. 177–191. New York: Ann. NY Acad. Sci., 1993.
48. Nishino, H. Cancer prevention by natural carotenoids. *J. Cell Biochem.*, 27 (Suppl.): 86–91, 1997.
49. Neveu, M., and Bertram, J. S. Gap junctions and neoplasia. In: E. L. Hetzberg and E. E. Bittar, (eds.), *Gap Junctions*, pp. 221–262, Greenwich, CT: JAI Press, 2000.
50. Tsai, H., Werber, J., Davia, M. O., Edelman, M., Tanaka, K. E., Melman, A., Christ, G. J., and Geliebter, J. Reduced connexin 43 expression in high grade, human prostatic adenocarcinoma cells. *Biochem. Biophys. Res. Commun.*, 227: 64–69, 1996.
51. Mehta, P. P., Lokeshwar, B. L., Schiller, P. C., Bendix, M. V., Ostenson, R. C., Howard, G. A., and Roos, B. A. Gap-junctional communication in normal and neoplastic prostate epithelial cells and its regulation by cAMP. *Mol. Cell. Biochem.*, 15: 18–32, 1996.
52. Mehta, P., Perez-Stable, C., Nadji, M., Mian, M., Asotra, K., and Roos, B. A. Suppression of human prostate cancer cell growth by forced expression of *connexin* genes. *Develop. Gene.*, 24: 91–110, 1999.
53. Cohen, P., Peehl, D. M., and Rosenfeld, R. G. The IGF axis in the prostate. *Horm. Metab. Res.*, 26: 81–84, 1994.
54. Cohen, P., Peehl, D. M., Lamson, G., and Rosenfeld, R. G. 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, 1991.
55. Jones, J. I., and Clemmons, D. R. Insulin-like growth factors and their binding proteins: biological actions. *Endocr. Rev.*, 16: 3–34, 1995.
56. Li, Y., Upadhyay, S., Bhuiyan, M., Sarkar, F. H. Induction of apoptosis in breast cancer cells MDA-MB-231 by genistein. *Oncogene*, 18: 3166–3172, 1999.
57. King, T. J., Hieber, A. D., Fukushima, L., and Bertram, J. S. Role of connexin 43 expression and junctional communication in growth control. *Proc. Am. Assoc. Cancer Res.*, 38: 585, 1997.
58. Khachik, F., Beecher, G. R., Goli, M. B., Lusby, W. R., and Smith, J. C. Separation and identification of carotenoids and their oxidation products in extracts of human plasma. *Anal. Chem.*, 64: 2111–2122, 1992.
59. Khachik, F., Spangler, C. J., Smith, J. C., Jr., Canfield, L. M., Pfander, H., and Steck, A. Identification, quantification, and relative concentrations of carotenoids, their metabolites in human milk and serum. *Anal. Chem.* 69: 1873–1881, 1997.
60. Khachik, F., Askin, F. B., and Lai, K. Distribution, bioavailability, and metabolism of carotenoids in humans. In: W. R. Bidlack, S. T. Omaye, M. S. Meskin, D. Jahner (eds.), *Phytochemicals, A New Paradigm*, pp. 77–96. Lancaster, PA: Technomic Publishing, 1998.
61. Khachik, F., Beecher, G. R., and Whittaker, N. F. Separation, identification, and quantification of the major carotenoid and chlorophyll constituents in the extracts of several green vegetables by liquid chromatography. *J. Agric. Food Chem.*, 34: 603–616, 1986.
62. Khachik, F., Englert, G., Daitch, C. E., Beecher, G. R., Lusby, W. R., and Tonucci, L. H. Isolation and structural elucidation of the geometrical isomers of lutein and zeaxanthin in extracts from human plasma. *J. Chromatogr. B Biomed. Appl.*, 582: 153–166, 1992.
63. Khachik, F., Englert, G., Beecher, G. R., and Smith, Jr., J. C. Isolation, structural elucidation, and partial synthesis of lutein dehydration products in extracts from human plasma. *J. Chromatogr. B Biomed. Appl.*, 670: 219–233, 1995.
64. Hengartner, U., Bernhard, K., Meyer, K., Englert, G., and Glinz, E. Synthesis, isolation, and NMR spectroscopic characterization of fourteen (Z)-isomers of lycopene and of some acetylenic dihydro- and tetrahydrolycopenes. *Helv. Chim. Acta*, 75: 1848–1865, 1992.
65. Khachik, F., Steck, A., Niggli, U. A., and Pfander, H. Partial synthesis and structural elucidation of the oxidative metabolites of lycopene identified in tomato paste, tomato juice and human serum. *J. Agric. Food Chem.*, 46: 4874–4884, 1998.
66. Sakr, W. A. Prostatic intraepithelial neoplasia: a marker for high-risk groups and a potential target for chemoprevention. *Eur. Urol.*, 35: 474–478, 1999.
67. Pateau, I., Khachik, F., Brown, E. D., Beecher, G. R., Kramer, T. R., Chittams, J., and Clevidence, B. A. Chronic ingestion of lycopene-rich tomato juice or lycopene supplements significantly increases plasma concentrations of lycopene and related tomato carotenoids in humans. *Am. J. Clin. Nutr.*, 68: 1187–1195, 1998.
68. Lee, S. W., Tomasetto, C., and Sager, R. Positive selection of candidate tumor suppressor genes by subtractive hybridization. *Proc. Natl. Acad. Sci. USA*, 88: 2825–2829, 1991.
69. Omori, Y., and H. Yamasaki. Mutated connexin43 proteins inhibit rat glioma cell growth suppression mediated by wild-type connexin43 in a dominant-negative manner. *Int. J. Cancer*, 78: 446–453, 1998.
70. Hossain, M. Z., and Bertram, J. S. Retinoids suppress proliferation, induce cell spreading, and up-regulate connexin43 expression only in postconfluent 10T1/2 cells: implications for the role of gap junctional communication. *Cell Growth Diff.*, 5: 1253–1261, 1994.
71. King, T. J., Fukushima, L. H., Hieber, A. D., Shimabukuro, K. A., Sakr, W. A., and Bertram, J. S. Reduced levels of connexin 43 in cervical dysplasia: inducible expression in a cervical carcinoma cell line decreases neoplastic potential with implications for tumor progression. *Carcinogenesis (Lond.)*, 21: 1097–1109, 2000.
72. Hossain, M. Z., Jagdale, A. B., Ao, P., LeCiel, C., Huang, R. P., and Boynton, A. L. Impaired expression and posttranslational processing of connexin 43 and downregulation of gap junctional communication in neoplastic human prostate cells. *Prostate*, 38: 55–59, 1999.