cis-trans Lycopene Isomers, Carotenoids, and Retinol in the Human Prostate[1]

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Abstract

An evaluation of the Health Professionals Follow-Up Study has detected a lower prostate cancer risk associated with the greater consumption of tomatoes and related food products. Tomatoes are the primary dietary source of lycopene, a non-provitamin A carotenoid with potent antioxidant activity. Our goal was to define the concentrations of lycopene, other carotenoids, and retinol in paired benign and malignant prostate tissue from 25 men, ages 53 to 74, undergoing prostatectomy for localized prostate cancer. The concentrations of specific carotenoids in the benign and malignant prostate tissue from the same subject are highly correlated. Lycopene and all-*trans* β-carotene are the predominant carotenoids observed, with means ± SE of 0.80 ± 0.08 nmol/g and 0.54 ± 0.09, respectively. Lycopene concentrations range from 0 to 2.58 nmol/g, and all-*trans* β-carotene concentrations range from 0.09 to 1.70 nmol/g. The 9-*cis* β-carotene isomer, α-carotene, lutein, α-cryptoxanthin, zeaxanthin, and β-cryptoxanthin are consistently detectable in prostate tissue. No significant correlations between the concentration of lycopene and the concentrations of any other carotenoid are observed. In contrast, strong correlations between prostate β-carotene and α-carotene are noted (correlation coefficient, 0.88; $P < 0.0001$), as are correlations between several other carotenoid pairs, which reflects their similar dietary origins. Mean vitamin A concentration in the prostate is 1.52 nmol/g, with a range of 0.71 to 3.30 nmol/g. We further evaluated tomato-based food products, serum, and prostate tissue for the presence of geometric lycopene isomers using high-performance liquid chromatography with a polymeric C_{30} reversed phase column. All-*trans* lycopene accounts for 79 to 91% and *cis* lycopene isomers for 9 to 21% of total lycopene in tomatoes, tomato paste, and tomato soup. Lycopene concentrations in the serum of men range between 0.60 and 1.9 nmol/ml, with 27 to 42% all-*trans* lycopene and 58 to 73% *cis*-isomers distributed among 12 to 13 peaks, depending upon their chromatographic resolution. In striking contrast with foods, all-*trans* lycopene accounts for only 12 to 21% and *cis* isomers for 79 to 88% of total lycopene in benign or malignant prostate tissues. *cis* Isomers of lycopene within the prostate are distributed among 14 to 18 peaks. We conclude that a diverse array of carotenoids are found in the human prostate with significant intra-individual variation. 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 prostate cancer risk associated with the consumption of tomato-based foods. The future identification and characterization of geometric lycopene isomers may lead to the development of novel agents for chemoprevention studies.

Introduction

Carotenoids are a diverse group of over 600 structurally related compounds synthesized by bacteria and plants. Carotenoids are relevant to human nutrition since some can undergo oxygen-dependent central cleavage to retinal, providing an important source of vitamin A in certain populations (1). The ability of many carotenoids to quench singlet oxygen and function as antioxidants has led to the generation of hypotheses concerning their role in preventing disease processes thought to be related to chronic oxidative damage, such as cancer and cardiovascular disease. However, evolving evidence suggests that carotenoids may modulate processes related to mutagenesis, cell differentiation, and proliferation independently of their role as antioxidants or precursors of vitamin A (2–5).

The frequent association between diets rich in fruits and vegetables and the reduced risk of several malignancies (6, 7) has led many to postulate that the carotenoids found in fruits and vegetables contribute to these relationships. The role of carotenoids in prostate carcinogenesis has not been extensively evaluated in epidemiological studies, and no definitive data has been derived in animal models. Estimated intake of β-carotene has been associated with increased risk (8), decreased risk (8–10), or no effect on prostate cancer risk (11–14). Rats and mice exhibit limited absorption of β-carotene and are rapid converters of β-carotene to vitamin A, making it difficult to evaluate the role of dietary carotenoids in available rodent models of prostate carcinogenesis (15). The data base for carotenoid content in the food supply has only recently been expanded to include carotenoids other than β-carotene (16).

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The new data base has been applied to an evaluation of estimated carotenoid intake and prostate cancer risk in the Health Professionals Follow-Up Study of over 50,000 men completing a dietary assessment and disease incidence report every 2 years since 1986 (14). The greater consumption of tomatoes and tomato-containing food products, such as tomato paste and pizza, was associated with a significant reduction in prostate cancer risk (14). For example, the combined intake of tomatoes and tomato products was inversely associated with risk of prostate cancer (multivariate RR = 0.65 and 95% confidence interval = 0.44–0.95 for consumption frequency of >10 *versus* <1.5 servings/week; *P*, trend = 0.01) and advanced (stage C and D) prostate cancer (multivariate RR = 0.47 and 95% confidence interval = 0.22–1.00; *P*, trend = 0.03). In another study, the intake of tomatoes was significantly associated with reduced risk of prostate cancer in a cohort of Seventh-Day Adventists (17). Among the many components of tomato products that may participate in cancer prevention is the nonprovitamin A carotenoid lycopene, which is responsible for the red color of tomatoes. In the Health Professionals Study, prostate cancer risk was 20% lower for men with the highest *versus* the lowest quintile of estimated lycopene intake (14). Lycopene is a potent antioxidant and quencher of singlet oxygen (18, 19) and a predominant carotenoid in the plasma and certain tissues of Americans (20–23). The possibility that lycopene may influence prostate carcinogenesis in rodent models or modulate prostate cell function *in vitro* studies has not been assessed.

In contrast to carotenoids, a series of diverse investigations have clearly documented that vitamin A is an important regulator of prostate biology. The possibility that provitamin A carotenoids, such as β-carotene, can indirectly modulate prostate cancer risk via the metabolic conversion to vitamin A, perhaps within the gland, remains speculative. Studies completed over 40 years ago showed that vitamin A deficiency in rats produced a squamous metaplasia in the prostate, a histological finding associated with a predisposition to malignant transformation in many tissues (24). In an organ culture system, vitamin A was shown to reverse squamous metaplasia induced by a chemical carcinogen (25). Retinoid binding proteins have been documented in the prostate (26–28). A recent *in vitro* study with vitamin A showed that primary cultures of prostatic epithelial cells grown under serum-free conditions have a biphasic growth response to vitamin A (29). Retinoic acid at 3 nM or higher inhibited proliferation, whereas lower concentrations were stimulatory. Retinoids inhibit testosterone-induced hyperplasia of mouse prostate explants (30). It has been hypothesized that this effect could be mediated by the ability of retinoic acid to inhibit the conversion of testosterone to dihydrotestosterone by 5 α-reductase (30, 31) or the proliferative response to growth factors (32). The synthetic retinoid 4-hydroxyphenol retinamide has been shown to be cytotoxic to cultured prostate cancer cells (33). 4-Hydroxyphenol retinamide also has been reported to inhibit carcinogen- and testosterone-induced (34), but not spontaneous, prostate cancer in rats (35).

Although the laboratory-based investigations indicate that normal prostate growth and function, as well as tumorigenesis, are modulated by vitamin A, the epidemiological data concerning vitamin A and prostate cancer are often termed "conflicting" or "contradictory" (36, 37). Higher estimated vitamin A intake was associated with a slightly increased risk of prostate cancer in some studies and lower risk in others (9, 10, 13, 38–44). Several studies have reported increased risk of prostate cancer with lower concentrations of serum retinol (11, 45, 46). It is reasonable to conclude that the cell culture and rodent studies clearly show that vitamin A and synthetic retinoids modulate prostate function and risk of carcinogenesis. However, the complexities of vitamin A nutrition in free-living men and the interactions with other dietary components (such as provitamin A carotenoids), genetic factors, and endocrine status indicate that more basic and epidemiological research is needed to define the role of vitamin A in prostate cancer.

The present study characterized the concentration and pattern of carotenoids and vitamin A in paired normal and malignant human prostate tissue. We documented the presence of a spectrum of carotenoids in the prostate and significant intra-individual variation, which probably reflects unique patterns of intake and metabolism. It has been established that the isomers of vitamin A (47), and perhaps carotenoids (48), are related to molecular mechanisms of action and warrant investigation in human biological specimens. We, therefore, used HPLC[3] technology that identified a diverse array of *cis* lycopene isomers in the prostate and human serum, although tomato-based foods contained primarily all-*trans* lycopene. This information provides a baseline for future epidemiological and laboratory studies designed to characterize the roles of lycopene isomers, other carotenoids, and vitamin A in the normal and diseased prostate.

Materials and Methods

Prostate Tissue and Serum Collection

Tissue samples were obtained from radical prostatectomy specimens at the Mayo Clinic (Rochester, MN) and stored at −70°C until processed or stored on dry ice during shipping. Prostatectomy specimens were initially examined by a pathologist, and tissue samples were taken from areas containing tumor and areas thought to be nonmalignant. Thirty paired specimens, randomly selected from the tissue bank, from 29 Caucasian males and one Native American were evaluated in these studies (n = 25 for carotenoid patterns and n = 5 for lycopene isomers). The median age was 66 years, with a range of 53 to 74. Preoperative serum prostate-specific antigen ranged from 0.2 ng/ml to 62.3 ng/ml with a median of 7.9, and only two patients exhibited values over 20 (Abbott IMx method). Preoperative CT scans, bone scans, and liver function tests showed no evidence of metastatic disease. Pathological staging revealed two specimens with microscopic node positively, two with seminal vesicle invasion, five with a focus of extracapsular extension, and the remaining lesions were confined to the prostate gland. Gleason sums ranged from 4 to 8, with 84% of the specimens graded as 5, 6, or 7. With a median follow-up of 26 months, three patients relapsed with biochemical (prostate-specific antigen) failure, and one died due to causes unrelated to prostate cancer. Among the patient population, 16% reported current smoking, 48% consumed alcoholic beverages of some type, and only three reported consumption of vitamin supplements. One or more prescription medications were consumed by 16 men. Serum samples (n = 10) for lycopene isomer measurement were obtained from men undergoing initial evaluation at the Dana-Farber Cancer Institute for localized prostate cancer and stored at −70°C until analysis. Median age was 57 years, with a range of 48 to 80. Median prostate-specific antigen at diagnosis was 9 ng/ml, with a range of 3 to 64. One smoked cigarettes, four reported occasional alcohol intake, and one consumed a multivitamin.

[3] The abbreviation used is: HPLC, high-performance liquid chromatography.

HPLC Analysis of Prostate Carotenoid Patterns and Retinol

Extraction. Tissue homogenization was carried out in subdued light. Extraction and analysis were performed under yellow lights to prevent sample degradation by photooxidation. Prostate tissue was weighed, minced, and homogenized (Brinkman Polytron, Westbury, NY) in 50-ml glass centrifuge tubes on ice containing 5 ml ethanol/butylated hydroxy toluene solution (0.1%). The samples were saponified with saturated KOH (1 ml) for 30 min at 70°C. Distilled water (2 ml) was added to each sample and cooled on ice to improve the extraction efficiency of retinol. Carotenoids and retinol were extracted by adding equal volumes of hexane, vortexing thoroughly, and removing the hexane epilayer after phase separation. The hexane extraction was repeated twice, and the combined extract was dried with a Savant AS 160 Speedvac (Farmington, NY). Reconstitution for reversed phase HPLC analysis was in methylene chloride or a mixture of methylene chloride and mobile phase.

Instrumentation and Chromatography. The HPLC system consisted of a Milton-Roy Constametric II solvent delivery system (Rivera Beach, FL) and a Bio-Rad model 170 (Richmond, CA) or Waters model 486 UV-Vis detector (Millipore Corporation, Milford, MA) at a wavelength of 450 nm for carotenoids and 325 nm for retinol. Shimadzu CR601 Chromatopac integrators (Kyoto, Japan) provided chromatograms and peak integration values. A Vydac 201TP54 C_{18} reversed phase column (The Separations Group, Hesperia, CA) was used for carotenoid analysis, whereas a Supelcosil LC18 (Supelco, Bellefonte, PA) column was used for retinol analysis. Mobile phases were 88% methanol, 9% acetonitrile, and 3% water, with the addition of 2,2,4-trimethyl pentane as a solvent modifier, and 47% methanol, 47% acetonitrile, and 6% chloroform for the Vydac and Supelcosil columns, respectively.

Peak Identification. Peak confirmation and quantitation were determined with authentic standards for lutein, lycopene, β-carotene, and retinol (Sigma Chemical Co., St. Louis, MO; Fluka Chemical Co., Ronkonkoma, NY). Standard curves were prepared daily for quantitative analysis. Carotenoid identification was also based upon expected elution order, the relative retention times of carotenoids compared to β-carotene, and absorption spectra obtained by photodiode array detection (Waters 991 photodiode array system; Millipore Corp., Milford, MA). To estimate the concentrations of the more polar carotenoids, zeaxanthin, and α- and β-cryptoxanthins, the standard curve for lutein was used because the extinction coefficients for these hydroxylated carotenoids are similar. Similarly, the standard curve for β-carotene was used to estimate the concentrations of α-carotene. Typical coefficients of variation for our HPLC analysis are 3–5% for β-carotene and 6–7% for α-carotene.

HPLC Analysis of Prostate, Serum, and Tomato Lycopene Isomers

Extraction. Handling, extraction, and analysis was carried out in subdued light. Tissue samples were weighed and transferred to 50 ml polypropylene centrifuge tubes containing 2.5 ml each of distilled water and ethanol containing 2% butylated hydroxy toluene. Samples were homogenized and saponified by the addition of 5 ml of 10% NaOH in methanol, followed by incubation for 30 min at 60°C. Fresh tomato and serum, extracted according to this procedure using either 10% NaOH in methanol or 100% methanol (without incubation), were used as controls to insure that the saponification step did not induce isomerization of lycopene. Samples were cooled in an ice bath, and 5 ml of distilled water added. Carotenoids were extracted by incorporating 5 ml of hexane and then removing the hexane epilayer after phase separation. Extractions were repeated twice, and the three hexane extracts were combined. Serum samples (2 ml) were transferred to polypropylene centrifuge tubes for carotenoid extraction, and 2 ml of ethanol were added. Each sample was extracted three times with 2.5 ml of a 1:2 acetone:hexane mixture. Contaminating water was removed by passing prostate and serum extracts through sodium sulfate. Each sample was concentrated to a volume of 2 ml with nitrogen gas. To simplify chromatographic analysis, the prostate and serum extracts were prefractionated using alumina Sep Pak cartridges (Type N; Millipore, Milford, MA) to remove certain carotenoids. Fractionation was done as follows: (*a*) 3.5 ml of hexane were passed through the alumina; (*b*) each 2-ml extract was loaded; (c) 3.5 ml of hexane were used to wash the extract; (*d*) 3.5 ml of 10% acetone in hexane were used to elute α- and β-carotenes; and (*e*) 3.5, 7.0, and 10.5 ml of 40, 70, and 100% acetone in hexane, respectively, were used to elute lycopene and the xanthophylls for subsequent HPLC analysis of lycopene isomers. Carotenoids were extracted from commercially available fresh tomatoes, tomato soup, and tomato paste according to a procedure reported previously (49). All fractions and extracts were dried under nitrogen gas, stored at −20°C, and analyzed by HPLC within 18 h.

Instrumentation and Chromatography. The HPLC system consisted of a Waters model 501 solvent delivery system and U6K injector (Millipore) and an Anspec model SM 95 UV-Vis detector from Linear Instruments (Reno, NV). A Dionex AC-1 advanced computer interface (Dionex Corp., Sunnyvale, CA) was coupled with a Dramen computer (Raleigh, NC) for data acquisition. Dionex AI-450 chromatography software (release 3.30) was used to integrate chromatographic peaks. Separations of geometric lycopene isomers were achieved using polymeric C_{30} reversed phase HPLC columns (250 × 4.6 mm) prepared at the National Institute of Standards and Technology (Gaithersburg, MD) according to Sander *et al.* (50). The mobile phase was 38% methyl-*t*-butyl ether in methanol, flowing at a rate of 1.1 ml/min for the prostate samples and 1.0 ml/min for serum and tomato extracts. Column effluent was monitored at 460 nm. Tissue and serum extracts were dissolved in 200 μl of mobile phase, and injection volumes were 100 and 20–50 μl, respectively.

Peak Identification. Quantification of geometric lycopene isomers in prostate and serum was facilitated by a standard curve of all-*trans* lycopene (Sigma). Tentative identification of chromatographic peaks as geometric (*cis-trans*) isomers of lycopene was based upon: (*a*) retention characteristics on alumina and C_{30} stationary phases; (*b*) electronic absorption spectra obtained by photodiode array detection using a Waters model 996 system (Millipore); and (*c*) comparison of chromatograms with those obtained for an isomerized lycopene standard and standards of common xanthophylls. The possibility that one or more peaks tentatively identified as a geometric lycopene isomer could be an oxidized form of lycopene was addressed using electrospray mass spectrometry. This was done by collecting all chromatographic peaks resulting from C_{30} chromatography of a photo-isomerized lycopene standard as four fractions according to ranges of retention time. The molecular weight of each fraction was then determined by electrospray mass spectrometry. The peak corresponding to the all-*trans* isomer of lycopene was identified by cochromatography of an authentic standard of all-*trans* lycopene added to a prostate tissue extract as well as the electronic absorption spectrum of this peak. Quantitation of pooled *cis* and all-*trans* lycopene was done using a

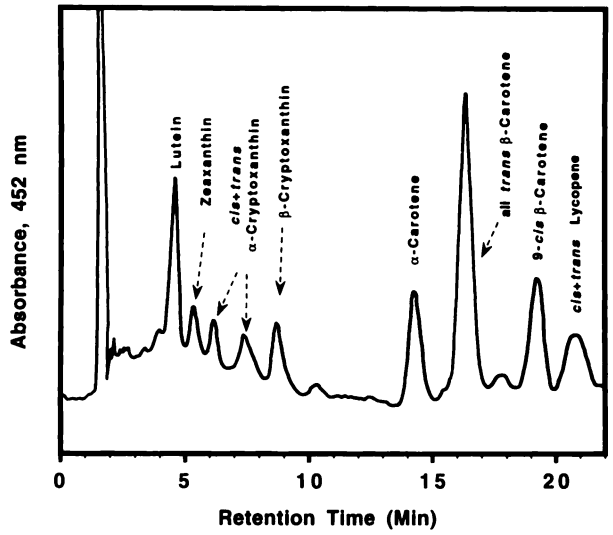

Fig. 1. Representative HPLC separation of carotenoids in human prostate tissue using a C_{18} reversed phase column.

published molar absorptivity value (ϵ) for the all-*trans* isomer, although it is known that *cis* lycopene isomers have lower molar absorptivities than does the all-*trans* form (51). This approach was necessary because the specific absorptivity values are not known individually or collectively for the *cis* isomers of lycopene. Thus, the relative contributions of pooled *cis* and all-*trans* lycopene to total lycopene are based on the assumption that molar absorptivity values for the various isomers are equal. It should be noted that this approach gives an underestimation of the contribution of *cis* lycopenes to total lycopene.

Statistical Analysis

Initial descriptive analysis of prostate carotenoids was computed and presented as box plots to reveal useful distribution and outlier characteristics of the data set. The concentrations of individual carotenoids in paired samples of normal and cancer-containing prostate tissue was evaluated by pairwise *t* testing. Associations between various carotenoids in the prostate tissue were evaluated by correlation analysis, which generated the correlation coefficient, *P*, and 95% confidence interval for each pair of carotenoids. The concentrations of pooled *cis* isomers and all-*trans* lycopene are presented as individual values for each sample. All statistical evaluations were completed using StatView 4.5 (Abacus Concepts, Inc., Berkeley, CA).

Results

A diverse array of carotenoids are detected by HPLC analysis of human prostate tissue (Fig. 1). Lycopene was detected in the highest concentration in 64% of patients, all-*trans* β-carotene in 28%, and lutein in 8%. Most of the chromatograms show a broad lycopene peak which is often detected with a shoulder and occasionally resolved as two peaks. The partial separation of two lycopene peaks, tentatively identified in a previous report as a *cis* isomer peak followed by the all-*trans* peak (23), suggests the predominance of *cis* geometric forms and the possibility that multiple geometric isomers of lycopene may occur in the prostate. We observed β-carotene in three peaks. All-*trans* β-carotene and 9-*cis* β-carotene were observed in all samples. Another peak, which is probably a mixture of 13-*cis*, 15-*cis* and perhaps other *cis* isomers of β-carotene, was occasionally observed. Prostate vitamin A (retinol plus retinol esters) concentrations ranged from 0.71 to 3.30 nmol/g, with a median of 1.52 nmol/g (Fig. 2). In addition to lycopene and β-carotene, we consistently detected α-carotene, lutein, zeaxanthin, β-crytoxanthin, and α-cryptoxanthin isomers in prostate tissue (Fig. 2). Significant person-to-person variations in concentrations were observed for each carotenoid and retinol.

The concentrations of specific carotenoids in the paired benign and malignant tissues were positively correlated (Table 1). For example, a correlation coefficient of 0.86 was observed with a 95% confidence interval of 0.70 to 0.94 for all-*trans* β-carotene in the paired samples ($P < 0.0001$). Although strongly correlated, the absolute concentrations of lycopene, all-*trans* β-carotene, and total carotenoids are slightly greater in the malignant sample compared to the noncancerous sample. Benign prostate tissue is composed of significant amounts of extracellular matrix, fibrous connective tissue, and nonepithelial cells. We speculate that, relative to cancerous tissue, the normal prostate is also less metabolically active and may show a lower uptake of serum carotenoids.

A correlation matrix illustrating the relationships between different carotenoids in the prostate is shown in Table 2. Lycopene content was unrelated to the concentrations of other carotenoids, such as all-*trans* β-carotene (Fig. 3). In contrast, significant associations between several carotenoids were observed (Table 2). For example (Fig. 3), the prostatic concentrations of all-*trans* β-carotene and α-carotene are strongly associated (correlation coefficient, 0.88; $P < 0.0001$).

Among the 25 men evaluated for complete carotenoid profiles in the prostate, there were 13 reporting current consumption of alcoholic beverages. We observed a lower mean lycopene concentration ($P = 0.04$) in the prostates of men reporting alcohol intake (0.57 ± 0.08 nmol/g) relative to those who deny alcohol consumption (0.95 ± 0.15 nmol/g). The mean concentrations of all other carotenoids were less in those reporting alcohol intake, although no others showed significant difference based upon statistical evaluation of this small data set.

Our observation that high concentrations of lycopene are present in the prostate and the previous studies suggesting that lycopene may exist in human serum or tissue samples as forms other than all-*trans* led us to further investigate the chemistry of prostatic lycopene (23, 52, 53). The analysis of tomatoes, serum, and prostate tissue for geometrical lycopene isomers using a recently developed polymeric C_{30} column in conjunction with reversed phase HPLC disclosed multiple *cis* isomers (Fig. 4). These C_{30} separations of geometrical lycopene isomers are clearly unique compared to those obtained using other HPLC columns. UV-Visible absorption spectra of representative geometrical lycopene isomers are illustrated in Fig. 5. All spectra possess the characteristic fine structure observed for lycopene. In traces A-C, the spectra of six different peaks illustrate increased absorbance in the 360-nm region, and the wavelengths of maximum absorbance are shifted (relative to the all-*trans* isomer), indicating the presence of *cis* bonds. The spectra in traces D and E are indistinguishable, although it should be noted that these spectra are not pure because the corresponding peaks were only partially resolved during chromatography. Trace D is the spectra of all-*trans* lycopene, the peak of which was identified by cochromatography. The isomer corresponding to trace E likely possesses a peripheral (*e.g.*, 5-*cis*) *cis* bond. Evidence for the presence of a peripheral *cis*

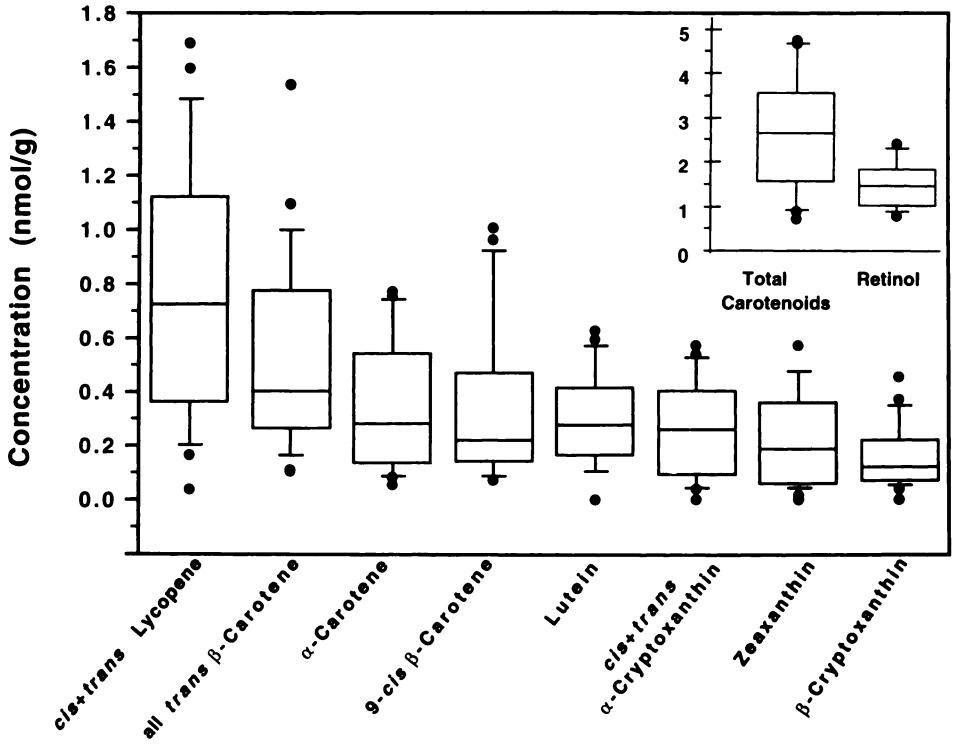

Fig. 2. The concentrations and pattern of individual carotenoids and vitamin A in human prostate tissue from 25 men undergoing prostatectomy for localized prostate cancer. A single value for each prostate (n = 25) was derived from the average of the HPLC analysis of the "normal" and malignant tissue. The *box plots* represent a description of the data showing the 10th and 90th percentiles of data (*ends of the whiskers*), the 25th and 75th percentiles (*ends of the box*), and the 50th percentile (*line within the box*). Any outlier points are depicted as individual values.

Table 1 The comparison of carotenoid concentrations in paired "normal" and cancer tissue from prostatectomy specimens from 25 men treated for localized prostate cancer

	"Normal" (nmol/g) mean ± SE	Cancer (nmol/g) mean ± SE	Pairwise comparison
Lycopene	0.63 ± 0.09	0.91 ± 0.13	$P < 0.03$
all-*trans* β-Carotene	0.48 ± 0.06	0.60 ± 0.08	$P < 0.02$
9-*cis* β-Carotene	0.38 ± 0.06	0.40 ± 0.07	NS[a]
α-Carotene	0.35 ± 0.06	0.35 ± 0.05	NS
Lutein	0.26 ± 0.05	0.33 ± 0.05	NS
cis + *trans* α-Cryptoxanthin	0.22 ± 0.03	0.29 ± 0.03	NS
Zeaxanthin	0.19 ± 0.04	0.29 ± 0.06	NS
β-Cryptoxanthin	0.14 ± 0.02	0.18 ± 0.03	NS
Total carotenoids	2.65 ± 0.25	3.35 ± 0.32	$P < 0.02$

[a] NS, not significant.

bond in this isomer comes not only from the similarity of its absorption spectra to that of the all-*trans* isomer (*i.e.*, low absorbance in the 360-nm region) but also from the retention characteristics of other carotenoid isomers on the C_{30} column. For example, the 9- and 9′*cis* isomers of α-carotene have been shown to elute after the all-*trans* isomer (54), as have the peripheral *cis* isomers of other common carotenoids (55). In contrast, geometrical isomers with centrally located *cis* bonds elute before the all-*trans* isomer during C_{30} chromatography (54, 55). Using electrospray mass spectrometry, all peaks identified as geometric lycopene isomers in the isomerized standard gave a single molecular ion peak corresponding to a molecular weight of 536 (data not shown). Molecular ions were not detected at higher m/z values for the same fractions, indicating that oxygen-addition products of lycopene were either absent or present below the limits of detection. Because the chromatographic profiles of prostate, serum, and tomato lycopene are qualitatively similar to that obtained for isomerized lycopene, it is unlikely that peaks identified as geometric lycopene isomers in extracts of biological tissues are oxidation products of lycopene. The only other hydrocarbon carotenoids of molecular weight of 536 detected in prostate tissue were α- and β-carotenes, and these were eliminated from prostate extracts during the alumina fractionation step prior to C_{30} chromatographic analysis. Based upon the information derived from these studies, we feel that those peaks are correctly identified as *cis* isomers, rather than oxidation products of lycopene forming during fractionation.

Very little is known about the structural identities of these previously unresolved *cis* lycopene peaks. Identification of the various *cis* lycopenes is currently under investigation by our laboratories. To summarize the data from individual chromatograms, we have pooled the peak areas for the *cis* isomers and presented the relative proportion and concentrations of the pooled *cis* isomers and all-*trans* lycopene in foods, serum, and prostate tissue. The proportion of lycopene distributed among all-*trans versus cis* isomers varies significantly between food products, human serum, and prostate tissue (Figs. 6 and 7). All-*trans* lycopene is predominant in tomatoes and tomato-based foods, accounting for 79 to 91% of total lycopene, with the remainder distributed among several small *cis* isomer peaks (Figs. 6 and 7). In contrast, all-*trans* lycopene accounted for only 12 to 21% and *cis* isomers for 79 to 88% of total lycopene in prostate tissue (Figs. 6 and 7), which ranged from 0.88 to 3.82 nmol/g (Fig. 7). *cis* isomers were distributed among 14 to 18 peaks in prostate tissue and 12 to 13 in serum, depending upon their chromatographic resolution (Fig. 4). Among individual lycopene isomers, the all-*trans* form of lycopene was the second most abundant in prostate, in contrast to the tomato and serum samples, where the all-*trans* configuration was predom-

Table 2 Correlation matrix illustrating the relationships between prostate carotenoids

Values represent the correlation coefficients, the 95% confidence interval for the pairwise evaluations, and the level of statistical significance. Only those associations with $P < 0.01$ are presented with confidence intervals.

	cis + *trans* Lycopene	all-*trans* β-Carotene	α-Carotene	9-*cis* β-Carotene	Lutein	*cis* + *trans* α-Cryptoxanthin	Zeaxanthin
all-*trans* β-Carotene	0.27 NS[a]						
α-Carotene	0.16 NS	0.88 0.74–0.95 $P < 0.0001$					
9-*cis* β-Carotene	0.18 NS	0.94 0.86–0.98 $P < 0.0001$	0.86 0.73–0.95 $P < 0.0001$				
Lutein	0.17 NS	0.44 NS	0.30 NS	0.69 0.36–0.86 $P < 0.0006$			
cis + *trans* α-Cryptoxanthin	0.21 NS	0.66 0.35–0.84 $P = 0.0002$	0.61 0.28–0.81 $P = 0.0009$	0.78 0.53–0.91 $P < 0.0001$	0.80 0.60–0.91 $P < 0.0001$		
Zeaxanthin	0.32 NS	0.57 0.23–0.79 $P = 0.002$	0.70 0.41–0.86 $P < 0.0001$	0.73 0.43–0.85 $P = 0.0002$	0.43 NS	0.69 0.36–0.86 $P = 0.0003$	
β-Cryptoxanthin	0.28 NS	0.12 NS	0.14 NS	0.30 NS	0.34 NS	0.44 NS	0.41 NS

[a] NS, not significant.

inant. Lycopene concentrations in the serum ranged between 0.59 and 1.93 nmol/ml with 27 to 42% all-*trans* lycopene and 58 to 73% *cis* isomers (Figs. 6 and 7). The serum concentrations of total lycopene, all-*trans* lycopene, or pooled *cis* isomers show significant interindividual heterogeneity. Interindividual patterns for serum and prostate lycopene isomers are very similar qualitatively. Differences in the number of lycopene peaks within the sets of serum and prostate samples reflect the difficulty in reproducibly separating lycopene peaks that are only partially resolved, rather than large interindividual differences in lycopene isomer patterns. Slight differences in the chromatographic resolution of lycopene isomers did not, however, affect the quantitative results because the *cis* isomers were pooled. Quantitative interindividual results of the proportion of *cis versus* all-*trans* lycopene varied slightly, but all serum samples and prostate tissues followed a similar pattern in this regard (Fig. 6).

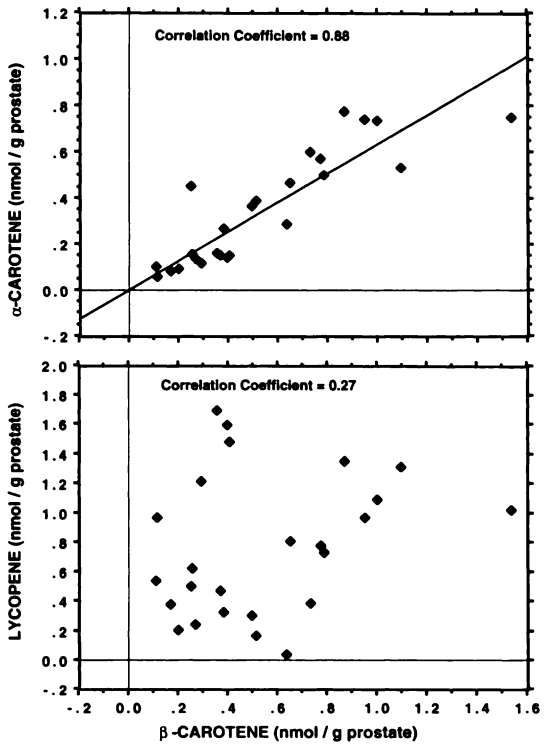

Fig. 3. The relationship between all-*trans* β-carotene and α-carotene or all-*trans* β-carotene and lycopene in 25 human prostates. The content of all-*trans* β-carotene and α-carotene are strongly correlated with a correlation coefficient of 0.88 (95% confidence interval of 0.74 to 0.95, $P < 0.0001$). The *line* represents the linear relationship between the two measurements in a regression analysis ($P < 0.0001$) and is described by the equation: $Y = 0.0x + 0.635$ with $R^2 = 0.93$. In contrast, prostate lycopene was not associated with the content of all-*trans* β-carotene or any other carotenoid.

Discussion

Variation in Prostate Carotenoids. Our studies reveal a diverse array of carotenoids in human prostate tissue. To our knowledge, there are no studies that have simultaneously examined prostate tissue and other organs to contrast their relative carotenoid contents. However, comparing our data to other reports, the prostate contains concentrations of total carotenoids similar to organs such as the lung, ovary, kidney, and adipose tissue (20–23). We observed approximately a 10-fold variation for total carotenoids and most individual carotenoids in prostate tissue. Within other tissues, the concentrations of individual carotenoids are also reported to show significant intraindividual variation (20–22). For example, Schmitz *et al.* (23) reported from an autopsy study that total carotenoids in the liver, kidney, and lung varied 30-, 60-, and 85-fold, respectively. Autopsy studies may report greater intraindividual variation due to the effects of terminal disease processes on dietary intake, metab-

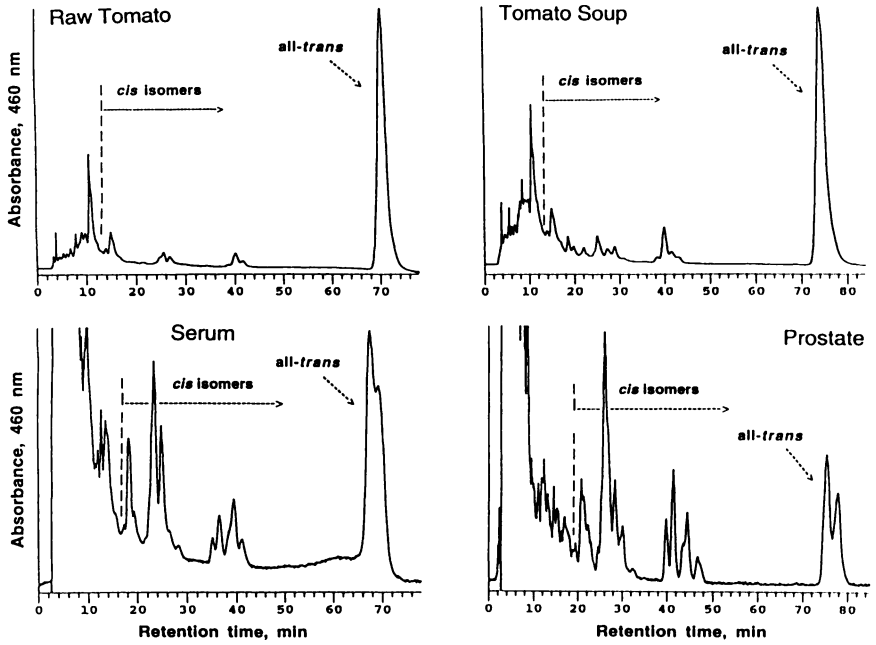

Fig. 4. Representative chromatograms illustrating *cis* and *trans* isomers of lycopene in fresh tomato, tomato soup, human serum, and the human prostate. HPLC used a C_{30} reversed phase column. The lycopene isomers elute after a retention time of 12 min. These chromatograms are typical of multiple samples of commercially available tomato products, serum, and prostate tissue evaluated.

olism, and tissue distribution in a population having a broader age distribution and complicated by undefined male/female differences. The men undergoing prostatectomy for early-stage prostate cancer are a select group having overall good health, particularly relative to cardiovascular disease. Men with medical conditions that add significantly to surgical risk or whom are thought to have reduced overall life expectancy are advised to undergo other forms of primary treatment, such as radiation therapy, hormone therapy, or observation rather than surgery for their organ-confined disease. Sixty % of the men in our study were taking prescription medications, the majority of which were a broad array of antihypertensives. This sample is too small to allow an assessment of drug effects on prostate carotenoid concentrations. Similarly, only 4 of the 25 men report recent smoking histories, and it is not possible to assess the effects of tobacco use on prostate carotenoid concentrations.

Tissue Specificity in Carotenoid Patterns and Concentrations. Previous studies indicate that the pattern of carotenoids in various tissues is not random, and the median levels of specific carotenoids vary dramatically between different tissues within an individual (20–22). For example, the zeaxanthin/lutein combined peak, β-carotene, and lycopene showed 10-, 20-, and 100-fold variations between different tissues within individuals (20). Zeaxanthin illustrates a striking tissue-specific distribution and accounts for 35–45% of total carotenoids in adipose tissue and the ovary but less than 5% in the adrenal or testes (20). The macular pigment of the human eye appears to selectively accumulate lutein and zeaxanthin (56, 57). Others have reported that lycopene accounted for 60 to 75% of total carotenoids in the adrenal and testes but only 10 to 40% in eight other tissues (20). We observed that lycopene accounted for an average of 30% of total carotenoids in the prostate, although the variation was very large with a range of 2–61%.

The mechanisms that account for tissue specificity in carotenoid patterns have not been established. It can be hypothesized that differential transport of individual carotenoids by lipoproteins and tissue-specific patterns of lipoprotein receptors may contribute to these observations. Evidence suggests that, under fasting conditions, the majority of β-carotene and lycopene are transported in the low density lipoprotein fraction, with the remaining associated with high density lipoprotein and to a lesser degree with very low density lipoprotein (15, 58). In contrast, lutein is almost equally distributed between the low density lipoprotein and high density lipoprotein fractions (58). In general, the tissues with the highest density of low density lipoprotein receptors and highest rates of reported lipoprotein uptake (adrenals, testes, and liver) contain the higher concentrations of carotenoids (59). However, lipoprotein receptor density alone will not completely explain the nonrandom and nonparallel patterns of carotenoids observed in different tissues. The accumulated evidence suggests that there may also be selective tissue-specific mechanisms for the cellular uptake of individual carotenoids. In addition, unique tissue-dependent intracellular metabolic processes subsequent to either a passive or selective uptake of carotenoids may further contribute to the specific patterns observed.

Diet and Prostate Carotenoids. How closely the patterns and concentrations of carotenoids in the prostate are related to dietary intake is unknown. The overall pattern of carotenoids that we observed in the human prostate generally reflects the carotenoid availability in the American diet (14, 60). However, correlations between estimated dietary intake of specific carotenoids using a food frequency questionnaire and serum concentrations have been reported as 0.11 for lycopene, 0.46 for lutein, and 0.41 for β-carotene (61). In contrast, the serum and tissue relationships may be more closely related. Peng *et al.* (62) reported correlations for paired plasma and skin concentrations of major carotenoids of 0.6 to 0.7. However, as is commonly observed, between-person variation is large. Overall, it appears that tissue concentrations are defined by many interacting variables including seasonal dietary composition (63), food preparation practices, meal composition, factors affecting lipid absorption, lipoprotein metabolism, and poorly understood mechanisms controlling tissue uptake and metabolism (15).

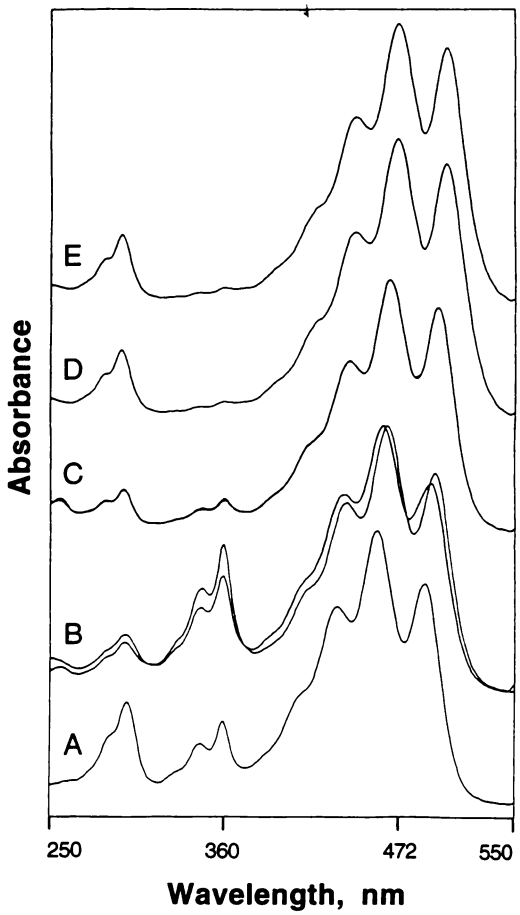

Fig. 5. Representative UV-Visible absorption spectra of geometrical lycopene isomers. The peaks and retention times correspond to the chromatogram of serum in Fig. 4. Spectra were obtained in the C_{30} mobile phase (38:64 methyl-tertiary-butyl ether:methanol) during chromatography and are normalized and superimposed within each trace. *A*, a single peak eluting at 18.5 min, λ_{max} = 459 nm; *B*, two superimposed peaks at 23.5 min, λ_{max} = 465 nm, and 25 min, λ_{max} = 462 nm; *C*, three superimposed peaks at 35 min, λ_{max} = 466nm, 36.5 min, λ_{max} = 466 nm, and 39.5 min, λ_{max} = 466nm; *D*, the single peak at 68 min, λ_{max} = 472nm; and *E*, the peak at 70 min, λ_{max} = 472 nm. Peak D is the spectra of all-*trans* lycopene, which was identified by cochromatography. The six different peaks contained in A-C have increased absorbance in the 360-nm region, and the wavelengths of maximum absorbance are shifted (relative to the all-*trans* isomer in D), indicating the presence of *cis* bonds. The spectra of D and E are indistinguishable, although it should be noted that these peaks are not pure since they were only partially resolved during chromatography. The isomer that gave trace E probably possesses a peripheral (*e.g.*, 5-*cis*) *cis* bond (see "Results").

Geometric Lycopene Isomers in Serum and Prostate Tissue. The chemical structure of β-carotene, lycopene, and most carotenoids suggests that they can theoretically occur in a large variety of geometrical configurations. Isomerization *in vivo* may be the result of interconversion by light, heat, or chemical reactions including the quenching of singlet oxygen, and possibly by specific enzymes (48, 64). However, little is known about the formation, distribution, and biological relevance of the *cis* isomers of carotenoids in human tissues. A few *cis* isomers of β-carotene have been documented *in vivo* including all-*trans*, 9-*cis*, 13-*cis*, and 15-*cis*. The chromatographic techniques used in this study revealed the presence of all-*trans*, 9-*cis*, and a combined 13-*cis* and 15-*cis* peak in human prostate tissue. Previous studies have failed to detect significant concentrations of 9-*cis* β-carotene in serum, even when high concentrations are in the diet, which has been attributed to poor absorption, rapid distribution and uptake by tissues, or isomerase activity processing 9-*cis* to all-*trans* β-carotene by the intestine (65). Although 9-*cis* is not observed in serum, it has been reported in tissues (52). The recent discovery of a geometrical isomer of retinoic acid, 9-*cis* retinoic acid, binding specific receptors to regulate gene expression suggests that β-carotene isomers warrant further investigation. A role for tissue 9-*cis* β-carotene as a precursor of 9-*cis* retinoic acid is supported by recent data suggesting the presence of enzymatic processes mediating this conversion (48). The possibility that isomers of β-carotene, such as 9-*cis*, may be locally converted to specific isomers of retinoic acid in the prostate to exert receptor-specific biological effects within the prostate is under investigation by our laboratory.

Previous reports indicate that lycopene is detected in several geometrical configurations in plasma and tissues (23, 52, 53, 66). In these studies, investigators were able to separate as many as four lycopene peaks, which were tentatively identified by retention times and diode array data as the all-*trans*, 9-*cis*, 13-*cis*, and 15-*cis* isomers (23, 52, 53, 66). In one report, Stahl and Sies (53) observed that processed tomato juice contained a small amount of 9-*cis* lycopene in addition to the predominant all-*trans* configuration, but the serum of humans contained significant amounts of 9-*cis* lycopene, leading to the speculation that there are *in vivo* isomerization mechanisms. Although existing HPLC columns have revealed the presence of a few geometrical isomers of lycopene and the predominance of *cis* lycopenes in blood and certain tissues (52, 66), none of the established techniques has provided adequate separation of these isomers to closely examine their number and relative abundance. As a result, little is known about the patterns and possible physiological or molecular roles of lycopene isomers in biological tissues.

The development of the C_{30} HPLC column for improved reversed phase HPLC separations of carotenoids (50) and the recent demonstration of its excellent selectivity toward geometrical isomers of several common carotenoids (54, 55) has greatly enhanced the ability of investigators to evaluate the pattern of lycopene isomers in foods and biological samples. With the C_{30} column, we were able to separate 12 to 13 geometric lycopene isomers in serum extracts and 15 to 18 isomers in prostate extracts, exposing the complex composition of geometrical lycopene isomers in these samples. This is the first application of the C_{30} column to HPLC separations of lycopene isomers in biological tissues. Results of the present research provide a basis for further studies on the separation and identification of geometrical lycopene isomers in other tissues, as well as future investigations on the metabolism and potential molecular roles of lycopene and its geometric isomers.

The significance of the structural heterogeneity of lycopene in tissues remains speculative. At this time, no specific lycopene receptors or interactions between lycopene and other members of the steroid receptor superfamily have been identified. Furthermore, there is no evidence for stereospecific functions of lycopene or metabolites *in vivo*. The mechanisms whereby the all-*trans* lycopene, which accounts for the vast majority of lycopene in tomatoes, ultimately leads to a progressively increased proportion of *cis* isomers in serum and tissue remain a mystery. Perhaps some of the heterogeneity may occur during cooking due to heat or during digestion prior to absorption. Differential absorption, transport, and uptake of specific stereoisomers can also be hypothesized. The extent to which isomerization may occur *in vivo* due to prolonged exposure to

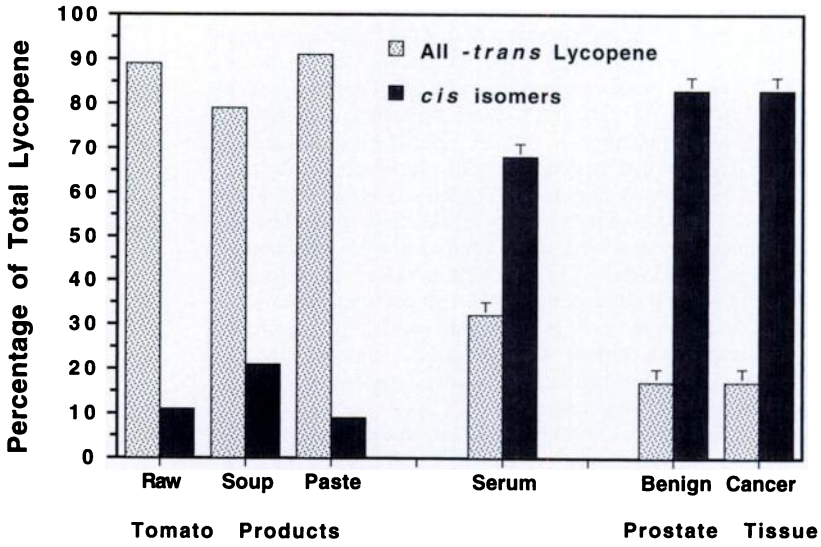

Fig. 6. The proportion of lycopene as pooled *cis* or the all-*trans* isomer in tomato products, human serum, and the prostate tissue. Measurements are based upon the assumption that absorptivities (ϵ) of the lycopene isomers are equal (see "Methods"). *Bars,* SE.

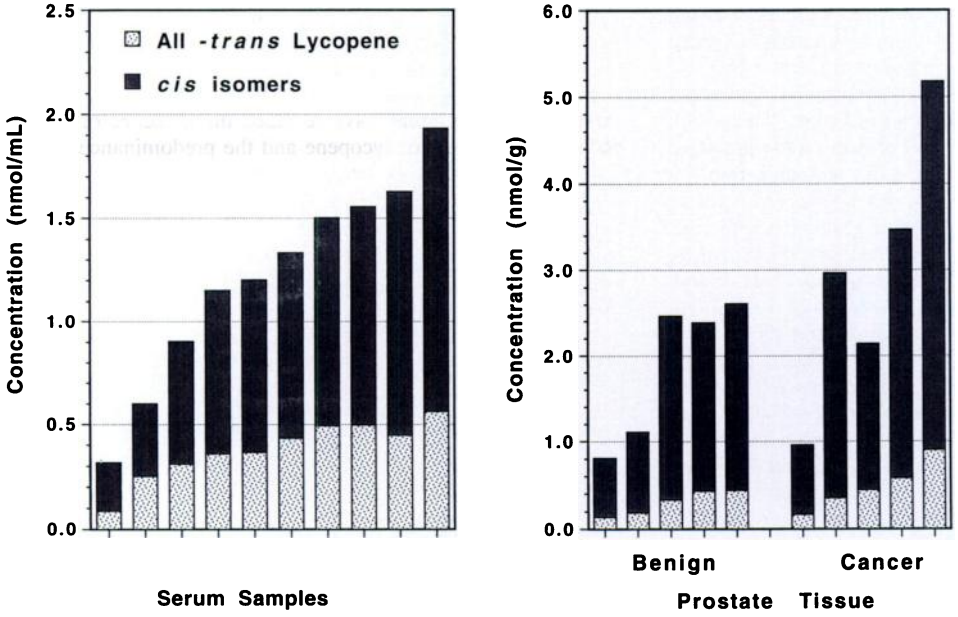

Fig. 7. The concentrations of all-*trans* and pooled *cis* isomers of lycopene in 10 human serum samples and 5 prostatectomy specimens divided into "normal" and malignant samples. Measurements are based upon the assumption that absorptivities (ϵ) of the lycopene isomers are equal (see "Methods").

temperatures of 37°C is unknown (67). Although speculative at this time, perhaps isomer patterns reflect the participation of lycopene in antioxidant reactions (68). Further efforts to elucidate the structures of geometric lycopene isomers and biological functions in the prostate may lead to the development of novel chemopreventive agents.

Alcohol and Prostate Carotenoids. We observed that men reporting current alcohol consumption had reduced prostate concentrations of lycopene and total carotenoids. This association should be viewed with caution since the sample size is limited to 25 men. Furthermore, this data set is too small to allow further evaluation of beverage type, frequency of consumption, or quantity of intake relative to prostate carotenoid content. The relationship of alcohol intake to tissue carotenoids has not been extensively evaluated in previous studies. In one report, liver biopsy specimens showed that those with alcoholic and nonalcoholic liver dysfunction have 4–25-fold lower concentrations of β-carotene than normal subjects (69). The lack of dietary data in our study population limits our ability to differentiate between a metabolic effect of ethanol, reduced fruit and vegetable intake in association with greater alcohol intake, or other unknown mechanisms.

Lycopene and the Etiology of Prostate Cancer. If lycopene is a factor contributing to the reduced prostate cancer risk in men consuming tomato-rich diets, the mechanism is unknown. Lycopene cannot be converted to vitamin A and metabolites, which eliminates a hypothetical mechanism of action. Scavenging reactive oxygen species is one potential biological activity of lycopene that may be relevant to the pathogenesis of prostate cancer (64). Singlet oxygen ($^1O_2$), which is an excited form of ground state (triplet) oxygen (70), is produced by activated immune cells (71) that are involved in prostatitis.

Singlet oxygen can produce mutations in DNA (72–75) and damage lipids (76) and enzymes (77). Other reactive oxygen species are also produced by cells. For example, it is estimated that 10^{12} O_2 molecules are processed by a cell per day, and the leakage of partially reduced oxygen molecules is about 2%, yielding approximately 2×10^{10} O_2^- and H_2O_2 molecules per cell per day (78). Carotenoids, especially lycopene, are the most efficient biological quenchers of $^1O_2$ known (64). In addition to their roles as antioxidants, investigators have proposed that carotenoids may modulate other cellular processes associated with the cancer cascade. These include increased gap junctional intercellular communication (3, 79), reduced mutagenesis (2), inhibition of tumor cell proliferation (4, 5), and improved antitumor immune responses (80). However, evidence is not yet available supporting any of these or other hypotheses relative to lycopene and prostate cancer risk.

In conclusion, the epidemiological observations and laboratory investigations suggest that diets rich in certain carotenoids or vitamin A may modulate prostate function and disease processes. However, our understanding of the potential mechanisms underlying these associations are entirely speculative. Certainly, provitamin A carotenoids may indirectly modulate prostate function via providing retinol to the host. The possibility that provitamin A carotenoids and their isomers, such as 9-*cis* β-carotene, may be cleaved to vitamin A and its isomers within the prostate is an important question for future investigation since this pathway could conceivably provide significant quantities of retinoids directly to cellular receptors. The possibility that carotenoids may have direct effects on the prostate, such as modulating gap junctions and cell communication (3, 79) or act as antioxidants (18, 19, 64), have not been investigated in the prostate. We have no data concerning the ability of dietary assessment tools to predict prostate tissue concentrations of carotenoids. Furthermore, the degree to which blood carotenoid patterns or concentrations reflect those of the prostate tissue is not known. Information concerning diet/serum/tissue relationships is critical for defining hypotheses whereby carotenoids in the diet may modulate prostate cancer. Much is to be learned about mechanisms of carotenoid absorption, deposition in the prostate, local metabolism, mechanisms of removal, factors modulating tissue concentrations, and the value of dietary assessment or blood levels in defining prostate concentrations. Despite the limitations of our knowledge, our data showing the presence of carotenoids in the prostate over a range of concentrations that may have biological consequences support the hypothesis that dietary carotenoids may be related to prostate function and disease processes.

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