

# Tomatoes, Lycopene, and Prostate Cancer: Progress and Promise

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Prostate cancer has emerged as a major public health problem in nations that have an affluent culture with an aging population. The search for etiologic risk factors and an emphasis on the development of chemopreventive agents has gained momentum over the last decade. Among the landmark epidemiologic findings during this period has been the association between the consumption of tomato products and a lower risk of prostate cancer. The traditional reductionist scientific approach has led many investigators to propose that lycopene, a carotenoid consumed largely from tomato products, may be the component responsible for lowering the risk of prostate cancer. Thus, many laboratory and clinical studies are now underway with the goal of assessing the ability of pure lycopene to serve as a chemopreventive agent for prostate and other malignancies. The focus on lycopene should continue, and an improved understanding of lycopene absorption, distribution, role in antioxidant reactions, and metabolism is critical in the quest to elucidate mechanisms whereby this compound could possibly reduce prostate cancer risk. In contrast to the pharmacologic approach with pure lycopene, many nutritional scientists direct their attention upon the diverse array of tomato products as a complex mixture of biologically active phytochemicals that together may have anti-prostate cancer benefits beyond those of any single constituent. These contrasting approaches will continue to be explored in clinical, laboratory and epidemiologic studies in the near future, providing hope that the next generation will benefit from this knowledge and experience a lower risk of prostate cancer. *Exp Biol Med* 227:869–880, 2002

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**P**rostate cancer is the most common visceral malignancy in American men, with over 198,000 new cases per year, and is directly responsible for 31,500 deaths each year (1). The expense of screening programs, diagnos-

tic tests, initial therapies, management of therapeutic complications, and the treatment of metastatic disease add significantly to our national health care budget for aging men. It is important to recognize that many men cured of prostate cancer suffer lifelong incontinence and sexual dysfunction, the two major complications of surgery and radiotherapy for localized disease. It is well documented that more men die "with" prostate cancer than "of" prostate cancer. This phenomenon is expected for a relatively slow-growing cancer, often diagnosed after the sixth decade of life when other illnesses such as cardiovascular disease and diabetes also begin to take their toll. However, many men who die of other causes have suffered significantly with slowly progressive prostate cancer and receive many years of sequential interventions with hormonal therapy, radiation, chemotherapy, and novel biological therapies. Even for the majority of men who do not die of prostate cancer, a significant decrement in the quality of life is common.

The ultimate goal is the elimination of prostate cancer through safe and effective prevention strategies. The key for prevention is to define interventions that will lower risk, but are also convenient and exhibit acceptable toxicity or risk. A male with only average risk of prostate cancer may not be willing to incur side effects of a chemopreventive agent that must be consumed for years or decades and may only partially reduce his risk of prostate cancer, a disease that may only strike in his senior years. For example, finasteride the 5- $\alpha$ -reductase inhibitor, which is currently being tested the Prostate Cancer Prevention Trail (PCPT) of 18,000 men, may prove to lower risk over the 7-year duration of exposure. However, the frequency of sexual dysfunction associated with disruption of androgen metabolism may preclude the widespread use of this or similar drugs unless a man perceives that his risk of prostate cancer is great.

There are several strategies to reduce the risks of interventions while enhancing efficacy. Perhaps one of the most important is the combination of chemopreventive agents, each of which acts through different mechanisms of action,

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and together have noninteractive or nonaccumulating toxicity profiles. Nutritional scientists have an important role to play in the development of chemopreventive agents. For example, many individual phytochemicals, including lycopene from tomatoes, are worthy of consideration as candidate chemopreventive agents and will need extensive pre-clinical development and translation into human Phase I, II, and III studies. Another strategy is to combine promising chemopreventive regimens with nutritional interventions. Traditionally, investigators pursuing chemopreventive strategies have been trained in pharmacology, carcinogenesis, or related fields with little opportunity to interact with nutritional scientists who are focusing on cancer prevention. It is imperative that barriers to interaction be identified so that transdisciplinary projects can be rapidly moved from concept into human trials.

In summary, prostate cancer is an enormous societal and personal burden because of the lives lost and the morbidity of treatments in those cured as well as those suffering from recurrent disease. Furthermore, the costs of screening, diagnosis, localized therapy, and treatment of metastatic disease add significantly to our health care expenditures in an aging population. Research from a variety of fronts, ranging from molecular biology to epidemiology, strongly implicates a role for diet and nutrition in prevention. Tomato products and lycopene, the focus of this review, are among the most provocative lines of evidence recently uncovered (2, 3). Opportunities for nutritional scientists to contribute significantly to the development of effective interventions should be encouraged through increased research activity and interactions with clinical and basic investigators.

## **The Epidemiology of Tomato Products, Lycopene, and Prostate Cancer**

In recent decades, we have seen an accumulated body of evidence strongly supporting the conclusion that diets rich in fruits and vegetables are associated with a lower risk of many malignancies, although an association with prostate cancer has not been as strong in comparison with other malignancies (4, 5). However, epidemiologic studies using diet-assessment tools that have the ability to examine the specific role of tomatoes and tomato products in cancer risk is relatively new, with the vast majority of published reports occurring over the last decade. A detailed review of the epidemiologic data regarding tomato products and cancers of a variety of tissues is included in the review by Giovannucci as part of this symposium. One of the first studies to suggest this relationship was conducted in the late 1970s in the Seventh-day Adventist population (6). A food-frequency questionnaire was used to evaluate the diet of approximately 14,000 men. After 6 years of follow-up, 180 men were diagnosed with prostate cancer. The risk of

prostate cancer was found to be significantly lower in men consuming five or more servings of tomatoes or tomato products per week compared with men who consumed less than one serving of tomatoes or tomato products per week ( $RR = 0.60$ ; 95%  $CI = 0.37-0.97$ ,  $P$  for trend = 0.02). In addition, there was a statistically significant inverse relationship with the consumption of beans, lentils, and peas (6). This study is in agreement with a smaller ( $n = 669$ ) case-control study completed at approximately the same time that found a lower risk of prostate cancer in men consuming tomatoes and/or tomato products  $\geq 14$  times per month compared with those consuming less than three servings per month ( $OR = 1.41$  for  $<3$  servings/month vs  $\geq 14$  servings/month, 95%  $CI$  not reported; 7). Conversely, in 1991, Le Marchand *et al.* (8) published a case-control study of diet and prostate cancer in a multiethnic Hawaiian cohort. They found no association between raw tomato consumption or estimated lycopene intake and prostate cancer risk. It is not clear, however, whether fresh and processed tomato products were included in the analysis.

The study providing the strongest evidence thus far concerning tomatoes and prostate cancer prevention was published in 1995 (9). The dietary habits of over 47,000 men enrolled in the Health Professionals Follow-Up Study (HPFS) initially established in 1986 were examined. Dietary intake was estimated using a 131-item food-frequency questionnaire that was completed twice during the initial 6-year follow-up period. There were 773 cases of prostate cancer (nonstage A1) diagnosed during the follow-up period (1986-1992). The only fruits or vegetables found to be associated with a reduced risk of prostate cancer were raw tomatoes ( $RR = 0.74$  for zero servings vs 2-4 servings/week, 95%  $CI = 0.58-0.93$ ,  $P$  for trend = 0.03), tomato sauce ( $RR = 0.66$  for zero servings vs 2-4 servings/week, 95%  $CI = 0.49-0.90$ ,  $P$  for trend = 0.001), pizza ( $RR = 0.85$  for zero servings vs 2-4 servings/week, 95%  $CI = 0.57-1.10$ ,  $P$  for trend = 0.05) and strawberries ( $RR = 0.80$  for zero servings vs 1 serving/week, 95%  $CI = 0.57-1.10$ ,  $P$  for trend = 0.005; 9). In men who had more advanced prostate cancer (defined as either stage C or D), consuming 10 servings of tomato products per week compared with less than 1.5 servings per week was significantly protective ( $RR = 0.47$ , 95%  $CI = 0.22-1.00$ ,  $P$  for trend = 0.03). The data derived from this study are considered the most powerful linkage between tomato products and a lower risk of prostate cancer because of the large size of the cohort and the prospective collection of dietary data with a validated assessment tool.

Four recent case-control studies evaluating the link between tomatoes and prostate cancer incidence have been published with only one demonstrating a protective effect of tomato products. A study, conducted in Greece, compared the dietary habits of 320 men with prostate cancer to 246 controls and found that consumption of cooked tomatoes was inversely associated with prostate cancer risk ( $P =$

0.005; 10). The intake of raw tomatoes alone was not significantly protective ( $P = 0.12$ ). From their data, the authors concluded that increasing cooked tomato intake from two times per week to eight times per week reduced the risk of prostate cancer by 15% (OR = 0.85, 95% CI = 0.75–0.97). In three other case-control studies, no relationship was found between raw tomatoes or cooked tomatoes and prostate cancer risk; however, two of the studies noted a significant protective effect of cruciferous vegetables (11–13). Cohen and colleagues (11) completed a nested case-control study in King County Washington with 628 patients and 602 control patients. Food-frequency questionnaires were completed and total fruit and vegetable intake was summarized. There were no protective effects of raw tomatoes (<1 serving/week vs  $\geq 3$  serving/week, OR = 1.22, 95% CI = 0.83–1.80,  $P$  for trend = 0.26), cooked tomatoes (<1 serving/week vs  $\geq 3$  servings/week, OR = 0.90, 95% CI = 0.57–1.42,  $P$  for trend = 0.68), or estimated lycopene intake (<4900  $\mu\text{g/day}$  vs  $\geq 9900$   $\mu\text{g/day}$ , OR = 0.89, 95% CI = 0.60–1.31,  $P$  for trend = 0.96). However, both total vegetable intake (<14 servings/week vs  $\geq 28$  servings/week, OR = 0.65, 95% CI = 0.45–0.94,  $P$  for trend = 0.01) and cruciferous vegetable intake (<1 serving/week vs  $\geq 3$  servings/week, OR = 0.59, 95% CI = 0.39–0.90,  $P$  for trend = 0.02) were significantly protective.

Although tomatoes and tomato products have many nutrients and phytochemicals that are proposed to inhibit carcinogenesis, lycopene has received the most intense focus. Giovannucci *et al.* (9) estimated lycopene intake in the HPFS cohort using the USDA Carotenoid Database. The estimated dietary intake of  $\beta$ -carotene,  $\alpha$ -carotene, lutein, and  $\beta$ -cryptoxanthin was not related to prostate cancer risk. However, dietary intake of lycopene (80% of which was derived from tomatoes and tomato products) was inversely related to risk when the highest quartile (>6.4 mg lycopene/day) was compared with the lowest quartile (<2.3 mg lycopene/day, RR = 0.79, 95% CI = 0.64–0.99,  $P$  for trend = 0.04). A few years later, a case-control study of 797 men in New Zealand found a weak, nonsignificant trend between lycopene intake and prostate cancer incidence when comparing the lowest quartile (<663  $\mu\text{g/day}$ ) of lycopene intake to the highest quartile (>1994  $\mu\text{g/day}$ , OR = 0.76, 95% CI = 0.53–1.26,  $P$  for trend = 0.30). Additionally, there was no association between dietary intake of raw tomatoes and prostate cancer (<13 g/day vs >35 g/day, OR = 1.01, 95% CI = 0.66–1.63,  $P$  for trend = 0.93) and only a weak, nonsignificant trend between processed tomato products and prostate cancer risk (<18.7 g/day vs >64.2 g/day, OR = 0.83, 95% CI = 0.53–1.26,  $P$  for trend = 0.30; 14). Interestingly, in this study the estimated median intake of lycopene was less than half of the median in the HPFS cohort (1.2 mg lycopene/day vs 3.4 to 4.6 mg lycopene/day, respectively).

The dietary intake of lycopene is difficult to precisely quantify for several reasons, thus reducing the sensitivity of an epidemiologic study to detect relationships with cancer

risk. Food diaries and food-frequency questionnaires provide an estimate of lycopene-containing foods consumed. The USDA database, or others, can then be applied to obtain an estimate of lycopene consumed. However, foods do not contain constant concentrations of lycopene. For example, the content of tomato sauce varies significantly between brands. Thus, it is proposed that the measurement of lycopene concentration in blood may provide a useful link between dietary lycopene intake and risk assessment in epidemiologic studies. Interestingly, serum lycopene is not strongly correlated with estimated dietary intake of lycopene with correlation estimates that range from 0.16 to 0.47 (15–17). Inaccurate estimation of dietary intake, variation in bioavailability among food sources of lycopene, inconsistency in absorption as a result of diet composition, and changes in uptake that may be related to age and genetics will contribute to the low correlations between estimated intake and blood concentrations. Two of three case-control studies have found weak, nonsignificant, inverse relationships between serum lycopene levels and risk of prostate cancer. One study suggested an inverse association between serum lycopene levels and aggressive prostate cancer (18–20). A nested case-control investigation was undertaken and involved the analysis of carotenoids in blood samples from men enrolled in the Physicians' Health Study, a randomized, placebo-controlled trial of aspirin and  $\beta$ -carotene. In this study, men in the highest quintile (>580.1 ng/ml) of serum lycopene levels had a significantly lower risk of prostate cancer compared with men in the lowest quintile of serum lycopene ( $\leq 261.7$  ng/ml, OR = 0.56, 95% CI = 0.34–0.92,  $P = 0.05$ ). The inverse association between serum lycopene and aggressive prostate cancer was particularly significant for men who were not consuming  $\beta$ -carotene supplements (OR for highest quintile versus lowest quintile = 0.40, 95% CI = 0.19–0.84,  $P$  for trend = 0.006; 18). Shortcomings of these and similar case-control studies evaluating serum carotenoids and cancer risk frequently include small sample size, possible lycopene degradation in samples because of factors such as exposure to light or long periods of storage before analysis, and the unknown ability of a single blood sample to represent lycopene concentrations over a longer time span. It is clear that more studies are necessary to draw any conclusions regarding serum lycopene and prostate cancer risk.

Mechanisms by which tomatoes and tomato products may reduce prostate cancer risk have also been investigated in an epidemiologic context. One focus of investigation is the relationship between diet and insulin-like growth factors and binding proteins (21). In a nested case-control study in Greece, Mucci and colleagues (22) collected sera and administered a food-frequency questionnaire to 112 cancer-free men. Consumption of cooked tomatoes was found to be significantly inversely associated with insulin-like growth factor-1 levels with a mean change of  $-31.5\%$  for each one serving increase per day. Blood lycopene concentrations were not quantitated in this study but would have been a

valuable component of the investigation.

## Clinical Studies

There are few human intervention studies investigating the role of tomatoes and/or lycopene on processes that are related to the development of prostate cancer. The most provocative observations have recently been published by Kucuk and colleagues (23) with additional details presented at this symposium and reviewed in this journal. The study involved 26 men diagnosed with presumed localized prostate cancer who were scheduled to undergo a radical prostatectomy. The men were randomized to consume 30 mg of lycopene per day from two tomato oleoresin capsules (Lyc-O-Mato; LycoRed Natural Products Industries, Beer-Sheva, Israel) or to continue their normal diet for 3 weeks before surgery. Post-surgical prostate tissue specimens were then compared between the two groups. Men consuming the lycopene supplement had 47% higher prostatic tissue lycopene levels than the control group ( $0.53 \pm 0.03$  ng/g vs  $0.36 \pm 0.06$  ng/g,  $P = 0.02$ ); however, plasma lycopene levels were not significantly different between the groups nor did they change significantly within each group. Men who consumed the lycopene supplement were less likely to have involvement of surgical margins (73% vs 18% of subjects,  $P = 0.02$ ). Additionally, they were less frequently found to have high-grade prostatic intraepithelial neoplasia (HGPIN) in the prostatectomy specimen (67% vs 100%,  $P = 0.05$ ). HGPIN is considered to be a premalignant lesion predisposing a man to prostate cancer. Additionally, the intervention group was found to have smaller tumors, a greater reduction in prostate-specific antigen (PSA) over the 3-week study period, and a higher expression of connexin 43; however, none of these differences were statistically significant.

Readers should use extreme caution in the interpretation of case reports. A recent example describes a 62-year-old man with hormone refractory prostate cancer who failed multiple treatment regimens, including leuprolide, bicalutamide, ketoconazole and hydrocortisone, doxorubicin, vinorelbine, and prednisone (24). His PSA had increased to 365 ng/ml when he began taking 10 mg of lycopene per day and 300 mg of saw palmetto three times each day. One month after beginning these dietary supplements, his PSA reportedly decreased to 139 ng/dl and remained between 3 and 8 ng/ml for at least 18 months. The authors attributed this dramatic improvement to the lycopene supplements rather than the saw palmetto (24). There are some reports, however, that saw palmetto can influence the prostate and exhibit effects similar to finasteride (a 5- $\alpha$ -reductase inhibitor) so it is difficult to discount the possible effects of saw palmetto (25, 26). Additionally, the source of lycopene was not given and lycopene content and stability within a supplement can vary widely. Although intriguing, this case report should be viewed with great skepticism.

**Tomatoes, Lycopene, and Experimental Prostate Cancer.** Several laboratories are conducting rodent studies of prostate carcinogenesis. A recently published investigation using the DMAB and PhIP-induced rat prostate cancer models failed to detect a chemopreventive effect of lycopene provided as an extract of 99.9% purity from LycoRed Ltd (27). Our laboratory has recently completed a series of studies with a lycopene oleoresin in mice bearing human xenografts of PC-3, DU145, and LNCaP human cell lines and observed no major anti-tumor effects (preliminary data). Our group has also completed a large rat study evaluating the ability of lycopene or freeze-dried tomato powder to inhibit survival in the *N*-nitrosomethylurea-androgen-induced prostate cancer model. In this system, we observed a very small beneficial trend for lycopene and a significant benefit of tomato powder (preliminary data). Thus far it appears that tomatoes may contain components in addition to lycopene that may inhibit prostate tumorigenesis.

## Lycopene and the Carotenoid Family

Lycopene and other carotenoids are natural pigments synthesized by plants and microorganisms. The most-established natural roles of carotenoids are to protect cells against photosensitization and to serve as light-absorbing pigments during photosynthesis (28). Some dietary carotenoids, such as  $\beta$ -carotene, provide an important source of vitamin A; however, the majority of carotenoids, including lycopene, do not exhibit provitamin A activity. Lycopene is a carotenoid present in high concentrations in tomatoes and tomato products and is responsible for the characteristic red color of these foods. The recent associations between tomato products, lycopene, and disease risk have stimulated a greater effort to understand these relationships through cell culture and animal studies, as well as human metabolic studies (14, 18, 29–34).

## Lycopene Chemistry

More than 600 carotenoids have been characterized and share common structural features, such as the polyisoprenoid structure and a series of centrally located conjugated double bonds (35, 36). The color and photochemical properties of each carotenoid are determined by its structure (36). In addition, the structure also contributes to the chemical reactivity of carotenoids toward free radicals and oxidizing agents, which may be relevant to *in vivo* biological functions in animals (36). Lycopene is a forty carbon ( $C_{40}H_{56}$ ) acyclic carotenoid with 11 linearly arranged conjugated double bonds. Lycopene lacks the  $\beta$ -ionone ring structure and is therefore devoid of provitamin A activity. Because of the highly conjugated nature of lycopene, it is particularly subject to oxidative degradation and isomerization. Chemical and physical factors known to degrade other carotenoids, including exposure to light, oxygen, elevated temperature, extremes in pH, and active surfaces, apply to lycopene as well (37–41).

As a polyene, lycopene readily undergoes a *cis-trans*

isomerization. As a result of the 11 conjugated carbon-carbon double bonds in its backbone, lycopene can theoretically be arranged in 2048 different geometrical configurations. Although a large number of geometrical isomers are theoretically possible for all-*trans* lycopene, Pauling and Zechmeister *et al.* (42, 43) have found that only certain ethylenic groups of a lycopene molecule can participate in *cis-trans* isomerization because of steric hindrance. Interconversion of isomers is thought to take place with exposure to thermoenergy, absorption of light, or by involvement in specific chemical reactions. *Cis* isomers of lycopene have chemical and physical characteristics distinctly different from their all-*trans* counterparts. Some of the differences observed as a result of a *trans*-to-*cis* isomerization reaction include lower melting points, decreased color intensity, a shift in the lambda max, smaller extinction coefficients, and the appearance of a new maximum in the ultraviolet spectrum (44). To avoid underestimating the quantitative measurement of lycopene *cis*-isomers, the appropriate wavelength maximum and extinction coefficient should be applied. Because of the difficulty in identifying individual *cis* forms, quantitative data for isomer content of biological samples are generally estimated values.

### Analytic Advances and Isomer Characterization

High performance liquid chromatography (HPLC) is the most commonly used method for the separation, quantitation, and identification of carotenoids found in plasma and biological tissues. Additional information regarding the chemistry, distribution, and metabolism of lycopene can be found in the symposium proceedings by Khachik and colleagues. Similarities in the structural characteristics of carotenoids causes difficulty when trying to adequately identify individual carotenoids using only fixed wavelength or retention time data. The use of photodiode array detection, allowing for the collection of spectral data across a wide range of wavelengths, has improved our ability to more accurately characterize individual carotenoids. However, measurements of retention time, peak resolution, and spectral data for individual absorbing species, in addition to the use of authentic standards for comparison of UV/VIS spectra and retention times, are required (45). Mass spectrometric and tandem mass spectrometric analyses, which provide molecular weight and characteristic fragmentation patterns, provide additional information that increases our confidence in the identification of various carotenoids (45). In addition, both electron impact and fast atom bombardment have been used in mass spectrometric analysis of carotenoids (46–48).

Lycopene is generally separated from other carotenoids using HPLC with reversed-phase C<sub>18</sub> columns. Variations in the properties of the silica packing material in terms of carbon load, particle size, porosity, end-capping technique, and polymerization can significantly alter the selectivity and sensitivity of lycopene analysis (49–52). Our ability to detect low levels of carotenoids in biological samples has been somewhat limited by methodology and detection that does

not adequately quantify carotenoid concentrations. The recent development of a C<sub>30</sub> reversed-phase gradient HPLC method coupled with a coulometric electrochemical (EC) array detector provides a much lower detection limit and a unique opportunity to quantify low levels of carotenoids in tissue samples and in the plasma chylomicron fraction (53). The improved sensitivity of this HPLC-EC method (1–10 fmol) allows for a reduced sample volume at each blood collection while comparing bioavailable carotenoids as a function of dietary fat level and postingestion time.

Compared with conventional C<sub>18</sub> reversed-phase and silica normal-phase columns, reversed-phase C<sub>30</sub> columns are frequently used to achieve superior selectivity of lycopene isomers (52, 54). The polymerically synthesized C<sub>30</sub> columns not only provide excellent separation of the all-*trans* lycopene isomers from the *cis* counterpart but they also display extraordinary selectivity among the individual *cis*-isomers (54, 55). A recent HPLC method using multiple columns in series has also been shown to similarly resolve *cis* and *trans* lycopene isomers (56). Identification and structure elucidation of isomeric carotenoids have been facilitated with the aid of high-resolution NMR spectroscopy. Hengartner *et al.* (57) reported the use of H- and C-NMR, UV/VIS, mass, and IR spectroscopy to fully characterize 15 (E/Z)-isomeric forms of lycopene.

The rapid improvement in analytic technology will significantly impact future investigations designed to elucidate the biological impact of lycopene and its isomers on tissues and organs. Investigators ranging from epidemiologists, clinical scientists, and those involved in rodent studies will be able to more precisely quantitate lycopene isomers in very small biological samples.

### Lycopene Profiles in Tomato Products

The presence of lycopene in human plasma and tissues primarily results from consumption of a variety of tomato products, such as tomatoes, spaghetti sauce, salsa, tomato soup, and ketchup (Table I). It is estimated that greater than 80% of lycopene consumed in the United States is derived from tomato products, although apricots, guava, watermelon, papaya, and pink grapefruit also provide a dietary source of lycopene (58–60). The lycopene content of tomatoes can vary considerably with variety and ripening stage of tomatoes. Lycopene concentrations in the red strains approach 50 mg/kg compared with only 5 mg/kg in yellow varieties (58). With very few exceptions, lycopene from natural plant sources exists primarily in the all-*trans* form, the most thermodynamically stable configuration (43, 61, 62).

### Food Processing and Lycopene Profiles

Consumers use the intensity of the red color as an index of quality for tomato products. Therefore, reducing the loss of lycopene throughout the production process and during storage has always been an important issue for food processors. Exposure to thermal treatments during food-

**Table I. Common Food Sources of Lycopene**

| Food            | Type                     | Amount per serving<br>(mg/100 g wet wt.) | (mg)       | Serving size    |
|-----------------|--------------------------|------------------------------------------|------------|-----------------|
| Apricots        | Fresh                    | 0.005 <sup>a</sup>                       | 0.007      | 140 g           |
| Apricots        | Canned, drained          | 0.065 <sup>a</sup>                       | 0.091      | 140 g           |
| Apricots        | Dried                    | 0.86 <sup>a</sup>                        | 0.34       | 40 g            |
| Chili           | Processed                | 1.08–2.62 <sup>a</sup>                   | 1.40–3.41  | 130 g           |
| Grapefruit      | Pink, fresh              | 3.36 <sup>a</sup>                        | 4.70       | 140 g           |
| Guava           | Pink, fresh              | 5.40 <sup>a</sup>                        | 7.56       | 140 g           |
| Guava juice     | Pink, processed          | 3.34 <sup>a</sup>                        | 8.35       | 240 mL (~250 g) |
| Ketchup         | Processed                | 16.60 <sup>a</sup>                       | 3.32       | 1 tbsp (~20 g)  |
| Papaya          | Red, fresh               | 2.00–5.30 <sup>b</sup>                   | 2.8–7.42   | 140 g           |
| Pizza sauce     | Canned                   | 12.71 <sup>a</sup>                       | 15.89      | 125 g           |
| Pizza sauce     | From pizza               | 32.89 <sup>c</sup>                       | 9.867      | slice (~30 g)   |
| Rosehip puree   | Canned                   | 0.78 <sup>a</sup>                        | 0.47       | 60 g            |
| Salsa           | Processed                | 9.28 <sup>d</sup>                        | 3.71       | 2 tbsp (~40 g)  |
| Spaghetti sauce | Processed                | 17.50 <sup>d</sup>                       | 21.88      | 125 g           |
| Tomatoes        | Red, fresh               | 3.1–7.74 <sup>c</sup>                    | 4.03–10.06 | 130 g           |
| Tomatoes        | Whole, peeled, processed | 11.21 <sup>c</sup>                       | 14.01      | 125 g           |
| Tomato juice    | Processed                | 7.83 <sup>c</sup>                        | 19.58      | 240 mL (~250 g) |
| Tomato soup     | Canned, condensed        | 3.99 <sup>c</sup>                        | 9.77       | 245 g           |
| Tomato paste    | Canned                   | 30.07 <sup>c</sup>                       | 9.02       | 30 g            |
| Watermelon      | Red, fresh               | 4.10 <sup>a</sup>                        | 11.48      | 280 g           |
| Vegetable juice | Processed                | 7.28 <sup>c</sup>                        | 17.47      | 240 mL (~250 g) |

<sup>a</sup> USDA. 1998. USDA-NCI Carotenoid Database for U.S. Foods. Nutrient Data Lab., Agric. Res. Service, U.S. Dept. of Agriculture, Beltsville Human Nutrition Research Center, Riverdale, MD.

<sup>b</sup> From Mangels *et al.* (59).

<sup>c</sup> Nguyen ML and Schwartz SJ. (69).

<sup>d</sup> Nguyen ML and Schwartz SJ. (109).

processing operations causes well-documented changes in the physiochemical stability of carotenoids. Boskovic and Cano *et al.* (63, 64) observed that processing and extended storage of dehydrated tomato products resulted in a loss of *all-trans* lycopene content by up to 20%. Food-processing techniques, such as canning and freezing, led to a significant reduction in lycopene and total carotenoid content of papaya slices. In contrast, many studies have found that hydrocarbon carotenoids such as lycopene,  $\alpha$ -carotene, and  $\beta$ -carotene in processed fruits and vegetables are fairly heat resistant (65, 66). According to Khachik *et al.* (65), most of these carotenoids remain stable after bench-top food preparation. Saini and Singh (67) also reported that thermal processing had no effect on the lycopene content in juices made from several high-yield tomato hybrids. Zanori *et al.* (68) recently reported that despite the oxidative and thermal severity of the drying process, reflected in the 5-hydroxymethyl-2-furfural and ascorbic acid values, lycopene displayed high stability during drying of tomato halves. Additionally, Nguyen and Schwartz (69) recently reported that processing does not have a significant effect on the stability of lycopene, independent of product type, moisture content, container type, tomato variety, and severity of heat treatments.

Although lycopene may be fairly stable during standard food processing procedures, less is known about its impact on isomerization. Studies have shown that heating tomato juice and bench-top preparation of a spaghetti sauce from canned tomatoes increases *cis*-isomer concentrations (56,

70). In contrast, Khachik *et al.* (66) observed that common heat treatments during food preparation, such as microwaving, steaming, boiling, and stewing, did not significantly change the distribution of carotenoids in tomatoes and green vegetables. Other studies have also reported low levels of lycopene *cis* isomers in thermally processed tomato products (69, 71). Recently, Nguyen and *et al.* (72) reported that during typical cooking of tomatoes, factors such as genotypic differences in overall carotenoid composition, the presence of oil, and physical changes to tomato tissues did not influence the thermal isomerization of *all-trans* lycopene, *all-trans*  $\delta$ -carotene, *all-trans*  $\gamma$ -carotene, or prolycopene. Additional information needs to be gathered on the thermal behavior of lycopene before definitive answers can be offered regarding its physical state and stability during processing and cooking. Nevertheless, it is evident that lycopene is more stable in native tomato fruit matrices than in isolated or purified form due to the protective effects of cellular constituents such as water (73).

### Bioavailability of Lycopene

Differences in bioavailability of lycopene may account, in part, for the relatively poor correlations between blood lycopene concentrations and estimated dietary intake. Carotenoids are strongly bound to intracellular macromolecules in many foods, and absorption therefore may be limited unless released from the food matrix (74). Heating tomato juice was shown to improve the uptake of lycopene in humans (70). Gartner *et al.* reported that lycopene bioavail-

ability from tomato paste, a processed product, was higher than from fresh tomatoes when both were consumed with corn oil (75). These observations seem to be the result of thermal weakening and disruption of lycopene-protein complexes, rupturing of cell walls, and/or dispersion of crystalline carotenoid aggregates. Likewise, various food-processing operations such as chopping and pureeing, which result in a reduction in physical size of the food particle, will also enhance lycopene bioavailability (76, 77). Lycopene bioavailability was recently studied after a single dose of fresh tomatoes or tomato paste by measuring carotenoid concentrations in the chylomicron fraction of the systemic circulation (75). Each source of lycopene (23 mg) was consumed with 15 g of corn oil. Tomato paste was found to yield a 2.5-fold greater total *all-trans* lycopene peak concentration and a 3.8-fold greater area under the curve than fresh tomatoes. When compared with fresh tomatoes, ingestion of tomato paste resulted in a significantly higher area under the curve for *cis* lycopene isomers. Recent data in our laboratory from a pilot clinical trial of lactating women showed greater concentration of lycopene in human milk for those consuming tomato sauces compared to fresh tomatoes (78). These observations support the conclusion that food processing and cooking enhances lycopene bioavailability.

Digestive processes will certainly influence lycopene bioavailability. Several factors affect initial carotenoid release from the physical food matrix and transfer and distribution into lipid droplets within the stomach and proximal duodenum (79). Perhaps of major importance, dietary lipids may serve a critical role in dissolution and subsequent absorption of a very hydrophobic carotenoid such as lycopene. Pancreatic lipases and bile salts act upon the carotenoid-containing lipid droplets entering the duodenum and form multilamellar lipid vesicles containing the carotenoids (80). The transfer of lycopene, like other carotenoids, from the micelle into the mucosal cells appears to occur via passive diffusion (81, 82). Factors such as the structural features of the carotenoid, the dietary fat content, fatty acid patterns, fiber, and other food components may influence the carotenoid content of micelles and subsequent mucosal transfer (80).

Chylomicrons are responsible for carrying carotenoids from the intestinal mucosa to the blood stream via the lymphatics (80). Little is known about how lycopene in chylomicrons is subsequently accumulated by the liver and other tissues, repackaged in lipoproteins, and returned to the circulation. Lycopene is carried in the plasma entirely by lipoproteins, and no other lycopene-specific binding or carrier proteins have been identified thus far (80, 83). Details of how hepatocytes, the initial source of circulating lipoproteins, transfer lycopene into specific secreted lipoproteins and how this process may be regulated is unclear. However, it is likely that dietary and pharmacologic agents that influence lipoprotein metabolism will influence circulating lycopene concentrations. The physical properties based on the carotenoid structure appear to add to the vary-

ing distribution of specific carotenoids among lipoprotein classes. It is hypothesized that very lipophilic carotenoids, such as lycopene, are present within the hydrophobic core of the lipoprotein particle.

## Metabolism and Geometrical Isomerization of Lycopene

Several studies have examined changes in serum lycopene concentrations that take place following variations in dietary intake. Elimination or restriction of dietary sources of lycopene causes a steady decline in plasma lycopene content that can be detected within days. In studies of healthy individuals consuming a low-carotenoid diet, the plasma depletion half-life of lycopene was estimated to be between 12 and 33 days (84). Others report a plasma half-life of approximately as little as 2–3 days compared to approximately 2 weeks (29, 85). Our laboratory has shown that healthy individuals consuming a lycopene free diet exhibit a decrease in total serum lycopene of 49% in 14 days (86). In general, we conclude that blood lycopene concentrations can change significantly in a period of days if dietary intake is altered. Thus, epidemiologic studies of blood lycopene provide an indicator of recent consumption, but questions remain regarding the utility of single blood samples to reflect long-term dietary intake.

It is now well known that between 10 and 20 *cis* isomer peaks are typically observed in human blood and together account for the majority of lycopene in serum (2, 71). Interestingly, we observed that the ratio of *cis:trans* isomers changes in those on a lycopene free diet (86). Plasma isomer concentrations exhibit a 61:39 ratio for *cis:all-trans* at the start of a lycopene-free diet whereas after 2 weeks, the ratio shifts to 70:30, which was highly significant. We have confirmed the shift in isomer ratio in a subsequent study over seven days (87). These studies suggest that the all *trans* lycopene content of serum is maintained through continuous dietary intake and that mobilization of *all-trans* lycopene from liver or other tissues, or reconversion of *cis* isomers to *trans* cannot maintain the *cis:trans* ratio. In addition, it is plausible to hypothesize that there is a biological preference for certain lycopene isomers to be cleared from serum, distributed to tissues, or participate in reactions that cause degradation.

Little is known about the metabolism or degradation of lycopene in mammals (29, 30). Few metabolites of lycopene have been identified in human tissues or plasma. For example, 5,6-dihydroxy-5,6-dihydro-lycopene has been detected by Khachik and colleagues (88). It is hypothesized that this compound may be a product of an *in vivo* oxidation reaction via a transitional lycopene epoxide. Much more information regarding lycopene metabolism is required to understand the role of lycopene in prostate cancer. How lycopene is metabolized and which tissues are able to participate in this process remain to be elucidated.

Two recent studies in rodents suggest that androgens, such as testosterone, can influence lycopene metabolism



(89, 90). Relative to prostate cancer, this could suggest an important dietary:hormonal interaction. Castrated rats were found to accumulate more than twice the amount of liver lycopene than intact males, with no effect on other tissues (89). Furthermore, an increase in *cis* isomer content of liver tissue was observed with castration (89). In a subsequent study, the administration of testosterone was found to reverse the effects of castration (90). These studies suggest that androgens may stimulate lycopene metabolism and degradation.

### Prostate Lycopene

Although data are still limited, it is apparent that carotenoids are not uniformly and equally dispersed in human tissues (29, 70, 91–93). The tissue-specific carotenoid patterns reported thus far suggest a process whereby certain carotenoids may exert unique biologic effects in one tissue but not in another (Table II). Thus far, there is no evidence for a specific receptor or enzymatic process that mediates lycopene uptake by the prostate or cells in any tissue. We therefore must assume that uptake in the prostate is related to lipoprotein metabolism. However, very little is known about lipoprotein uptake by benign and malignant prostate cells. The metabolism of the prostate is certainly regulated by the neuroendocrine axis and thus it is reasonable to postulate that these factors may also influence energy metabolism in a fashion that alters lipoprotein uptake.

In a study comparing the major carotenoid levels in normal versus malignant human prostate tissue, Clinton *et al.*

*al.* (71) reported that lycopene and other major carotenoids were present in higher concentration in the malignant prostate tissue than in normal prostate tissue. This may at first seem inconsistent with a protective effect of lycopene. However, if uptake is fairly nonspecific and related to blood flow and lipoprotein metabolism then a greater uptake by the metabolically more active and blood vessel rich cancer would be a logical explanation.

Lycopene has been shown to exist in over 15 different geometrical configurations in human prostate tissue, where the *cis* isomer content is even greater, at 80 to 90%, than observed in serum (71). The chemical and physiologic processes that account for the high proportion of *cis* isomers in tissue remains speculative. An intriguing hypothesis is that isomerization reflects the participation of lycopene in antioxidant reactions within the prostate. If so, the isomer pattern may provide a “chemical footprint” of oxidative stress in the prostate. Furthermore, isomerization changes the structure of lycopene in a fashion that could alter the intracellular distribution of lycopene within organelles and membrane structures that in turn could influence biological processes related to prostate carcinogenesis. These are hypotheses that will need additional investigation.

### Antioxidant and Biological Effects of Lycopene

Mammals have developed multiple defenses against reactive oxygen, some of which are genetically programmed,

**Table II.** Lycopene Concentrations in Human Tissue Using HPLC Technology as Reported in Several Publications

| Tissue   | Concentration<br>(nmol/g wet wt) |                            |                             |                          |                                                                                                   |
|----------|----------------------------------|----------------------------|-----------------------------|--------------------------|---------------------------------------------------------------------------------------------------|
|          | Kaplan <i>et al.</i> (91)        | Schmitz <i>et al.</i> (92) | Nierenberg and<br>Nann (93) | Stahl <i>et al.</i> (70) | Others                                                                                            |
| Adipose  | 1.30                             |                            |                             | 0.20                     |                                                                                                   |
| Adrenal  | 21.60                            |                            |                             | 1.90                     |                                                                                                   |
| Brain    |                                  |                            |                             |                          | 2.55 <sup>a</sup>                                                                                 |
| Breast   |                                  |                            | 0.78                        |                          | 0.43 <sup>b</sup>                                                                                 |
| Cervix   |                                  |                            |                             |                          | 0.18 <sup>b</sup>                                                                                 |
| Colon    |                                  |                            | 0.31                        |                          |                                                                                                   |
| Kidney   | 0.39                             | 0.62                       |                             | 0.15                     |                                                                                                   |
| Liver    | 2.45                             | 5.72                       |                             | 1.28                     | 0.65 <sup>b</sup>                                                                                 |
| Lung     |                                  | 0.57                       | 0.22                        |                          | 0.56 <sup>b</sup>                                                                                 |
| Ovary    | 0.28                             |                            |                             | 0.25                     |                                                                                                   |
| Prostate |                                  |                            |                             |                          | 0.12 <sup>c</sup> , 0.24 <sup>d</sup> , 0.36 <sup>e</sup> , 0.53 <sup>c</sup> , 0.63 <sup>f</sup> |
| Skin     |                                  |                            | 0.42                        |                          |                                                                                                   |
| Stomach  |                                  |                            |                             |                          | 0.20 <sup>g</sup>                                                                                 |
| Testes   | 21.36                            |                            |                             | 4.34                     |                                                                                                   |

<sup>a</sup> Craft NE *et al.* (110).

<sup>b</sup> Khachik F *et al.* (111).

<sup>c</sup> Rao AV *et al.* (112).

<sup>d</sup> Freeman, *et al.* (15).

<sup>e</sup> Kucuk, *et al.* (23).

<sup>f</sup> From Clinton *et al.* (71).

<sup>g</sup> From Clinton (1).



such as the enzymes glutathione peroxidase and superoxide dismutase, and others that are derived from nutritional substances such as vitamin E, vitamin C, selenium, and perhaps carotenoids (94). The ability of lycopene to act as an antioxidant and scavenger of free radicals is considered by most investigators as the most likely mechanism that could account for the hypothesized beneficial effects on human health (30, 95–100). As a result of having an extensive chromophore system of conjugated carbon-carbon double bonds, lycopene can accept energy from various electronically excited species. This is due to its ability to quench singlet oxygen (99), formed by energy transfer from a meta-stable excited photosensitizer (101). Although not technically a free radical, singlet oxygen ( $^1\text{O}_2$ ) is a very reactive high-energy and short-lived oxygen species produced in biologic systems that can react with biomolecules. Lycopene may also interact with reactive oxygen species such as hydrogen peroxide and nitrogen dioxide (102–104). There is some evidence that lycopene may serve as an antioxidant in biological systems. Lycopene may prevent oxidative damage to lipoproteins and DNA (2, 30). A protective effect against oxidative stress also was illustrated when lycopene was found to be preferentially destroyed relative to  $\beta$ -carotene when human skin was irradiated with UV light (105).

Lycopene is extremely hydrophobic and is most commonly located within cell membranes. Therefore, the interaction of lycopene with reactive oxygen molecules may be most profound in the hydrophobic inner core of the cellular membranes unless the lycopene is associated with specific transmembrane proteins extending to the surface and interacting with the aqueous environment. Isomerization may slightly alter the physiochemical interactions between lycopene and subcellular structures. This in turn allows the lycopene to interact with a greater variety of components within the cell and participate in reactions that may be specific for subcellular compartments. However, at this point in time, almost no data has been generated using prostate cells or human prostate tissue to support these hypotheses. The review by Heber in this issue provides additional information regarding mechanisms whereby lycopene may influence biological systems.

The role of oxidative damage in the initiation or promotion of prostate cancer remains to be defined. Although many scientists, including the authors of this review, feel that reactive oxygen produced during metabolism of cells is a prime candidate for DNA-damaging agents, absolute proof has not been generated. Another source of reactive oxygen in the prostate comes from inflammatory infiltrates. Prostatitis is a common inflammatory condition of the prostate that is associated with bacterial or viral infections. In many men prostatitis becomes a chronic or recurring condition that may lead to long-term exposure to reactive oxygen species.

## **Tomatoes and Prostate Cancer: The Future Agenda**

The relationship between tomato products and a reduced risk of prostate cancer remains one of the most provocative and exciting developments within the field during recent years. However, in contrast to media and advertising hype, this hypothesis requires additional research to establish a causal relationship. A randomized, double-blinded intervention study to assess the ability of tomato products to prevent prostate cancer would be considered the definitive study. However, as is true for many dietary interventions, a study of this type is impossible in humans. Tomato products are widely consumed, blinding is impossible, and the media reporting and marketing of tomato products or components can influence food selection behavior over time. Furthermore, it is most likely that tomato products must act over a long period of time, perhaps decades to alter risk. In addition, the costs of an intervention study are prohibitive. Thus, we must establish causality based upon accumulated data from a variety of sources including epidemiology, clinical investigation, animal models, and cell biology. Furthermore, a research focus emphasizing lycopene as a mediating factor in a protective relationship should continue but not without strong consideration given to other phytochemicals found in tomato products.

Additional epidemiologic research in a variety of different cohorts will be helpful in confirming the associations already identified in a broader array of men living in diverse cultures, especially those of different ethnic groups. In addition, epidemiologic studies focusing upon the relationships between biomarkers of tomato product intake, such as serum lycopene or other phytochemicals, and biomarkers of risk are critically needed. An emphasis by investigators and funding agencies on well-designed studies with adequate statistical power to answer key questions is necessary.

Clinical studies currently underway at several programs will provide key links between the laboratory bench and population studies. Clinical studies should focus upon healthy men as well as those with premalignant conditions and those with established prostate cancer. Healthy men will be useful in studies designed to better understand the effects of different tomato products on the absorption, distribution, and bioactivity of phytochemicals found in tomatoes. Some men present to the clinic with an elevated PSA and a normal digital rectal exam. These men often undergo a biopsy that shows no evidence of cancer or perhaps a premalignant condition such as HGPIN. These men are potential candidates for intervention studies with diet or chemopreventive agents with follow-up evaluation of serum and prostate biomarkers. Although prostate biopsy specimens are small, enough tissue is available for assessment of some immunohistochemical and molecular outcomes. Men with prostate cancer also provide several opportunities to intervene with dietary regimens and obtain important new data relevant to tumor progression. For example, in men with disease

thought to be localized to the gland, the period between initial diagnosis and prostatectomy is often many weeks or months. This period of time is a window of opportunity for investigators to provide interventions with diet or chemopreventive agents and obtain information regarding alterations in biomarker expression. Specifically, we need to evaluate tissue biomarkers of proliferation and the cell cycle, apoptosis, angiogenesis, invasion and metastases, and the expression of growth factors and inhibitors within the prostate.

The expanding array of new rodent models of prostate carcinogenesis provides a means to evaluate dietary interactions with specific genetic and molecular determinants of prostate carcinogenesis. Several transplantable systems are useful in the assessment of tumor biomarkers related to progression. There is a rapid growth in the characterization of novel prostate cancer models using transgenic and knockout technology (106). Several systems have been developed that employ hormonal and chemical carcinogenic stimuli that can also be applied to the preclinical assessment of dietary and chemopreventive agents. The rodent models are extremely valuable because of the precision with which an investigator can control a vast array of potentially interacting and confounding variables. Genotype, age, diet composition, and environment are also held constant in a well-designed rodent study. Although of enormous value in demonstrating biological plausibility, rodent studies should not be overly emphasized but rather placed in perspective with data derived from a variety of sources. In parallel with carcinogenesis studies it is important to investigate the similarities and differences between rodents and humans in the absorption, distribution, and metabolism of phytochemicals derived from tomato products. Together, these experiments will provide essential mechanistic data regarding the potential efficacy of different tomato products, extracts, concentrates, or pure phytochemicals to alter the different phases of prostate carcinogenesis. This data will subsequently provide a foundation for human intervention trials, the products to be evaluated, the biomarkers to quantitate, the target population, and ultimately provide definitive safety and efficacy data in a timely fashion.

Studies with carefully characterized cell culture systems can provide considerable insight into the mechanisms whereby phytochemicals may influence cellular and molecular processes involved in carcinogenesis and tumor promotion. Biomarkers of activity can be characterized and tools established for assessment in rodent and human studies. It is also important that investigators evaluating components of tomato products in cell culture establish appropriate delivery vehicles that ensure stability and uptake by cells. In addition, the delivery vehicle for lipophilic compounds such as carotenoids *in vitro* must also be carefully evaluated and monitored for biological effects. For example, many carotenoids such as lycopene and  $\beta$ -carotene, may be relatively unstable or metabolized under cell culture conditions leading to the formation of metabolic or degra-

dation products that may have biological activity *in vitro* but uncertain relevance to *in vivo* conditions (107, 108). The development of new prostate cancer cell lines and prostate epithelial primary cultures will further enhance our ability to understand how phytochemicals from tomatoes may influence prostate biology.

In summary, we have many questions concerning the role of tomato products in prostate and other cancers. However, the tools and technology available to a wider array of investigators ensures that progress in the next few years will be dramatic. In addition, barriers to transdisciplinary research, as illustrated in this symposium, are gradually being dismantled and cooperative research activity between various disciplines ensures that the maximum amount of information will be derived from future studies.

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