Tomato Sauce Supplementation and Prostate Cancer: Lycopene Accumulation and Modulation of Biomarkers of Carcinogenesis

PHYLLIS BOWEN,[1]*¶ LONGWEN CHEN,*‡ MARIA STACEWICZ-SAPUNTZAKIS,* CLAUDINE DUNCAN,* ROOHOLLAH SHARIFI,† LUNA GHOSH,‡ HYUNG-SOOK KIM,* KONSTANTIN CHRISTOV-TZELKOV,§ AND RICHARD VAN BREEMEN¶

*Departments of *Human Nutrition, †Urology, ‡Pathology, §Surgical Oncology, and ¶Medicinal Chemistry and Pharmacognosy, University of Illinois and Westside Veterans' Administration Medical Center, Chicago, Illinois 60612*

As part of a randomized placebo-controlled study to evaluate the effect of lycopene supplementation on DNA damage in men with prostate cancer, a nonrandomized 5th arm using tomato sauce was included and reported here. Thirty-two patients with localized prostate adenocarcinoma consumed tomato sauce-based pasta dishes for 3 weeks (30 mg of lycopene/day) before their scheduled radical prostatectomy. Prostate tissue was obtained as biopsies at baseline and as resected tissue at the time of the prostatectomy. Serum and prostate lycopene, serum prostate specific antigen (PSA) concentrations, and leukocyte DNA 8-OH-deoxyguanosine/deoxyguanosine (8OHdG) were measured at baseline and at the end of the intervention. Cancer cells in paraffin sections of prostate biopsies and postintervention resected tissue were compared for 8OHdG staining and for apoptosis. Adherence to the daily consumption of tomato-based entrees was 81.6% of the intended dose, and serum and prostate lycopene concentrations increased 1.97- and 2.92-fold ($P < 0.001$), respectively. Mean serum PSA concentrations decreased by 17.5% ($P < 0.002$) and leukocyte 8OHdG decreased by 21.3% ($P < 0.005$) after tomato sauce consumption. Resected tissues from tomato sauce-supplemented patients had 28.3% lower prostate 8OHdG compared with the nonstudy control group ($P < 0.03$). Cancer cell 8OHdG staining of Gleason Score-matched resected prostate sections was reduced by 40.5% in mean nuclear density ($P < 0.005$) and by 36.4% in mean area ($P < 0.018$) compared with the presupplementation biopsy. Apoptotic index was higher in hyperplastic and neoplastic cells in the resected tissue after supplementation. These data taken as a whole indicate significant uptake of lycopene into prostate tissue and a reduction in DNA damage in both leukocyte and prostate tissue. Whether reduction in DNA damage to prostate cancer cells is beneficial awaits further research, although reduction in serum PSA concentrations is promising. Exp Biol Med 227:886–893, 2002

Key words: prostate cancer; lycopene isomers; tomatoes; 8 hydroxydeoxyguanosine; prostate-specific antigen; DNA damage; apoptosis

The lower risk of prostate cancer associated with tomato product consumption observed in some U.S. cohort studies (1, 2) has raised the possibility that tomato products or lycopene, their most visible component, may be useful in the prevention or control of prostate cancer. The experience of the β-carotene intervention trials for the prevention of lung cancer, where increased risk was found among current smokers (3, 4), has prompted a more cautious program for the development of lycopene as a promising chemopreventive agent. Among the questions that must be answered before lycopene is seriously considered for clinical trials are: whether lycopene reaches the prostate in sufficiently high concentrations to be functionally active; what are the possible mechanisms of action in the prevention or reversal of carcinogenesis; what activities may lead to adverse effects; and what are the best biomarkers to follow efficacy in a clinical trial? To that end, we embarked on a research program to characterize lycopene uptake in the prostate and to explore one of the most plausible mechanisms of action, the possibility that lycopene acts as an *in vivo* prostate antioxidant. Ongoing coordinated studies involve the pharmacokinetics of multiple doses of lycopene delivered as a tomato-based product or as supplement-based lycopene, prostate cell line studies of lycopene uptake, DNA damage and apoptosis, and short-term clinical studies using a presurgical or biopsy model to evaluate lycopene uptake, isomerization, and reductions in DNA damage. This preliminary report summarizes a tomato sauce intervention in one of the clinical studies.

Lycopene outperforms a number of tested carotenoids and vitamin E in several *in vitro* antioxidant systems, especially those that generate singlet oxygen (5), but it is more likely to be a pro-oxidant in peroxide-generating systems.

[1] To whom requests for reprints should be addressed at Department of Human Nutrition and Dietetics, m/c 517, 1919 West Taylor Street, University of Illinois, Chicago, IL 60612. E-mail: pbowen@uic.edu

1535-3702/02/22710-0886$15.00
Copyright © 2002 by the Society for Experimental Biology and Medicine

(6) Short-term tomato product feeding in humans has led to decreases in lipid peroxides (measured as thiobarbituric acid-reactive substances [TBARS]) (7), DNA strand breaks in circulating lymphocyte DNA (8, 9), and a trend toward decreased nucleoside damage in the form of 8 hydroxydeoxyguanosine/2′deoxyguanosine (8OHdG/dG) in circulating leukocytes (7, 10), pointing to *in vivo* antioxidant activity.

The role of a pro-oxidative state, through the formation of oxidative products of DNA, in the forwarding of carcinogenesis is hardly questioned, despite the largely circumstantial flood of reports and observations (11–13). Of the several approaches to the measurement of oxidative DNA damage, one of the most appreciated is the measurement of 8OHdG, which can be formed in DNA by several reactive species. It has been shown to be mutagenic and is quickly removed from DNA and excreted in the urine (14). Therefore, its residual presence in the DNA of normal and neoplastic tissues is the result of the imbalance between oxidative attack and DNA repair. The prostate may be particularly vulnerable to oxidative attack because of greater chronic inflammation of prostate epithelial cells and faster cell turnover and its lower levels of DNA repair enzymes (15) compared with other tissues.

Here, we review a series of preliminary studies on blood and prostate tissue collected from patients with prostate cancer who received tomato sauce in the form of pasta entrees prior to surgery to explore the *in vivo* role of lycopene, or some other tomato ingredient, as an antioxidant capable of protecting DNA from oxidative damage.

Study Design and Methods

The reported study constituted a 5th unrandomized arm of an ongoing trial of 60 men with adenocarcinoma of the prostate (clinical stage T1 or T2) who have elected prostatectomy as their treatment and 60 men with serum prostate specific antigen (PSA) concentrations of ≥4 ng/ml but are biopsy negative for adenocarcinoma. Each group is randomized to receive either 30 mg/day lycopene as tomato oleoresin (Lyc-o-Mato; Lyco Red-Bodura, New York, NY) or placebo for a 3-week period, and is double-masked. This portion of the study is ongoing. The 5th arm was composed of 32 men with adenocarcinoma who received 30 mg/day lycopene as tomato sauce baked into pasta entrees. This preliminary report analyzes data before and after treatment for only this whole-food intervention.

Study Subjects. All the men had newly diagnosed stage T1 or T2 adenocarcinoma and had not received hormone therapy. Because there was insufficient biopsy material to measure both lycopene and 8OHdG/dG by electrochemical analysis, resected prostate tissue was collected from seven prostatectomy patients from the same patient population who were not study participants and from 50 men with >4 ng/ml serum PSA concentrations but biopsy negative for prostate cancer or who were not candidates for prostatectomy, constituting two additional comparison groups. All patients gave informed consent prior to study participation. Both studies were approved by the University of Illinois at Chicago and the West Side VA Hospital Institutional Review Board.

Whole-Food Intervention. Sufficient tomato sauce (200 g of Hunt's Spaghetti Sauce, Hunt-Wesson, Irvine, CA) was incorporated into four different recipes of spaghetti, lasagna, and pasta shells designed to deliver 30 mg of lycopene, and roughly-equivalent amounts of fat, carbohydrate, and protein. The average energy content of the entrees was 771 kcal. Men could select from these entrees, which were cooked and frozen and required 10 to 15 min reheating in a microwave oven. They consumed one entree per day for the 3 weeks prior to their scheduled prostatectomy. The amount and frequency of entree consumption was recorded by shading in diagrams (25%, 50%, 75%, and 100%) of the pasta dishes on a daily calendar. Three days of 24-hr diet recalls were collected by telephone prior to the whole-food intervention, and another 3 days of diet intake recalls were obtained during the 2nd week of supplementation.

Outcome Measurements. Blood samples were collected at baseline (approximately 2–3 weeks postprostate biopsy) and 1–2 days prior to prostatectomy for lycopene, total PSA concentration, and leukocyte 8OHdG/dG determination. Body composition was assessed by bioelectric impedance, and body weight was obtained before and after supplementation. As part of patient recruitment, two additional fresh prostate needle biopsies were obtained at the time of the diagnosing biopsy, and they were snap frozen and stored at −80°C until analysis for lycopene content. Resected prostate tissue was collected at the time of prostatectomy and was snap frozen. Slides from biopsy and surgical paraffin blocks for each patient were evaluated for Gleason score by a clinical pathologist, and paraffin blocks of biopsy and resected tissue from each patient were matched for Gleason score for slide preparation, for histochemical 8OHdG, and for apoptotic index comparisons. An assay for 8OHdG staining using the monoclonal antibody N45.1, which recognizes both the hydroxy function of 8OHdG and the 2′ portion of deoxyribose, was developed and validated for human prostate after the manner of Toyokuni *et al.* (16) Staining density was subjectively evaluated by two independent pathologists and was quantitated by using a CAS-200 image analyzer (Cell Analysis Systems, Becton Dickinson, San Jose, CA), which performs high-speed digital image processing. Twenty high-power fields containing prostate adenocarcinoma were selected from each slide and were measured as the sum of the density, or areas of staining, as a function of total nuclear area, and expressed as a percentage. Apoptotic index was assessed on deparaffinized prostate tissue sections by the TUNEL assay (Apop Tag Peroxidase kit; Intergen, Purchase, NY). Lycopene and its isomers were separated by high-performance liquid chromatography (HPLC) in tomato sauce, serum, and prostate samples and were measured by UV/vis detection (17, 18) with an interassay reliability of 7.4% for total lycopene. Isomers were separated using a

Suplex pKb-100 C18, 250 × 4.6 mm column (Supelco, Bellefonte, PA) and were isocratically eluted with methanol:acetonitrile:isopropanol (54:44:2, v/v). Lycopene isomers were confirmed using liquid chromatography-mass spectrometry (LC-MS) in the laboratory of Dr. Richard van Breemen. Leukocyte and prostate 8OHdG/dG were measured by HPLC separation and were quantitated by electrochemical detection in our laboratory (19), but with modifications that improved the extent of hydrolysis and reduced artifactual 8OHdG. Interassay reliability was 7.6%. Nutrient and lycopene intakes from self-selected diets were calculated using the Nutrition Data System For Research, Database 29, version 4.01 (Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN) augmented with lycopene values from the U.S. Department of Agriculture Carotenoids Data Base (www.usda.gov). Total serum PSA was measured using a Microparticle Enzyme Immunoassay (Abbot Laboratories, Abbot Park, IL) by the University of Illinois Hospital Pathology Laboratory. Changes in measured values were assessed by paired *t* test with complete data sets. Leukocyte and prostate DNA 8OHdG/dG ratios and apoptotic index and PSA concentrations were square root or log transformed to overcome skewness. Associations between variables were evaluated by simple regression analysis and by categorized means. The statistical package SAS (version 7.1, 1997; Cary, NC) was used for analysis. Differences of $P < 0.05$ were considered statistically significant.

Results

Of the 32 men who completed the study, 27 biopsy samples were collected and 28 out of the 32 had prostatectomy at the end of the study. One patient was excluded from the DNA damage analyses because his sample was 2 SDs above the mean at baseline, but the sample taken at the end of the study showed markedly lower 8OHdG/dG. He was found to be a drug user who abstained prior to his surgery. Table I presents patient characteristics, consumption of pasta entrees, and changes in lycopene intake from their self-selected diets. The Westside VA serves a primarily lower socioeconomic African-American population, which was reflected in the patients who ranged from 60 to 74 years of age. Intervention days varied due to fluctuations in surgery scheduling and averaged 19.8 ± 0.6 days.

Adherence to a Whole-Food Intervention and Prostate Uptake of Lycopene. The large amount of tomato sauce (3/4 cup) was well tolerated, with only three out of 32 patients reporting minor gastrointestinal problems, which resolved within a few days. Men consumed at least 25% of a pasta entrée on nine out of 10 days on the study and 81.6% of the intended total dose of lycopene (Table I). Because they reduced their mean intake of lycopene from their self-selected diet during the intervention, the mean increase in lycopene consumption was 21.8 mg/day. Energy intake increased by a mean of 242 kcal/day, which meant that an average of 529 kcal/day of the 771 kcal/day supplied by the pasta entree was off-set by a down-regulation of energy intake in their self-selected diet. This was verified by body weight maintenance during the 3-week intervention period. Macronutrient diet composition did not change, although there was an increase in cholesterol intake due to additional cheese and sausage consumption as part of the pasta entrees.

Self-reported adherence to pasta entree consumption was consistent with the observed doubling and tripling of serum and prostate total lycopene concentrations, respectively (Table II). Figure 1A shows a reasonable correlation between plasma and prostate total lycopene concentrations

Table I. Patient Characteristics and Adherence to the Whole Food Intervention

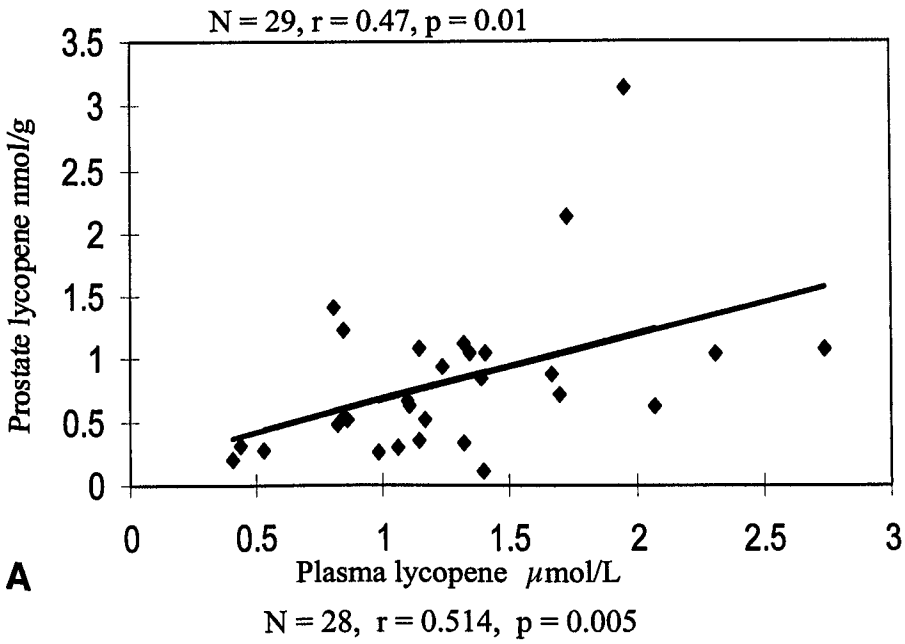
Variable	Before intervention	During intervention	P-value[c]
Number	32		
Age (yr)	63.7 ± 6.1[a]		
Weight (kg)	87.3 ± 3.2[a]	86.8 ± 3.2[a]	
BMI (kg/m^2)	28.0 ± 4.9[a]		
Body fat (%)		20.9 ± 5.4[a]	
Ethnicity			
% African-American	75.0		
% Other	25.0		
Intervention Days		19.8 ± 0.6[b]	
% Adherent Days		90.0 ± 2.0[b]	
% Adherent to Dose		81.6	
Self-selected diet lycopene (mg/d)	5.0 ± 7.3[b]	1.0 ± 0.02[b]	
Total lycopene intake (mg/d)		26.8 ± 2.2	
Total energy intake	2096 ± 132[b]	2338 ± 122[b]	0.04
Fat intake (en%)	38.7 ± 1.4[b]	39.8 ± 0.9[b]	
Protein intake (en%)	15.4 ± 0.6[b]	16.1 ± 0.6[b]	
Cholesterol intake (mg/d)	341 ± 39.2[b]	472 ± 33.1[b]	0.0002

[a] Values are means ± standard deviations
[b] Values are means ± standard error of the mean
[c] Two-tailed paired t tests performed on log-transformed data

Table II. Serum and Prostate Uptake of Lycopene in Response to the Whole Food Intervention

Analyte/tissue	Before/without intervention	After intervention	P-value
Serum total lycopene (nM)	638 ± 60[a]	1258 ± 95	0.0001
Prostate total lycopene (nmol/g)	0.279 ± 0.450	0.820 ± 0.119	0.001
Pasta entree % *trans* lycopene		68.5	
Serum % *trans* lycopene	43.2 ± 6.4	42.9 ± 6.8	NS
Prostate % *trans* lycopene	22.5 ± 1.5[b]	28.7 ± 4.2	<0.001

[a] Means ± SD
[b] 10 assays of pooled biopsies (5 in pool) representing men with high serum PSA but not eligible for study participation.



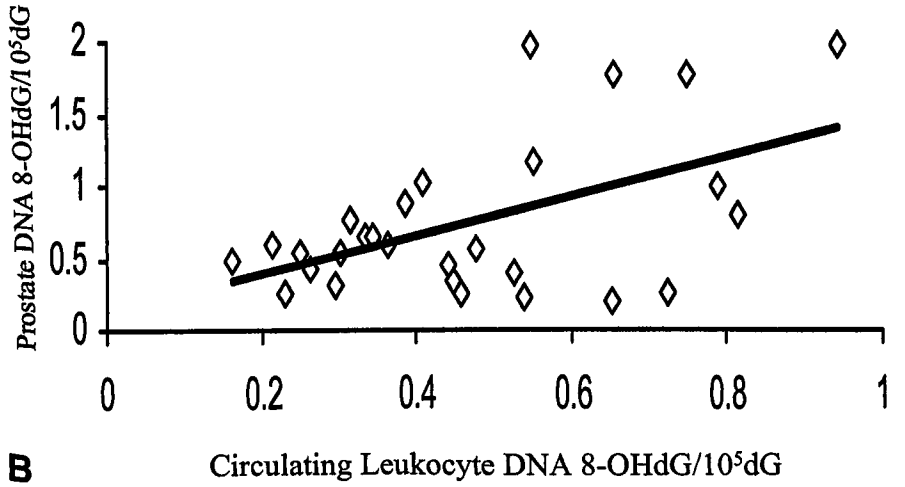


Figure 1a and 1b. Regression scatterplots of the relationship between (1A) prostate and plasma total lycopene concentrations and (1B) prostate and circulating leukocyte 8OHdG/dG ratios from resected prostate tissue and blood samples obtained after 3 wks of tomato sauce supplementation.

postintervention and baseline correlations were similar (r = 0.46, P = 0.01). Consistent with previous observations (20), there appeared to be a successive conversion of *trans* to *cis* isomers moving from food to serum to prostate where 70% of the lycopene was in the *cis* form, whereas the baked and reheated pasta entree contained only 30% lycopene *cis* isomers (Table II). There was insufficient biopsy prostate tissue to adequately quantitate the isomers. Therefore, prostate biopsies obtained from men screened for the study but found to be ineligible, either because they were biopsy negative for cancer or were not candidates for prostatectomy, were pooled in groups of five and were evaluated in 10 separate assays (mean percentage represented 50 subjects). The percentage of lycopene *trans* isomer was higher in the men who consumed tomato sauce, indicating a possible enrichment of the all *trans* isomer due to recent tomato product consumption.

Chemical Evaluation of Oxidative DNA Damage by 8OHdG/dG Ratio. Leukocyte 8OHdG/dG decreased by 21.3% by the 3rd week of tomato sauce supplementation. Prostate 8OHdG/dG was 58.3% higher than leukocyte ratios postintervention, which supports the hypothesis that the prostate of patients with cancer may be oxidatively stressed compared with other tissues. Oxidative DNA damage was 28% lower in the resected prostates of the tomato sauce-supplemented group compared with the reference group who did not participate in the study (Table III). There was no correlation between serum lycopene concentrations and leukocyte 8OHdG/dG at either time period, or when data from both time periods were considered together. Leukocyte lycopene concentrations were not evaluated because the sample was not sufficient for both measurements. There was a good correlation between leukocyte and prostate 8OHdG/dG (Fig. 1B), but there was no association between prostate lycopene concentration and prostate 8OHdG/dG postintervention.

Evaluation of Prostate Adenocarcinoma Cells for DNA Damage by 8OHdG Staining. Prostate tissue

Table III. Leukocyte and Prostate DNA Damage (8OHdG/dg) and Its Localization in Prostate Cancer Cell Nuclei (8OHdG staining), Apoptotic Index and Serum PSA Concentration

Measurement	Before intervention	After intervention	P-value
Leukocyte 8OHdG/dG × 10^5 n = 30	0.61 ± 0.05[a]	0.48 ± 0.04	0.0006[c]
Prostate 8OHdG/dG × 10^5 n = 29		0.76 ± 0.10[b]	
Reference Group Prostate 8OHdG/dG × 10^5 n = 7		1.06 ± 0.17[b]	0.03[b]
Cancer cell nuclei 8OHdG Immunodensity % optical density n = 26	22.1 ± 2.7	13.2 ± 2.7	0.005[c]
Cancer cell nuclei 8OHdG Immunodensity % positive area n = 26	12.5 ± 1.7	7.9 ± 1.6	0.009[c]
Carcinoma Apoptic Index % n = 24	0.8 ± 0.7[d]	2.7 ± 3.0[d]	0.01[c]
Hyperplasia Apoptic Index % n = 24	0.7 ± 0.5[d]	1.4 ± 1.6[d]	0.05[c]
Serum PSA ng/mL n = 30	10.9 ± 1.1	8.7 ± 0.9	0.001

[a] Values are means ± SEM
[b] Compared on square-root transformed data by unpaired t test
[c] Compared by paired t test on log-transformed data
[d] Values are means ± SD

is composed of a variety of cell types, which include epithelial cells (normal, hyperplastic, and neoplastic), stromal cells, and capillary endocytes. If DNA damage is different among cell types, then the 8OHdG/dG values obtained from fresh tissue samples could be a reflection of the proportion of cell types in the sample. To explore this possibility, an histochemical assay for 8OHdG was adapted for prostate tissue using a highly specific monoclonal antibody. Resected and biopsy prostate tissue showed differential 8OHdG staining for various cell types. Stromal nuclei were rarely stained, whereas hyperplastic cell nuclei varied from none to moderate staining. Cancer cells had the highest density staining, which indicated that they were subject to the greatest DNA damage. Two independent pathologists evaluated 8OHdG-stained slides and scored only cancer areas using a + to +++ system, and both found shifts from more staining to less staining for each patient's biopsy and resected tissue slides, respectively, indicating a decrease in DNA damage in cancer cells after tomato sauce consumption (Fig. 2). The subjective evaluation of the pathologists was verified by digital image processing using a light microscope. Mean (sum of 20 fields/slide) cancer cell nuclear 8OHdG immunodensity decreased by 40.3% as percentage optical density, and 36.8% as percentage positive area with tomato sauce supplementation (Table III). There was a trend toward a positive correlation between postintervention resected prostate 8OHdG/dG by HPLC-EC analysis and paraffin section prostate cancer cell 8OHdG immunodensity ($r = 0.38$, $P = 0.06$). Taken together, these data indicate that the oxidative DNA damage in cancer cells decreased in response to short-term tomato sauce supplementation.

Changes in Serum PSA Concentrations and Prostate Cell Apoptosis. Serum total PSA concentrations decreased 17.5% in these men (10.9 ± 1.1 to 8.7 ± 0.9 ng/ml, $P < 0.001$) in the 5–6 weeks between measurements (Table III). Apoptotic index was 2- and 3-fold higher in the resected prostate tissue compared with biopsies in hyperplastic and neoplastic cells, respectively.

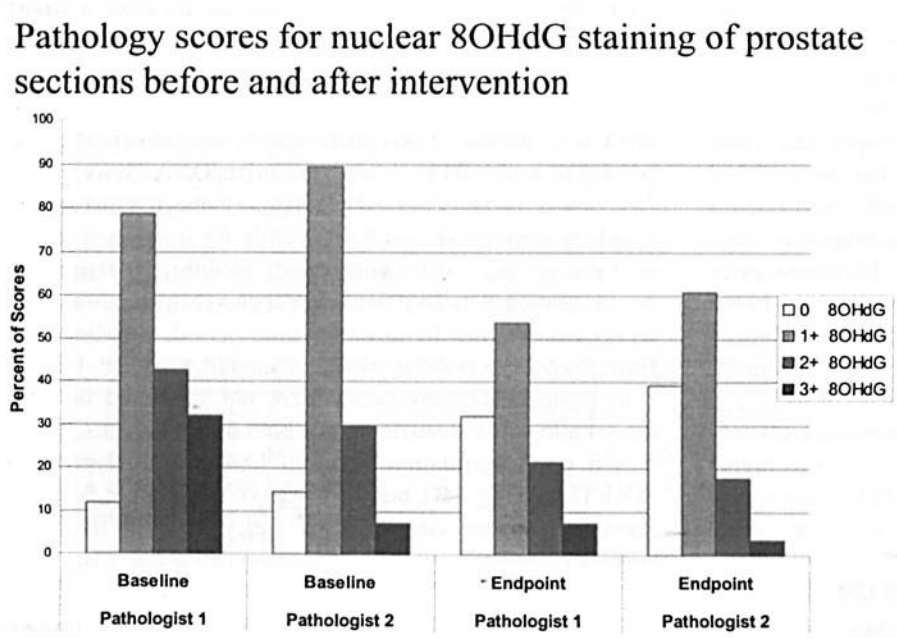


Figure 2. Percent of scores for degree of 8OHdG nuclear staining assigned by two pathologists to biopsy (baseline) and resected prostate tissue (endpoint). Scoring is 0 = negligible, + = light, ++ = moderate, +++ = heavy staining.

Discussion

The selection of a preoperative model for the evaluation of lycopene uptake into the prostate was predicated on the availability of resected prostate tissue for the measurement of lycopene uptake and assessment of oxidative DNA damage. Although the time for intervention was limited, there was a significant increase in prostate lycopene content with consistent decreases in prostate cell DNA damage, as measured by 8OHdG, both chemically and histochemically. Cancer cells were responsive to the intervention. Furthermore, leukocyte and prostate 8OHdG/dG were positively correlated, meaning that routine measurement of leukocyte 8OHdG/dG might be useful as a prognostic biomarker of oxidative stress and is worthy of further investigation. The assumption that lycopene alone caused the decrease in oxidative DNA damage is premature, especially because there was no inverse linear correlation between plasma or prostate lycopene content and 8OHdG/dG in leukocytes or prostate tissue. However, such correlations may not be expected because the lycopene content of leukocytes was not measured. Porrini *et al.* (21) provided 7 mg of lycopene/day as tomato puree to healthy women for 2 weeks and found a 44% increase in lymphocyte lycopene and a 50% increase in their resistance to oxidative DNA damage via the measurement of strandbreaks. Both plasma and lymphocyte lycopene concentrations were highly correlated with resistance to oxidative DNA damage (21). Their mean plasma lycopene concentration was 0.55 ± 0.21 μM compared with our 1.26 ± 0.95 μM after tomato sauce supplementation. Because their subjects were placed on a low-carotenoid diet prior to the study, their mean baseline plasma lycopene was only 0.13 ± 0.06 μM, whereas our mean baseline value was 0.64 μM. Furthermore, they were more directly measuring the ability of lycopene contained in lymphocytes to protect DNA from an *ex vivo* hydrogen peroxide insult, whereas we measured *in vivo* DNA damage. Rao *et al.* (7) found that 1 week of either lycopene or tomato product supplementation of healthy subjects produced a trend toward a decrease in leukocyte 8OHdG/dG, indicating that lycopene may be an important tomato antioxidant.

There was no association between prostate lycopene concentration and prostate 8OHdG/dG measured chemically in whole tissue or histochemically in cancer cells after intervention. Whether lycopene or some other component of tomato sauce or a combination of phytochemicals are the active components must await the completion of the remainder of the clinical trial where lycopene alone is supplemented. Tomato products contain a number of candidate phytochemicals beside lycopene such as phenolic compounds that are equivalent to 50% to 100% of the lycopene content in various tomato products. Water-soluble fractions of tomato products containing the phenolics were shown to have more antioxidant activity in hydrophilic *in vitro* antioxidant detection systems than the lipid-soluble fraction containing lycopene. (22) The combination of phenolic compounds and lycopene may compose an efficient system that keeps lycopene in an antioxidant state in cell membranes. Pastori *et al.* (23) found that lycopene alone did not inhibit the growth of two cancer cell lines, but did so quite effectively when combined with α-tocopherol in the cell medium. Another candidate that may modify cell proliferation, apoptosis, and necrosis is the tomato glycoalkaloid, tomatine, which forms complexes with cholesterol in membranes and has been shown to cause disruption of intestinal epithelial cells (24). Because large amounts of cholesterol are secreted during food digestion and form indigestible complexes with tomatine, little is thought to be absorbed in humans (25). However, no direct measurement of tomatine in plasma of subjects consuming large quantities of tomatoes has been made. Green tomatoes contain far greater quantities of tomatine (26).

The delivery of lycopene (or other bioactive tomato components) in the form of a variety of delicious food products was highly successful in this population of predominantly African-American, lower socioeconomic status patients. African-American men have 130% higher age-adjusted death rates from prostate cancer compared with Euro-Americans (27). Segments of this population are suspicious of participation in research, as evidenced by a higher refusal rate for the lycopene capsule compared with the whole-food intervention, where refusal was negligible. The disadvantage of food-based delivery, besides difficulty in logistics, is that individuals decrease tomato product consumption in their self-selected diet. Patients consuming the highest amounts of lycopene prior to the study decreased their consumption the most, which dilutes the effect of the intervention for these patients and must be taken into consideration when planning future food-based intervention trials. A main course entrée was chosen as the most acceptable vehicle for tomato product delivery, to incorporate sufficient tomato sauce to contain 30 mg of lycopene, but the entrees constituted approximately 34% of the patients' total daily energy intake. The men displaced most of this energy without jeopardizing their macro- or micronutrient consumption except for the increased cholesterol intake. However, their self-reported daily energy intake increased by an average of 242 kcal/day, which, if sustained over a 12-month period of time, would theoretically result in a net weight gain of 12.4 lbs.

The increase in *cis* isomers of lycopene in prostate tissue compared with plasma has been noted by Clinton *et al.* (28), and tumor cells appear to have more *cis* isomers. Plasma enrichment of *cis* over *trans* isomers was noted by several investigators (29, 30) and has been explained by destabilization of lycopene crystalline structure by bile, allowing for slow thermally induced *cis-trans* isomerization (the required energy level is lower for lycopene compared with β-carotene [31]) with subsequent selectively advantageous micelle, and perhaps chylomicron, incorporation of *cis* lycopene (32). There may also be selective uptake of *cis* isomers into prostate cells. Alternatively, lycopene isomer-

ization could occur as a result of prostate metabolism via the formation of a radical through oxidation or by action of the monooxygenase system, which is known to be modulated by carotenoids (33).

Decreased DNA damage in preneoplastic cells might be assumed to decrease the probability of carcinogenic mutations, whereas decreased DNA damage in neoplastic cells could lead to one of two possibilities: it could confer increased probability of cancer cell survival or it could mean that there are fewer viable cancer cells due to the induction of apoptosis or necrosis. Preliminary explorations of apoptotic index point to the second possibility because there was a significant increase in apoptotic index in both hyperplastic and neoplastic cells when comparing biopsy with the resected prostate tissue collected postintervention. However, these changes may have been due to sampling bias in the collection of the biopsy tissue. β-Carotene at high cell medium concentrations has been shown to induce apoptosis in a dose- and time-dependent manner in colon adenocarcinoma cells grown in culture, accompanied by an enhanced production of intracellular reactive oxygen species. Both actions were blocked by the addition of vitamin E to the culture medium (34). The capacity of carotenoids, including lycopene, to act as pro-oxidants at high concentrations and as antioxidants at low concentrations has been noted by others (35–37). High carotenoid concentrations in cell media may not be associated with high intracellular concentrations because lycopene uptake by cells is relatively low (38), which would favor an antioxidant role for lycopene *in vivo* as observed in this study.

The observed decrease in serum PSA concentrations is consistent with a decrease in the number of PSA-secreting cells (39). Serum PSA concentrations, along with biopsy Gleason score, are the most important predictors of clinically important cancer of the prostate (40). PSA concentrations were measured 2–3 weeks after biopsy, and previous PSA concentrations in these patients were no different than the baseline PSA concentration obtained for this study. Therefore, the postbiopsy rise in PSA concentrations would have subsided (41, 42) and could not explain the observed decreases with tomato sauce supplementation. A few studies have shown that lycopene, at physiological concentrations, can inhibit prostate cancer cell growth (43, 44), and lycopene supplementation in mice modestly inhibited benzo(a)pryene-induced mutagenesis in prostate, but enhanced it in lung and colon tissue (45). The mechanism of action may or may not be linked to a pro- or antioxidant action because the growth inhibitory effects of lycopene on the MCF7 mammary cell line were not due to the toxicity of lycopene, but rather to interference in IGF-1 receptor signaling and cell cycle progression.

These data demonstrate that lycopene or tomato phytochemicals can modulate several biomarkers associated with prostate carcinogenesis in patients with cancer, and are consistent with a startling case report of a patient with hormone-refractory metastatic prostate cancer with extensive nodal disease and skeletal metastases who stopped all formal treatment regimens and transferred to hospice care, but then began alternative treatment with lycopene (10 mg/day) and saw palmetto (900 mg/day) supplements. His serum PSA concentrations were 365.0 ng/ml at the beginning of phytotherapy, dropped to 139.6 ng/ml in 1 month, and then to 8.1 ng/ml at the end of the 2nd month, and remained between 3 and 8 ng/ml for 18 months. His bone scan showed improvement in bone metastases and he has remained asymptomatic at the last follow-up and has continued on phytotherapy (46). Saw palmetto has not been shown to lower serum PSA concentrations in clinical trials (47), pointing to lycopene as the most likely active compound. Although these results appear promising, the lack of a control group for these preliminary studies limits their clinical value and more robust comparisons await the completion of our randomized placebo-controlled trial, where lycopene alone can be compared with tomato sauce and with placebo cross-sectionally. If the results are consistent with these preliminary studies, it may indicate the possible use of lycopene as complementary therapy for prostate cancer, and further studies into mechanism of action, efficacy, and safety for homogeneous patient populations should be encouraged.

1. Giovannucci E, Ascherio A, Rimm EB, Stampher MJ, Colditz GA, Willett WC. Intake of carotenoids and retinol in the relation to risk of prostate cancer. J Natl Cancer Inst **87:**1767–1776, 1995.
2. Gann PH, Ma J, Giovannucci E, Willet W, Sacks FM, Hennekens CH, Stampfer MJ. Lower prostate cancer risk in men with elevated plasma lycopene levels: results of a prospective analysis. J Cancer Res **59:**1225–1230, 1999.
3. The α-Tocopherol B-CCPSG. The effect of vitamin E and β-carotene on the incidence of lung cancer and other cancers in male smokers. N Engl J Med **330:**1029–1035, 1994.
4. Omenn GS, Goodman GE, Thornquist MD, et al. Effects of a combination of β-carotene and vitamin A on lung cancer and cardiovascular disease. N Engl J Med **334:**1150–1155, 1996.
5. Di Mascio P, Kaiser S, Sies H. Lycopene as the most efficient biological carotenoid singlet oxygen quencher. Arch Biochem Biophys **274:**532–538, 1989.
6. Woodall AA, Britton G, Jackson MJ. Carotenoids and protection of phospholipids in solution or in liposomes against oxidation by peroxyl radicals: relationship between carotenoid structure and protective ability. Biochim Biophys Acta **1336:**575–586, 1997.
7. Rao AV, Agarwal S. Bioavailability and in vivo antioxidant properties of lycopene from tomato products and their possible role in the prevention of cancer. Nutr Cancer **31:**199–203, 1998.
8. Riso P, Pinder A, Santangelo A, Porrini M. Does tomato consumption effectively increase the resistance of lymphocyte DNA to oxidative damage? Am J Clin Nutr **69:**712–718, 1999.
9. Porrini M, Riso P. Lymphocyte lycopene concentration and DNA protection from oxidative damage is increased in women after a short period of tomato consumption. J Nutr **130:**189–192, 2000.
10. Rehman A, Bourne LC, Halliwell B, Rice-Evans CA. Tomato consumption modulates oxidative DNA damage in humans. Biochem Biophys Res Commun **262:**828–831, 1999.
11. Cerutti PA. Prooxidant states and tumor promotion. Science **227:**375–380, 1985.
12. Ames BN, Shigenaga MK, Park EM. DNA damage by endogenous oxidants as a cause of aging and cancer. In: Kelvin JA, Ed. Oxidative

Damage and Repair: Chemical, Biological and Medical Aspects. New York: Pergamon Press, pp181, 1990.
13. Loft S, Poulsen HE. Cancer risk and oxidative DNA damage in man. J Mol Med **74:**297–312, 1996.
14. Halliwell B. Why and how should we measure oxidative DNA damage in nutritional studies? How far have we come? Am J Clin Nutr **72:**1082–1087, 2000.
15. De Marzo AM, Coffey DS, Nelson WG. New concepts in tissue specificity for prostate cancer and benign prostatic hyyperplasia. Urology **53**(Suppl 3A):29–40, 1999.
16. Toyokuni S, Tomoyuki T, Hattori Y, Nishiyama Y, Yoshida A, Uchida K, Hiai H, Ochi H, Osawa T. Quantitative immunohistochemical determination of 8-hydroxy-2′-deoxyguanosine by a monoclonal antibody N45.1: its application to ferric nitrilotriacetate-induced renal carcinogenesis model. Lab Invest **76:**365–374, 1997.
17. Stacewicz-Sapuntzakis M, Bowen PE, Kikendall JW, Burgess M. Simultaneous determination of serum retinol and various carotenoids: their distribution in middle-aged men and women. J Micronut Analysis **3:**27–45, 1987.
18. Philips RW, Kikendall JW, Luk GD, Willis SM, Murphy JR, Maydonovitch C, Bowen PE, Stacewicz-Sapuntzakis M, Wong RKH. β-Carotene inhibits rectal mucosal ornithine decarboxylase activity in colon cancer patients. Cancer Res **53:**3723–3725, 1993.
19. Chen L, Bowen PE, Berzy D, Sapuntzakis M, Riley R. Diet modification affects DNA oxidative damage in health humans. Free Radic Biol Med **16:**111–115, 1994.
20. Clinton SK, Emenhiser C, Schwartz SJ, Bostwick DG, Williams AW, Moore BJ, Erdman JW. Cis-trans lycopene isomers, carotenoids and retinol in the human prostate. Cancer Epidemiol Biomarkers Prev **5:**823–833, 1996.
21. Porrini M, Riso P. Lymphocyte lycopene concentration and DNA protection from oxidative damage is increased in women after a short period of tomato consumption. J Nutr **130:**189–192, 2000.
22. Lavelli V, Peri C, Rissolo A. Antioxidant activity of tomato products as studied by model reactions using xanthine oxidase, myeloperoxidase, and copper-induced lipid peroxidation. J Agric Food Chem **48:**1442–1448, 2000.
23. Pastori M, Pfander H, Boscoboinik D, Azzi A. Lycopene in association with α-tocopherol inhibits at physiological concentrations the proliferation of prostate carcinoma cells. Biochem Biophys Res Commun **250:**582–285, 1998.
24. Keukens EAJ, de Vrije T, Jansen LAM, de Boer H, Janssen M, de Kroon APM, Jongen WMF, de Kruijff B. Glycoalkaloids selectively permeabilize cholesterol containing biomembranes. Biochem Biophys Acta **1279:**243–250, 1996.
25. Friedman M, Fitch TE, Yokayama WE. Lowering of plasma LDL cholesterol in hamsters by the tomato glycoalkloid tomatine. Food Chem Toxicol **38:**549–553, 2000.
26. Leonardi C, Ambrosino P, Esposito F, Foglioano V. Antioxidant activity and carotenoid and tomatine contents in different typologies of fresh consumption tomatoes. J Agric Food Chem **48:**4723–4727, 2000.
27. Hovenian MS, Deming CL. The heterologous growth of cancer of the human prostate. Surg Gynecol Obstet **86:**29, 1948.
28. Clinton SK, Emenhiser C, Schwartz SJ, Bostwick DG, Williams AW, Moore BJ, Erdman JW. Cis-trans lycopene isomers, carotenoids, and retinol in the human prostate. Cancer Epidemiol Biomarkers Prev **5:**823–833, 1996.
29. Schierle J, Bretzel W, Buhler N, Faccin D, Hess K, Steiner K, Schuep W. Content and isomeric ratio of lycopene in food and human blood plasma. Food Chem **59:**459–465, 1997.
30. Muller H, Bub A, Watzl B, Rechkemmer G. Plasma concentrations of carotenoids in healthy volunteers after intervention with carotenoid-rich foods. Eur J Nutr **38:**35–44, 1999.
31. Holloway DE, Yang M, Paganga G, Rice-Evans CA, Bramely PM. Isomerization of dietary lycopene during assimilation and transport in plasma. Free Radic Res **32:**93–102, 2000.
32. Boileau AC, Merchen NR, Wasson K, Atkinson CA, Erdman JW. Cis-lycopene is more bioavailable than trans-lycopene in vitro and in vivo in lymph-cannulated ferrets. J Nutr **129:**1176–1186, 1999.
33. Tan B, Chu FL. Effects of palm carotenoids in rat hepatic cytochrome P450-mediated benzo(a)pyrene metabolism. Am J Clin Nutr **53:**1071S–1075S, 1991.
34. Palozza P, Calviello G, Serini S, Maggiano N, Lanza P, Ranelletti FO, Bartoli GM. β-Carotene at high concentrations induces apoptosis by enhancing oxy-radical production in human adenocarcinoma cells. Free Radic Biol Med **30:**1000–1007, 2001.
35. Palozza P. Prooxidant actions of carotenoids in biological systems. Nutr Rev **56:**257–265, 1998.
36. Yeh S-L, Hu M-L. Antioxidant and pro-oxidant effects of lycopene in comparison with β-carotene on oxidant-induced damage of Hs68 cells. J Nutr Biochem **11:**548–554, 2000.
37. Schwartz JL. The dual roles of nutrients as antioxidants and prooxidants: their effect on tumor cell growth. J Nutr **126:**1221S–1227S, 1996.
38. Xu X, Wang Y, Constantinou A, Stacewicz-Sapuntzakis M, Bowen P, van Breemen R. Solubilization and stabilization of carotenoids using micelles: delivery of lycopene in cell culture. Lipids **34:**1031–1036, 1999.
39. Stenman UH, Hakama M, Knekt P, Aromaa A, Teppo L, Leiononen J. Serum concentrations of prostate specific antigen and its complex with α1-antichymotrypsen before diagnosis of prostate cancer. Lancet **344:**1594–1598, 1994.
40. Abbas F, Scardino PT. The natural history of clinical prostate carcinoma. Cancer **80:**827–833, 1997.
41. Dew T, Coker C, Saadeh F, Mulvin D, Coptcoat MJ, Sherwood RA. Influence of investigative and operative procedures on serum prostate-specific antigen concentration. Ann Clin Biochem **36:**340–346, 1999.
42. Ornstein DK, Rao GS, Smith DS, Ratliff TL, Basler JW, Catalona WJ. Effect of digital rectal examination and needle biopsy on serum total and percentage of free prostate specific antigen levels. J Urol **157:**195–198, 1997.
43. Levy J, Bosin E, Feldman B, Giat Y. Lycopene is a more potent inhibitor of human cancer cell proliferation than either α-carotene or β-carotene. Nutr Cancer **24:**257–266, 1995.
44. Countryman C, Bankson D, Collins S, Mar B. Lycopene inhibits the growth of the HL-60 promyelocytic leukemia cell line. Clin Chem **37:**1056, 1991.
45. Guttenplan JB, Chen M, Kosinska W, Thompson S, Zhao Z, Cohen LA. Effects of a lycopene-rich diet on spontaneous benzo(a)pyrene-induced mutogenesis in prostate, colon and lungs of the lacZ mouse. Cancer Lett **10**(164):1–6, 2001.
46. Matlaga BR, Hall CM, Stindt D, Torti FM. Response of hormone refractory prostate cancer to lycopene. J Urol **166:**613, 2001.
47. Marks LS, Partin AW, Epstein JL, Tyler VE, Simon I, Macairan ML, Chan TL, Dorey FJ, Garris JB, Veltri RW, Santos PB, Stonebrook KA, Dekernion JB. Effects of a saw palmetto herbal blend in men with symptomatic benign prostatic hyperplasia. J Urol **163:**1451–1456, 2000.