

Oxidative DNA Damage in Prostate Cancer Patients Consuming Tomato Sauce-Based Entrees as a Whole-Food Intervention

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Background: Human prostate tissues are vulnerable to oxidative DNA damage. The risk of prostate cancer is lower in men reporting higher consumption of tomato products, which contain high levels of the antioxidant lycopene. We examined the effects of consumption of tomato sauce-based pasta dishes on lycopene uptake, oxidative DNA damage, and prostate-specific antigen (PSA) levels in patients already diagnosed with prostate cancer. **Methods:** Thirty-two patients with localized prostate adenocarcinoma consumed tomato sauce-based pasta dishes for the 3 weeks (30 mg of lycopene per day) preceding their scheduled radical prostatectomy. Serum and prostate lycopene concentrations, serum PSA levels, and leukocyte DNA oxidative damage (ratio of 8-hydroxy-2'-deoxyguanosine [8-OHdG] to 2'-deoxyguanosine [dG]) were assessed before and after the dietary intervention. DNA oxidative damage was assessed in resected prostate tissue from study participants and from seven randomly selected prostate cancer patients. All statistical tests were two-sided. **Results:** After the dietary intervention, serum and prostate lycopene concentrations were statistically significantly increased, from 638 nM (95% confidence interval [CI] = 512 to 764 nM) to 1258 nM (95% CI = 1061 to 1455 nM) ($P < .001$) and from 0.28 nmol/g (95% CI = 0.18 to 0.37 nmol/g) to 0.82 nmol/g (95% CI = 0.57 to 1.11 nmol/g) ($P < .001$), respectively. Compared with preintervention levels, leukocyte oxidative DNA damage was statistically significantly reduced after the intervention, from 0.61 8-OHdG/ 10^5 dG (95% CI = 0.45 to 0.77 8-OHdG/ 10^5 dG) to 0.48 8-OHdG/ 10^5 dG (95% CI = 0.41 to 0.56 8-OHdG/ 10^5 dG) ($P = .005$). Furthermore, pros-

tate tissue oxidative DNA damage was also statistically significantly lower in men who had the intervention (0.76 8-OHdG/ 10^5 dG [95% CI = 0.55 to 0.96 8-OHdG/ 10^5 dG]) than in the randomly selected patients (1.06 8-OHdG/ 10^5 dG [95% CI = 0.62 to 1.51 8-OHdG/ 10^5 dG]; $P = .03$). Serum PSA levels decreased after the intervention, from 10.9 ng/mL (95% CI = 8.7 to 13.2 ng/mL) to 8.7 ng/mL (95% CI = 6.8 to 10.6 ng/mL) ($P < .001$). **Conclusion:** These data indicate a possible role for a tomato sauce constituent, possibly lycopene, in the treatment of prostate cancer and warrant further testing with a larger sample of patients, including a control group. [J Natl Cancer Inst 2001;93:1872-9]

Prostate cancer is the second leading cause of cancer-related death among U.S. men. The incidence of prostate cancer is higher in African-American men than in Euro-American men (1). Although the reasons for the high incidence are unknown, human prostate tissue may be particularly vulnerable to oxidative DNA damage by free radicals, which are thought to play a critical role in all stages of carcinogenesis (2,3). Several explanations have been put forth to explain the vulnerability of prostate tissue to oxidative DNA damage, including faster cell turnover, fewer DNA repair enzymes, and chronic inflammation of prostate epithelial cells (4).

Oxidative damage to the DNA base 2'-deoxyguanosine(dG) produces 8-hydroxy-2'-deoxyguanosine (8-OHdG). 8-OHdG is the most prevalent DNA damage product and, when incorporated into DNA, leads to a point mutation via an A to T substitution (5). Increased 8-OHdG levels have been observed in target tissues of several animal cancer models (6,7) and in human leukocytes from patients with various diseases associated with oxidative stress (8,9). Decreased 8-OHdG levels have also been reported in human leukocytes from patients receiving foods high in antioxidants (10,11). Such foods include those high in tomatoes, which contain lycopene.

Lycopene, a non-provitamin A carotenoid (i.e., the red pigment in tomatoes), is the most efficient singlet-oxygen (a reactive oxygen species) quencher among the natural carotenoids (12). Epidemiologic studies (13,14) have shown that, among the antioxidant nutrients, only

high lycopene intake or plasma concentrations are associated with a lower risk of prostate cancer. For example, consumption of four or five servings of tomato products per week was associated with a 40% lower risk of prostate cancer in U.S. men (13). The mechanism by which lycopene reduces prostate cancer risk is unclear. Lycopene has been shown to inhibit proliferation in various cancer cell lines (15-17), but the poor absorption of carotenoids by laboratory rodents has severely hampered the use of animal models in cancer prevention studies to evaluate the efficacy and mechanism of action of lycopene.

In this study, we conducted a high-lycopene, tomato sauce-based, whole-food intervention in a predominantly African-American population diagnosed with prostate cancer. We chose this study population who are undergoing prostatectomy because of the availability of prostate tissue collected at resection for the assessment of lycopene accumulation and DNA damage. We also assumed that these patients may be experiencing higher rates of oxidative DNA damage because of the disease process. We evaluated the adherence to the intervention, the accumulation of lycopene in prostate tissue, and the oxidative DNA damage measured by 8-OHdG/ 10^5 dG ratios in leukocyte and prostate DNA.

PATIENTS AND METHODS

Study Design and Patient Recruitment

The study used a human presurgery prostate cancer model and was a nonrandomized, whole-food intervention arm of an ongoing placebo-controlled clinical trial for the evaluation of lycopene as an

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in vivo antioxidant. For the purposes of this report, the data were evaluated for only the whole-food intervention, and each patient was his own control.

Patients were recruited through verbal invitation by medical personnel from the Urology Clinic of the Westside Department of Veterans Affairs (VA) Hospital, Chicago, IL, during the period from May 1998 through July 1999. Thirty-two patients completed the study. Each patient was diagnosed with clinical stage T1 or T2 adenocarcinoma of the prostate (18). Upon reviewing all of the treatment options with the chief physician of the clinic, those patients who were younger than 75 years and had selected prostatectomy as their treatment plan were eligible for the study. After pre-admitting tests (x-ray, electrocardiogram, and major blood chemistry tests), those subjects who did not have cardiovascular diseases, chronic obstructive pulmonary diseases, deep venous thrombosis, alcohol or drug intoxication, or other medical problems that would exclude them from surgery were invited to sign a consent form. Patients were instructed to stop smoking and drinking alcohol during the study as part of the requirement for prostatectomy. Exclusion criteria also included the following: allergies to tomatoes or tomato products; consumption of dietary supplements containing more than the recommended dietary requirement for vitamins C, E, and A and β -carotene; participation in alternative research studies; or a history of chronic diseases associated with increased oxidative stress (i.e., inflammatory bowel disease, cardiovascular disease, or presence of other cancers). Thirty-five men were recruited, two dropped out, one patient was excluded for not meeting inclusion requirements, and one patient was excluded from the DNA damage and prostate-specific antigen (PSA) data analysis because he was identified as a drug user and his baseline leukocyte 8-OHdG/ 10^5 dG ratio and PSA concentrations were two standard deviations (SDs) above the mean. In addition to the prostatectomy tissues obtained from the patients enrolled in this study, we obtained resected prostate tissues at prostatectomy from seven randomly selected patients from the same patient pool (average age \pm SD, 65.7 ± 2.1 years), but who did not participate in the study, as a reference group. All of the patients were asked to provide written informed consent before participating in the study, which was approved by the Institutional Review Boards of the University of Illinois at Chicago and the Westside VA Medical Center.

Patient Protocol

On the initial visit after recruitment, each patient was scheduled for the regular transrectal-ultrasound/prostate needle biopsy because of either rising serum PSA concentrations or an abnormality detected by digital rectal examination. The patient received a take-home brochure that explained the purposes, benefits, and risks of this study and gave informed consent to allow the collection of two additional biopsy tissues during his regular transrectal-ultrasound/prostate needle biopsy. The patient was also informed that participation in the study depended on the results of the transrectal-ultrasound/prostate needle biopsy. After the results of the patients' biopsy specimens were reviewed, all of the patients who were diagnosed with adenocarcinoma of the prostate were potential candidates for prostatectomy, which made them eligible for the study.

Ninety-one percent of the candidates agreed to participate in the entire clinical trial. Because the window for scheduling surgery was 3–5 weeks, the presurgery, tomato sauce-based, whole-food intervention period was set at 3 weeks.

Commercial spaghetti sauce (Hunt-Wesson, Inc., Fullerton, CA) from the same lot number was used as the source of lycopene (30 mg of lycopene in 200 g [or three fourths of a cup] of spaghetti sauce per day) and was incorporated into four different entrees: sausage lasagna, baked rigatoni, penne pasta, and stuffed shells. Study cooks in the Nutrition and Metabolism Research Laboratory at the University of Illinois at Chicago prepared the entrees. All of the entrees were kept frozen until used. Each patient was given a choice of one entree for lunch or dinner. Frozen meals were picked up once every week or 2 in the Urology Clinic at the Westside VA Medical Center by the patients or, on some occasions, were delivered to those unable to come to the clinic. All of the patients were provided microwaveable containers to reheat the entrees for 8–10 minutes before they were consumed. Patients self-selected the remainder of their diet.

During the baseline visit, patients provided a pre-intervention fasting blood sample, were measured for height and weight, and were interviewed by the investigators to obtain information on age, marital status, health history, employment history, educational level completed, daily intake of medications and supplements, smoking history, and history of alcohol consumption. The patients were trained to record their daily consumption of the tomato sauce entrees by shading the portion consumed on a diagram depicting the rectangular shape of the entree. In addition, three 24-hour food-intake recalls (2 weekdays and 1 weekend day) were obtained before the intervention began. For some patients with an accelerated surgery schedule, baseline food-intake recalls were obtained after they had recovered from the surgery. A second set of food-intake recalls, obtained during the second week of the intervention by telephone interview, was used to assess the patient's energy and lycopene displacement from, and compliance to, the whole-food intervention. We assessed lycopene intake by multiplying the portion size of each consumed food by its lycopene content, which was downloaded from the U.S. Department of Agriculture Internet site (www.usda.gov) for carotenoid content of foods.

Because tomato products have been suspected to cause symptoms of heartburn and gastrointestinal distress, each patient was provided with a one-page checklist on which to record his experience of gastrointestinal adverse effects that could arise during the dietary intervention. These effects included, but were not limited to, the following: constipation, burping, gas and/or flatulence, nausea, bloating, diarrhea, cramping, and heartburn. For individuals who required assistance, charts were completed via telephone or in-person interviews. For each patient, the frequency of a symptom was recorded as the total number of days that the symptom occurred.

After the 3-week dietary intervention, patients returned to the clinic 1–2 days before surgery with their completed diet-compliance diagram, the gastrointestinal adverse effect evaluation form, and a daily record of medications, including vitamin supplements and antacids. A postintervention fasting blood sample was collected. The patient's per-

cent body fat was measured by the Weight Manager Bioelectrical Impedance System (BIA-101A; RJL Systems Inc., Clinton Township, MI). The patient's weight was also ascertained.

Assessment of Lycopene Concentration in Tomato Sauce Entrees, Prostate, and Serum

Four different entrees and the spaghetti sauce alone were analyzed for lycopene concentration. Prepared entrees were weighed, the contents of each were removed with a rubber spatula and placed in a blender, and the empty container was weighed again. The weight of the clean container was used to determine the residue, which was less than 10 g. Food was then blended with an equal volume of methanol containing the antioxidant pyrogallol (Fisher Scientific, Pittsburgh, PA) (2% wt/vol; 1 mL of methanol to 1 g of food). Because of poor and variable lycopene recovery from the high-fat pasta entrees, repeat samples were blended again with 50:50 vol/vol of water:methanol containing 1% pyrogallol (using the proportion of 1:10, wt/vol food to liquid). Aliquots (1 mL) of the homogenate were analyzed in triplicate, after they were weighed to the nearest 0.0001 g. The blended food was mixed with 60% KOH (0.2 mL per 1-g aliquot of the blended food mixture) and incubated for 1 hour at 70 °C to remove triglycerides (saponification). The mixture was then extracted with hexane (2 mL) by vortexing for 1 minute until the lycopene upper hexane layer was visibly yellow. The process was repeated four or five times until the last fraction of hexane was devoid of color. The hexane fractions were combined, washed with water, evaporated to dryness in a vacuum centrifuge, and then reconstituted with 1 mL of peroxide-free diethyl ether and 3 mL of methanol:acetonitrile:tetrahydrofuran, 50:45:5 vol/vol/vol. This mixture of solvents (methanol:acetonitrile:tetrahydrofuran) was used as a mobile phase in an isocratic high-performance liquid chromatography (HPLC) system to separate the carotenoids. The reconstituted samples were placed in autosampler vials, and a 10- μ L volume was injected onto a Novapak C18 column (Waters Corp., Milford, MA) and eluted with the mobile phase described above at a flow rate of 1 mL/minute (19).

Prostate biopsy specimens were collected by transrectal-ultrasound/prostate needle biopsy, and prostatectomy tissues collected during the surgery were stored in 0.9% saline in a 1.5-mL Eppendorf tube (Waters Corp.), transferred on dry ice to the laboratory, and centrifuged (14 000 rpm at 4 °C, Microcentrifuge 5414C; Fisher Scientific) for 1 minute. After the saline was removed, the tissues were nitrogen purged and frozen at –80 °C. The fresh weight of the transrectal-ultrasound/prostate needle biopsy specimens ranged from 5 to 18 mg, and that of the prostatectomy specimens ranged from 7 to 103 mg. Each prostate tissue specimen was placed in a 15-mL glass tube with 1.0 mL of 50% methanol (1% pyrogallol) and homogenized at 4 °C by a Powergen 125 homogenizer (Fisher Scientific) at high power for 8–10 minutes. After the prostate tissues were homogenized, the extraction and analytical methods were identical to those described above for food samples (20), with the necessary adjustment for small samples. A whole-tissue homogenate was used for saponification and extraction. Hexane ex-

traction of each sample was repeated twice, and the final volume of reconstituted sample varied from 100 to 200 μL , depending on the weight of the tissue. The injection volume varied from 20 to 50 μL because the limit of detection was 0.1 ng of lycopene per injection.

Serum and plasma were isolated from the collected blood samples, and aliquots of 0.5 mL were stored at -80°C . For the determination of the lycopene concentration, duplicate aliquots of plasma were thawed, depleted of proteins with ethanol, extracted twice with 2 mL of hexane, and then analyzed by HPLC with the use of the method described by Stacewicz-Sapuntzakis et al. (19). The coefficient of variation was 7.4% for the total lycopene concentration, which was based on multiple, individual assessments of the concentration in control plasma. Our laboratory is a reference laboratory for the National Institute of Standards and Technology (Gaithersburg, MD) quality-assurance program for carotenoids (21).

Leukocyte and Prostate DNA 8-OHdG Measured by HPLC-Electrochemical Detection

For the examination of 8-OHdG/ 10^5 dG ratios in leukocyte and prostate DNA samples, at the beginning and the end of the study, 15 mL of blood was taken from patients by venipuncture. The blood was collected into heparinized vacutainer tubes. Plasma and blood cells were separated by centrifugation at 3000g at 4°C for 15 minutes. The vacutainer tubes of blood cells were placed in 50-mL Falcon tubes (Fisher Scientific, Pittsburgh, PA) and stored at -80°C for no more than 2 weeks before the extraction of the nuclei.

Nuclei were prepared from 5- to 10-mL samples of blood by the method of Ciulla et al. (22) and were purged with nitrogen gas so that the nuclei could then be stored at -80°C for at least 6 months. DNA was isolated by incubating the nuclei in 900 μL of 1% wt/vol sodium dodecyl sulfate-EDTA, 90 μL of 5 M NaCl, 200 μL of ribonuclease (RNase) A (73 kU/mg in 2 mg/mL of 10 mM Tris [pH 5.0]), and 10 μL of RNase T1 (7500 U/mL) at 37°C for 30 minutes. Next, 75 μL of proteinase K (12 U/mg; 40 mg/mL) (Sigma Chemical Co., St. Louis, MO) was added to the solution for incubation at 60°C for 30 minutes. After the addition of 180 μL of 5 M NaCl and 10 μL of 1 M Tris (pH 8.0), DNA samples were extracted with an equal volume of n-butanol (99.8%) and precipitated with cold absolute ethanol. The DNA was pelleted by centrifugation at 4°C (14 000g) for 20 minutes, washed twice with 70% ethanol, and dissolved in 200 μL of 50 mM ammonium acetate containing 0.2 mM ZnCl_2 at pH 5.2. For the determination of the concentration and purity of the DNA, 5 μL of DNA was mixed with 495 μL of 5 mM bis-Tris, i.e., 2-[bis(2-hydroxyethyl)-imino]-2(hydroxy-methyl)-1,3-propanediol (Kodak, Rochester, NY), and 1 mM EDTA, and the absorbance at 260 nm (A_{260}) and 280 nm (A_{280}) was measured spectrophotometrically. A DNA sample with high purity would be expected to have an A_{260}/A_{280} ratio of 2.0. The ratios of A_{260}/A_{280} of all of the DNA samples were between 1.6 and 1.9.

The DNA from all prostate tissues was extracted by the method of Haeghele et al. (23). Approximately 200 mg of tissue was mixed with 1.0 mL of 7.5 M

ammonium acetate in a 13-mL polypropylene screw-cap tube and homogenized by a Powergen 125 homogenizer at "high" speed at 4°C for 10 minutes, after which 3 mL of proteinase K (500 $\mu\text{g}/\text{mL}$) and 5 μL of 26.4 mg/mL butylated hydroxytoluene were added and incubated at 50°C for 4 hours. After cooling for 5 minutes, the suspension was thoroughly mixed and centrifuged at 14 636g for 15 minutes at 4°C . The supernatant was removed and extracted twice with 1 mL of n-butanol (99.8%) and centrifuged at 5111g for 10 minutes at 4°C to separate the organic and aqueous phases. The aqueous phase was collected, and the DNA was precipitated by adding 3 mL of isopropyl alcohol and inverting the tube repeatedly. The precipitated DNA was transferred to an Eppendorf tube containing 500 μL of 100% ethanol. The DNA was washed twice with 70% ethanol, dried under vacuum for 5 minutes, and then reconstituted with 50 mM ammonium acetate containing 0.2 mM ZnCl_2 at pH 5.2 and stored at -80°C until analysis.

For the measurement of 8-OHdG/ 10^5 dG ratios, DNA was hydrolyzed by mixing 100 μL of DNA (1 $\mu\text{g}/\mu\text{L}$) with 10 μL of Nuclease P1 (Sigma Chemical Co.) (280 U/mL in 20 mM sodium acetate [pH 5.2]) and 5 μL of alkaline phosphatase (1 U/ μL of 50 mM Tris [pH 7.4]) and incubated at 37°C for 90 minutes. The hydrolysate was filtered by a 30 K micro-centrifuge filter (Osmonics Inc., Minnetonka, MN) and stored at 4°C before HPLC analysis within 48 hours. The quantity of free dG bases liberated from known concentrations of salmon sperm DNA was measured by HPLC analysis to assess the completeness of hydrolysis reaction and was consistently around 80%. The HPLC-electrochemical detector system consisted of a 712 WISP autoinjector (Waters Corp.), a model 580 solvent delivery system (ESA Inc., Bedford, MA) equipped with pulse damper, a Coulochem II detector (from ESA Inc.) equipped with a 5010 dual flow-cell, and a model 490 Waters UV detector. The mobile phase (100 mM sodium acetate and 5% methanol [pH 5.2]) was made with HPLC-grade chemicals, filtered, and degassed with helium. The column used to isolate 8-OHdG was SUPELCO (LC-18, 15 cm \times 4.6 mm; Supelco Co., Bellefonte, PA) operating at a flow rate of 1.0 mL/minute.

Aqueous stock solutions of dG and 8-OHdG (both from Sigma Chemical Co.) were prepared and stored at -20°C . These stock solutions were diluted with mobile phase and used as working standards. For the HPLC-electrochemical detector detection of 8-OHdG, the first electrode (guard cell) removed inherent electroactive impurities from the mobile phase and was set at +450 mV. The peak potential of 8-OHdG was +350 mV. The best operating conditions were 150 mV for cell 1 (conditioning cell) and 400 mV for cell 2 (analytical cell). dG was assessed simultaneously by UV light detection at 260 nm, and the number of 8-OHdG molecules was expressed as a ratio to 10^5 unoxidized dG molecules. The approximate limit of detection was 0.01 ppm (1×10^{-5} ng/ μL). The coefficients of variation for intra-assay and inter-assay were 4.5% ($n = 10$) and 7.6% ($n = 7$), respectively.

Serum PSA Concentrations

Serum PSA concentrations were analyzed with the use of the Micro-particle Enzyme Immunoassay

technology (Abbott Laboratories, Abbott Park, IL) in the Pathology Laboratory of the University of Illinois at Chicago Hospital. The within-run and between-run coefficients of variation were 4.4% and 4.6%, respectively. The detection limit was 0.1 ng/mL. Quality-control serum samples were assayed each time, along with the study samples, to check the accuracy of the test.

Pathology

Gleason scores (24) were obtained from the patients' medical records for prostate biopsy samples taken both before the tomato sauce intervention and at the time of the prostatectomy after the intervention.

Statistical Analysis

The changes in serum lycopene, prostate lycopene, and serum PSA concentrations as well as in leukocyte and prostate DNA 8-OHdG/ 10^5 dG ratios were evaluated by two-sided paired Student's *t* tests. Only complete sets of data were analyzed. Leukocyte DNA 8-OHdG/ 10^5 dG ratios were square root transformed to overcome skewness. Simple regression analysis was used to evaluate the correlation between plasma and prostate lycopene concentrations and leukocyte and prostate 8-OHdG/ 10^5 dG ratios as well as PSA. The statistical package SAS (version 7.1, 1997; SAS Institute, Inc., Cary, NC) was used for analyses. Differences of $P < .05$ were considered to be statistically significant. All statistical tests were two-sided.

RESULTS

To determine the effect of a diet high in lycopene (i.e., rich in tomato sauce-based whole foods) on men with prostate cancer, we used a human presurgery model of prostate cancer. Thirty-two men, aged 60–74 years, completed the study. Twenty-seven biopsy samples were collected, and 28 of 32 patients had prostatectomy. Table 1 presents the demographic characteristics of all 32 patients. The Westside VA Hospital serves a predominantly African-American population, and African-American men made up 75% of the patients enrolled in the study. All of the patients ate one tomato sauce-based entree per day. The mean number of intervention days \pm SD was 19.8 ± 0.6 days, with variation because of fluctuations in surgery scheduling. Only three of 32 patients reported minor gastrointestinal problems, which resolved within a few days. Overall, the patients consumed 81.7% (95% CI = 74.0% to 89.4%) of the total intended dose (630 mg) of lycopene in the tomato sauce entrees during the intervention period. The mean self-selected lycopene consumption reported before the study was 5.0 mg/day. Because

Table 1. Characteristics of the 32 patients and adherence to tomato sauce whole-food intervention

Characteristic	Mean \pm standard deviation
Age, y	63.7 \pm 6.1
Height, cm	175.7 \pm 7.7
Weight, kg	87.0 \pm 17.7
% of body fat	20.9 \pm 5.4
Body mass index, kg/m ²	28.0 \pm 4.9
Ethnicity	
African-American, %	75.0
Other, %	25.0
No. of intervention days	19.8 \pm 0.6
Compliant days,* %	90.0 \pm 2.0
Compliant to total dose,† %	81.7 \pm 21.5
Self-selected lycopene consumed before the study, mg/day	5.0 \pm 7.3
Self-selected lycopene consumed during the study, mg/day	1.0 \pm 0
Total lycopene consumed during the study, mg/day	26.8 \pm 2.2

*Number of days \geq 25% of entree was consumed/number of days that patients received entrees.

†Total grams of lycopene consumed/total grams of lycopene received in entrees.

of the large amount of tomato sauce consumed as the intervention, the lycopene consumption from the remainder of the self-selected diet decreased to 1.0 mg/day ($P = .02$). The mean daily lycopene consumption for the tomato sauce entree was 22.2 ± 1.9 mg, and the mean total lycopene consumption was 26.8 mg/day.

Adherence to the whole-food intervention should be reflected in increased lycopene concentrations in serum and prostate tissues. We measured the mean plasma lycopene concentrations before and after the tomato sauce intervention. We found

that these concentrations increased 1.97-fold, from 638 nM (95% CI = 512 to 764 nM) to 1258 nM (95% CI = 1061 to 1455 nM), a statistically significant difference ($P < .001$) (Fig. 1, left panel), indicating excellent adherence to the diet. Compared with the lycopene concentration before the study, the mean prostate lycopene concentration after the intervention increased 2.92-fold, from 0.28 nmol/g (95% CI = 0.18 to 0.37 nmol/g) to 0.82 nmol/g (95% CI = 0.57 to 1.11 nmol/g), a statistically significant increase ($P < .001$) (Fig. 1, right panel), indicating

excellent accumulation of lycopene in prostate tissues. There were positive correlations between serum lycopene and prostate lycopene concentrations, both before (at baseline, $r = .46$; $P = .01$) and after (at endpoint, $r = .47$; $P = .01$) the intervention.

Because lycopene has been reported to reduce oxidative damage (25,26), we next measured the 8-OHdG/10⁵ dG ratios in leukocyte and prostate tissue DNA. Compared with ratios from leukocytes isolated before the intervention, the average leukocyte DNA 8-OHdG/10⁵ dG ratio of 31 prostate cancer patients decreased by 21.3% from 0.61 8-OHdG/10⁵ dG (95% CI = 0.45 to 0.77 8-OHdG/10⁵ dG) to 0.48 8-OHdG/10⁵ dG (95% CI = 0.41 to 0.56 8-OHdG/10⁵ dG) after the tomato sauce intervention, a statistically significant difference ($P = .005$) (Fig. 2). One patient was found to be a drug user after testing and before his scheduled surgery. He had a leukocyte DNA 8-OHdG/10⁵ dG ratio of 2.51 that decreased to 0.48 8-OHdG/10⁵ dG after eating the pasta meals, during which he abstained from drug use in accordance with the presurgery requirements. His data were not included in the analysis because the value was an extreme outlier that was more than 2 SDs above the mean. Although eight of 31 patients were current smokers (smoked 15 cigarettes per day before the study) and three of 31 were alcohol drinkers, the decrease in leukocyte 8-OHdG/10⁵ dG ratios after intervention did not statistically significantly differ between subgroups: smokers compared with nonsmokers, 27.1% [95% CI = 23.4% to 31.6%] versus 28.5% [95% CI = 24.1% to 31.7%]) and drinkers compared with nondrinkers, 16.9% [95% CI = 13.8% to 18.5%] versus 21.2% [95% CI = 17.2% to 23.1%]). The mean prostate DNA 8-OHdG/10⁵ dG ratio for the patients who received the tomato sauce-based, whole-food intervention was 0.76 8-OHdG/10⁵ dG (95% CI = 0.55 to 0.96 8-OHdG/10⁵ dG), 28.3% lower than for the reference group who did not receive the intervention (1.06 8-OHdG/10⁵ dG [95% CI = 0.62 to 1.51 8-OHdG/10⁵ dG]), a statistically significant difference ($P = .03$) (Fig. 3). The postintervention 8-OHdG/10⁵ dG ratio was statistically significantly higher in prostate tissue DNA (0.76 8-OHdG/10⁵ dG [95% CI = 0.55 to 0.96 8-OHdG/10⁵ dG]) than in leukocyte DNA (0.48 8-OHdG/10⁵ dG [95% CI = 0.41 to 0.56 8-OHdG/10⁵ dG]). The 8-OHdG/10⁵ dG

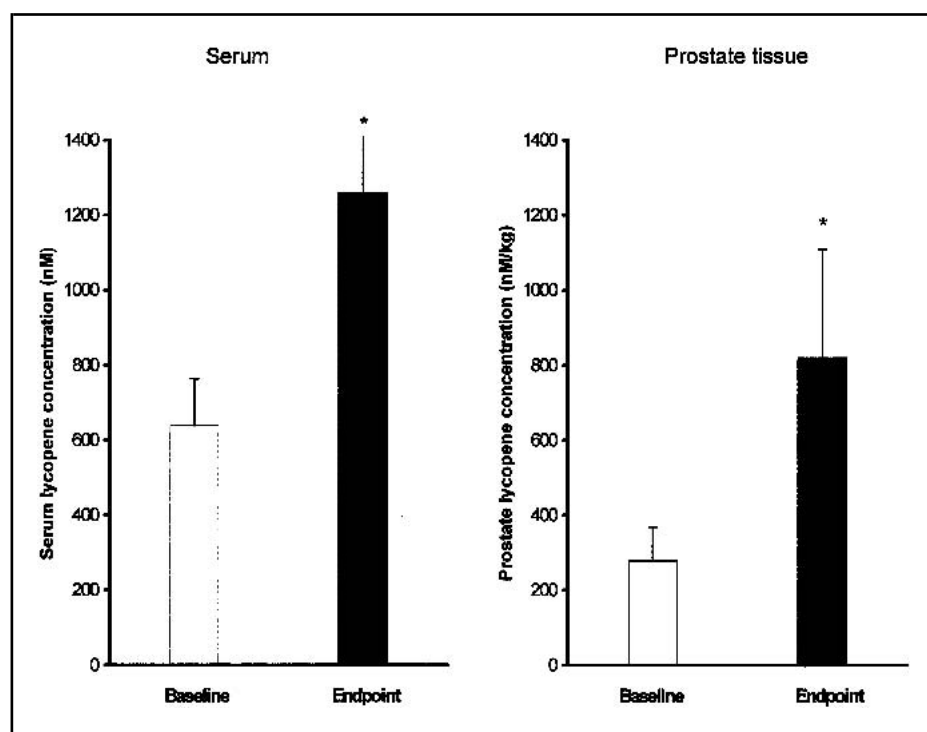


Fig. 1. Accumulation of lycopene in human serum and prostate tissue from patients diagnosed with prostate cancer after a 21-day, tomato sauce-based, whole-food intervention. **Left panel:** serum lycopene concentrations at baseline and after intervention ($n = 32$). **Right panel:** prostate lycopene concentrations at baseline and after intervention ($n = 25$). **Bars** represent the means and the 95% confidence intervals.

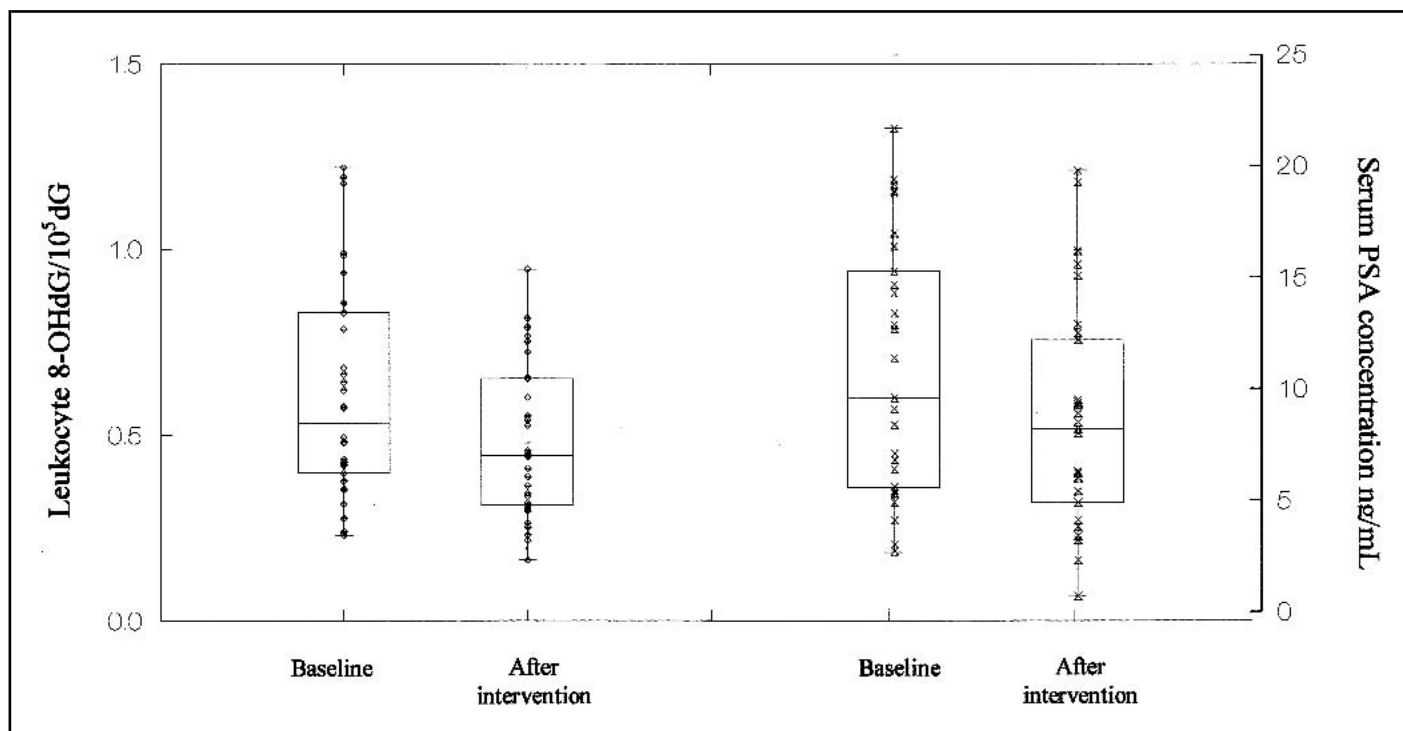
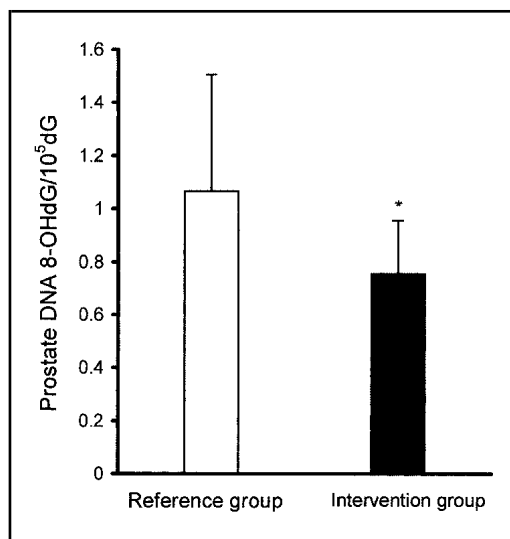


Fig. 2. Leukocyte oxidative DNA damage and serum prostate-specific antigen (PSA) levels in prostate cancer patients after 21-day, tomato sauce-based, whole-food intervention. Oxidative DNA damage was determined by high-performance liquid chromatography as the number of 8-hydroxy-2'-deoxyguanosine (8-OHdG) bases per 10^5 2'-deoxyguanosine (dG) bases. PSA levels were measured by microparticle enzyme immunoassay. Data are presented in a box plot, where each circle or cross represents the leukocyte DNA 8-OHdG/ 10^5 dG ratio ($n = 30$)

or serum PSA concentration ($n = 29$) of one patient. **Center horizontal lines** indicate the medians of the samples. The **length of each box** (interquartile range) indicates the range within which the central 50% of the values fell, with the box edges placed at the first and third quartiles. **Whiskers (the lines extending beyond the box)** show the range of the observed values that fell within 1.5 times the interquartile range.

Fig. 3. Prostate tissue oxidative DNA damage in prostate cancer patients after 21-day, tomato sauce-based, whole-food intervention. Oxidative DNA damage was determined by high-performance liquid chromatography as the number of 8-hydroxy-2'-deoxyguanosine (8-OHdG) bases per 10^5 2'-deoxyguanosine (dG) bases. The mean prostate DNA 8-OHdG/ 10^5 dG ratios for patients who participated in the study ($n = 32$) were compared with those of randomly selected prostate cancer patients who did not participate in the study ($n = 7$). **Bars** represent the means and the upper 95% confidence intervals.



ratios in the prostate tissue and leukocyte DNA were after the intervention positively correlated ($r = .514$; $P = .005$).

We also measured serum PSA levels before and after the whole-food intervention. Fig. 2 also shows that serum PSA concentrations decreased by 17.5%, from 10.9 ng/mL (95% CI = 8.7 to 13.2 ng/mL) to 8.7 ng/mL (95% CI = 6.8 to 10.6 ng/mL), a statistically significant differ-

ence ($P < .001$), after 20 days on the tomato sauce-based intervention. We found no correlation between baseline, end-point, or change in PSA and prostate lycopene concentration or leukocyte DNA 8-OHdG/ 10^5 dG ratios. The mean Gleason scores \pm SD of the biopsy specimens and resected prostate tissue were 6.6 ± 1.1 and 6.5 ± 1.1 , respectively, and were not correlated with prostate lycopene content,

DNA 8-OHdG/ 10^5 dG ratios, or serum PSA concentrations.

DISCUSSION

The use of a human presurgery prostate model to evaluate the modulation of the carcinogenic process by foods or chemopreventive agents has both benefits and drawbacks. The food or agent must be absorbed, must accumulate in target tissues, and must exert its effect within a short time. Because patients already have cancer, the presurgery model is not a true chemopreventive model. The window of opportunity between diagnosis and surgery is usually short, approximately 1 month. We previously found (27), however, that modifying the diet of healthy humans for 3 weeks was sufficient time to detect a decrease in leukocyte DNA oxidative damage.

In epidemiologic studies (13,14), tomato sauce consumption is associated with a lower risk of prostate cancer, in part because lycopene may be better absorbed from tomato sauce than from fresh tomatoes or because lycopene is not the only antioxidant in tomato products (28,29). Phenolic compounds account for

approximately 5130 $\mu\text{g/g}$ of the dry weight of tomato paste, whereas lycopene, other carotenoids, and ascorbic acid account for 1525, 72, and 433 $\mu\text{g/g}$, respectively, of the dry weight of tomato paste. When the antioxidant activities of lipid and water-based extracts of various tomato products were compared, the average dry weight of tomato paste required to produce 50% inhibition of oxidation was 2375 μg in hydrophilic antioxidant systems and 1143 μg in lipophilic antioxidant systems (30). These data suggest that protection against lipophilic oxidants was moderately more effective than protection against hydrophilic oxidants. Lycopene accounted for 55% of the measured tomato paste antioxidant in the lipophilic system (30). In our study, all of the tomato-based antioxidants may have contributed to the observed reduction in DNA damage; thus, the additive effects between the antioxidants cannot be excluded.

In this study, we took the novel approach of using a tomato sauce-based, whole-food entree as a way to increase dietary lycopene in human prostate tissue. This approach, which was well accepted by the men in our study, led to a 2.92-fold accumulation of lycopene in prostate tissue within a short time. Accumulation in prostate tissue did not exceed that found in plasma, 820 nmol/kg (95% CI = 570 to 1075 nmol/kg) versus 1258 nmol/L (95% CI = 1061 to 1455 nmol/L) (assuming that tissue is predominantly water and 1 L of water = 1 kg of water). Our results support the results reported by Boileau et al. (31), who also found that the mean lycopene concentration in rat prostate tissue did not exceed plasma concentrations after lycopene supplementation for 8 weeks. Our baseline prostate lycopene concentrations were consistent with those reported by Clinton et al. (32), which ranged from 0.63 to 0.91 nmol/g in 25 prostate adenocarcinoma patients undergoing prostatectomy.

Short-term dietary intervention with a tomato sauce-based entree decreased oxidative damage to DNA in prostate cancer patients by 21%, as measured by the mean leukocyte DNA 8-OHdG/ 10^5 dG ratios. These data are consistent with those obtained by Rao and Agarwal (11), who gave a placebo, spaghetti sauce (20.5 and 39.2 mg of lycopene), tomato juice (50.4 mg of lycopene), or tomato oleoresin (75 and 150 mg of lycopene) to 19 healthy subjects daily for 1-week periods

followed by 1-week washout periods and observed decreased lymphocyte DNA 8-OHdG/ 10^5 dG ratios (an average 20.7% decrease), although this decrease was not statistically significant. To explore the consistency of the reduction in leukocyte oxidative DNA damage among our patients, we calculated the slope of the line between baseline and endpoint of 8-OHdG/ 10^5 dG for each patient. Of 31 patients, 20 had negative slopes. These data were consistent with our previous finding (27) that short-term diet modification could lower leukocyte DNA damage.

The mean prostate DNA 8-OHdG/ 10^5 dG ratio after the tomato sauce-based, whole-food intervention was lower than that in seven resected prostate tissues from a reference group of patients who did not participate in the study. These data suggest that prostate cancer patients have high prostate DNA damage that may be reduced with tomato sauce consumption. These higher levels of prostate oxidative DNA damage may be linked to higher levels of androgen exposure, a recognized risk factor for prostate cancer, because Ripple et al. (33,34) found that exposure of human prostate cancer cells to androgen can induce the production of reactive oxygen species.

Although we found that the mean prostate DNA 8-OHdG/ 10^5 dG ratio was higher than the mean leukocyte 8-OHdG/ 10^5 dG ratio at the study endpoint, we found a correlation ($r = .514$) between prostate and leukocyte oxidative DNA damage. Farinati et al. (9) found that the 8-OHdG/ 10^5 dG ratio in circulating leukocytes correlated with that of liver tissue ($r = .618$) in 87 patients with chronic hepatitis B or C and suggested that the 8-OHdG/ 10^5 dG ratio in leukocytes was a reliable marker of oxidative stress occurring in the liver. Thus, it may be possible to use oxidative damage to leukocyte DNA as a surrogate marker for oxidative damage to prostate tissue to monitor the effectiveness of antioxidant interventions.

Although tomato paste contains many potential antioxidants, it is likely that lycopene is the most active compound because it is a potent singlet-oxygen quencher and also quenches other oxygen radicals. Singlet oxygen is a major generator of 8-OHdG (5). Furthermore, singlet oxygen has been shown to be the principal oxidant in myeloperoxidase-mediated bacterial killing by human polymorphonuclear leukocytes *in vitro*. Bac-

teria transformed to produce endogenous lycopene were eight times more viable than wild-type bacteria against this singlet-oxygen-generating system (35). Porrini et al. (36) gave 25 g of tomato puree daily (corresponding to 7 mg of lycopene) to 11 healthy females for 14 days and found that lycopene concentrations in human lymphocytes increased by 44% and that lymphocyte DNA resistance to oxidative damage (measured as DNA strand breaks in lymphocytes after exposure to oxidants) increased by 50%. Their data suggest that lycopene or tomato products may contribute to the protection of DNA from oxidative stress in human lymphocytes. Our study provides supportive evidence that lycopene may prevent leukocyte DNA from oxidative damage measured by 8-OHdG/ 10^5 dG ratios and suggest that the same protection may be afforded to prostate DNA.

Elevated serum PSA concentrations are used routinely to determine the risk of prostate cancer and the need for prostate needle biopsy as well as to monitor the success of treatment. It was surprising to find that the 3-week, tomato sauce-based dietary intervention decreased serum PSA concentrations because serum PSA concentrations are not tumor specific and may be affected by confounding factors, such as age, prostatitis, benign prostate hyperplasia, sexual activity, and prostate needle biopsy (37). For example, investigators (38) found that the average serum PSA level increased by 0.3 ng/mL 1 day after prostate needle biopsy and returned to prebiopsy levels 1 week after the procedure. In our study, serum PSA levels were measured at least 2–3 weeks after the prostate needle biopsy procedure and were found to be similar to previous PSA values. A more robust evaluation of whether PSA concentrations respond to tomato sauce or lycopene supplementation awaits the completion of the randomized, placebo-controlled lycopene supplement arms of our ongoing clinical trial, when serum PSA levels can be compared among various treatment groups. Nevertheless, the apparent decrease in PSA concentrations that we observed after the tomato sauce-based, whole-food intervention provides encouragement for future interventions with tomato products of longer time periods in men with high PSA levels.

Although lycopene appears to prevent oxidative DNA damage, whether it also has direct effects on prostate cancer cell

growth *in vivo* is unknown. Two studies (15,16) have shown that lycopene, in physiologic concentrations, can inhibit prostate cancer cell growth, and Karas et al. (39) demonstrated that lycopene treatment inhibited the growth of mammary cancer cells stimulated to rapid growth by insulin-like growth factor-I (IGF-I). Lycopene appeared to have an inhibitory effect on a number of factors associated with IGF-1 cell signaling. IGF-1 cell signaling is thought to be an important factor in prostate cancer risk, and epidemiologic studies have linked higher plasma levels of IGF-1 with increased prostate cancer risk (40,41).

In conclusion, a short-term, tomato sauce-based, whole-food intervention was well accepted by prostate cancer patients, with lycopene substantially accumulating in prostate tissue. Serum PSA levels and human leukocyte oxidative DNA damage decreased after the intervention. The correlation between leukocyte and prostate DNA 8-OHdG/10⁵ dG ratios and lower prostate oxidative DNA damage in men consuming the tomato sauce suggests a role for tomato sauce and possibly for lycopene in the prevention and treatment of prostate cancer. However, this study had a small sample size. A more robust analysis of the data awaits the completion of the lycopene and placebo supplementation arms of our clinical trial of patients with and without diagnosed prostate cancer.

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