Prostate Carcinogenesis in N-methyl-N-nitrosourea (NMU)–Testosterone-Treated Rats Fed Tomato Powder, Lycopene, or Energy-Restricted Diets

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Background: Consumption of tomato products or lycopene and energy restriction have been hypothesized to reduce the risk of human prostate cancer. We investigated the effects of these dietary variables in a rat model of prostate carcinogenesis. Methods: Male rats (n = 194) treated with N-methyl-N-nitrosourea and testosterone to induce prostate cancer were fed diets containing whole tomato powder (13 mg lycopene/kg diet), lycopene beadlets (161 mg lycopene/kg diet), or control beadlets. Rats in each group were randomly assigned to either ad libitum feeding or 20% diet restriction. Differences between Kaplan–Meier survival curves for diet composition or restriction were tested with the log-rank test. Cox proportional hazards models were developed to examine the combined effect of diet composition and restriction on survival. Statistical tests were two-sided. Results: Risk of death with prostate cancer was lower for rats fed the tomato powder diet than for rats fed control beadlets (hazard ratio [HR] = 0.74, 95% confidence interval [CI] = 0.59 to 0.93; P = .009). In contrast, prostate cancer–specific mortality of the control and lycopene-fed rats was similar (P = .63). The proportions of rats dying with prostate cancer in the control, lycopene, and tomato powder groups were 80% (95% CI = 68% to 89%), 72% (95% CI = 60% to 83%), and 62% (95% CI = 48% to 75%), respectively. Rats in the diet-restricted group experienced longer prostate cancer–free survival than rats in the ad libitum–fed group (HR = 0.68, 95% CI = 0.49 to 0.96; P = .029). The proportion of rats that developed prostate cancer was 79% (95% CI = 69% to 86%) for ad libitum–fed rats and 65% (95% CI = 54% to 74%) for rats fed restricted diets. No interactions were observed between diet composition and dietary restriction. Conclusions: Consumption of tomato powder but not lycopene inhibited prostate carcinogenesis, suggesting that tomato products contain compounds in addition to lycopene that modify prostate carcinogenesis. Diet restriction also reduced the risk of prostate cancer. Tomato phytochemicals and diet restriction may act by independent mechanisms. [J Natl Cancer Inst 2003; 95:1578–86]

Both prospective epidemiologic and case–control studies (1–5) have associated increased consumption of tomato products and greater blood concentrations of lycopene with a reduced risk of prostate cancer. These observations have led many to hypothesize that lycopene, the principal carotenoid in tomatoes, may be the active component in tomato products (6). Lycopene is found in human prostate tissue, further suggesting the plausibility of a direct effect on prostate biology (7–11). In addition, results of a recent case–control study (12) revealed that pre-diagnosis blood lycopene concentrations were lower in men who developed prostate cancer than in men who remained disease-free. Results of in vitro studies suggest that lycopene inhibits the growth of human prostate cancer cell lines (13), is a potent antioxidant (14,15), influences expression of gap junction proteins (16), and inhibits growth factor signaling (17,18). Two recent studies of men with prostate cancer who were given a lycopene-enriched supplement (8) or fed tomato products (9) for several weeks prior to prostatectomy demonstrated that lycopene concentrations in the prostate can change rapidly in response to dietary intake and that biomarkers of oxidative stress and tumor biology can be altered.

Although none of these studies alone establishes a causal relationship between tomato products or lycopene consumption and prostate cancer risk (19), they constitute a growing body of evidence supporting a continued research effort to further dissect these relationships (1–18). It is of particular interest to determine whether lycopene itself is associated with reduced risk or whether it is simply a biomarker that is indicative of exposure to tomato products that may contain other phytochemicals with anti–prostate cancer properties (20). A laboratory animal model of prostate carcinogenesis is an ideal system in which to address this question.

To assess the role of lycopene in an experimental animal model, it is important to consider the ability of the species used to achieve biologically relevant tissue concentrations of lycopene. Our laboratory (21,22) and others (23–25) have shown that rats accumulate dietary lycopene in prostate, blood, and other tissues at concentrations that overlap those reported for humans if the dietary concentrations are sufficient to compensate for the lower bioavailability of carotenoids by rodents compared with humans. We have also observed that the pattern of lycopene...
isomers in the rat prostate is indistinguishable from that in the human prostate (7,21,22), with the majority of lycopene present as multiple \textit{cis}-isomers (7,21). Thus, the rat appears to be a reasonable \textit{in vivo} model in which to evaluate the biologic actions of lycopene during prostate carcinogenesis.

Energy balance is another dietary variable that is linked to prostate cancer risk. For example, recent reports suggest that frequent exercise (26) and lower body mass index (27) are associated with reduced prostate cancer risk. However, human studies alone have not allowed investigators to precisely quantify the role of energy balance during prostate carcinogenesis due to the relative difficulty in measuring the key variables throughout the life cycle as well as the complex relationships among energy intake, sources of energy, basal energy expenditures, activity-related energy expenditures, body weight, and body composition. Experimental models have proven useful in this regard, because many interacting variables can be controlled. For example, a role for energy restriction in prostate tumor growth has been clearly demonstrated in transplantable rodent models (28,29). Both modest total diet restriction and selective limitation of energy intake from carbohydrate or lipid sources statistically significantly reduced the growth of the well-differentiated hormone-sensitive Dunning R3327H prostate adenocarcinoma (28).

Several new animal models of prostate cancer are being developed that have unique characteristics that may be relevant to specific aspects of the carcinogenic process or to subtypes of prostate cancer exhibiting specific molecular defects and/or biologic properties (30–32). One such model is the \textit{N}-methyl-\textit{N}-nitrosourea (NMU)--androgen-induced rat model of prostate cancer developed by Bosland (31,32) and used in several recent chemoprevention studies (33–35). Whereas many rodent prostate cancer models result in cancers that predominantly affect the seminal vesicle and ventral prostate (36,37), the NMU--androgen-induced model causes tumors of the dorsolateral and anterior prostate (33). These lobes of the rat prostate are generally considered homologous to the areas of the human prostate that are most susceptible to cancer (30–33). In this model, the incidence of prostate carcinomas approaches 75% by approximately 52 weeks, with many showing evidence of androgen dependence and histopathologic features similar to human prostate cancer (30–37). In addition, the host experiences limited toxicity and does not exhibit a high frequency of malignancies at nontarget sites (33). Thus, the NMU--androgen-induced rat model is an anatomically and physiologically relevant system for the preclinical evaluation of substances that are hypothesized to inhibit or enhance human prostate carcinogenesis (33–35).

In this study, we assessed the individual and interactive effects of precisely controlled dietary interventions on the survival of rats treated with NMU androgens to stimulate prostate carcinogenesis. One goal was to determine whether freeze-dried whole tomatoes (tomato powder) or pure lycopene could enhance survival in this model. A second goal was to assess the ability of diet restriction to enhance survival in a prostate carcinogenesis model as we have observed in transplantable systems (28,29). Finally, we aimed to determine whether interactions between energy intake and tomato powder or lycopene intake could be observed.

### MATERIALS AND METHODS

#### Animals and Diet Formulations

Male Wistar-Unilever rats (HsdCpb:Wu) \((n = 194)\) (Harlan, Indianapolis, IN) were obtained at 5 weeks of age and fed a standard AIN-93G--based diet for 1 week of adaptation. At 6 weeks of age, rats were randomly assigned to one of three semi-purified AIN-93G--based experimental diets (38) prepared according to our formulations (Dyets, Bethlehem, PA). One diet contained control beadlets (Hoffmann-La Roche, Basel, Switzerland) \((n = 64\) rats\), the second contained lycopene beadlets (Hoffmann-La Roche) \((n = 65\) rats\), and the third contained tomato powder (Armour Foods, Springfield, KY) \((n = 65\) rats\) (Table 1). The control beadlet diet was prepared by incorporating water-dispersible beadlets into the experimental diets at a concentration of 2.5 g of beadlets per kilogram of diet. The lycopene beadlet diet was prepared similarly. The tomato powder (Armour Foods) is a spray-dried product made from heat-processed tomato paste (prepared from whole tomatoes including seeds and skins) (Del Monte Foods, San Francisco, CA). The tomato powder contains 12.9 g of protein, 74.6 g of carbohydrate, and 0.44 g of fat per 100 g of powder. Diets were stored at 4 °C in the dark. Rats were weighed weekly. The University of Illinois Laboratory Animal Care Advisory Committee approved all animal procedures.

#### Dietary Restriction

All rats initially consumed one of the three diets, with unlimited access to food (defined as \textit{ad libitum}). When they reached 10 weeks of age (3 days after carcinogen administration; see below), the rats in each dietary group were further subdivided and randomized to \textit{ad libitum} or 20% total dietary restriction for the remainder of the study. Diet-restricted rats were fed daily a quantity of food equal to 80% of the average daily intake.

#### Table 1. The formulation and composition of AIN-93G-based diets containing control beadlets, lycopene beadlets, or tomato powder (g/kg)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Control beadlet</th>
<th>Lycopene beadlet</th>
<th>Tomato powder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>l-cysteine</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Corn starch</td>
<td>381.24</td>
<td>381.24</td>
<td>297.49</td>
</tr>
<tr>
<td>Dextrinized cornstarch</td>
<td>115.75</td>
<td>115.75</td>
<td>101.98</td>
</tr>
<tr>
<td>Cellulose fiber (sola floc)</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Mineral mix (AIN-93G-MX)</td>
<td>35</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Tertbutylhydroquinone</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>Vitamin mix (AIN-93G-VX)</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Placebo beadlets</td>
<td>2.5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lycopene beadlets</td>
<td>2.5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tomato powder</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Supplemental (total) vitamin E†</td>
<td>None</td>
<td>None</td>
<td>0.025</td>
</tr>
<tr>
<td></td>
<td>(0.095)</td>
<td>(0.095)</td>
<td>(0.095)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
</tr>
</tbody>
</table>

* Lycopene beadlets are 10% wt/wt lycopene.
† The freeze-dried tomato powder contains approximately 0.01% lycopene.
of ad libitum–fed rats, which was recalculated weekly until rats were 17 weeks of age, when food intake had stabilized. From that point on, food intake in the ad libitum–fed group was precisely measured every 4 weeks, and the amount of food provided to the diet-restricted groups was adjusted accordingly.

**Hormone and Carcinogen Treatment**

Starting at 6 weeks of age and continuing for the next 3 weeks, all rats received daily intraperitoneal injections of the luteinizing hormone–releasing hormone antagonist cyprotosterone acetate (CA) (50 mg/kg body weight) (Sigma Chemical, St. Louis, MO). CA inhibits androgen secretion from the testis, thereby causing atrophy of prosthetic epithelial cells. Starting on the day after the last injection of CA, the rats, which were then 9 weeks old, received daily subcutaneous injections of 100 mg of testosterone propionate (TP) (Sigma Chemical) per kilogram of body weight in 0.5 mL of soybean oil to maximally stimulate proliferation of prosthetic epithelial cells. On the day after the last TP injection, the rats were anesthetized with metofane and injected intravenously (via the tail vein) with the carcinogen NMU (Ashe Stevens, Detroit, MI) at a dose of 50 mg per kilogram of body weight. The NMU was initially wetted with 3% acetic acid and stored at –20 °C until use. Immediately prior to injection, the NMU was dissolved in saline at 10 mg/mL, yielding a final pH of 5.5. One week after NMU administration, rats received testosterone as two subcutaneous implants (1.0-mm inner diameter × 2.2-mm outer diameter × 2.54-cm-long silastic laboratory tubing; Dow Corning, Midland, MI), each containing crystalline testosteronone (Sigma Chemical) that had been drawn into the implants under vacuum pressure before the ends of the tube were sealed with silicone adhesive (Dow Corning). Implants were inserted subcutaneously in the dorsolumbar region of the back using sterile technique, and wounds were sealed with surgical glue.

**Survival and Necropsy**

All rats were monitored daily, and rats showing any signs or symptoms of morbidity, including reduced food intake or weight loss, were killed. The remaining rats were killed at 73 weeks of age, when the study was terminated. Death in healthy-appearing rats was rare, and such rats were necropsied immediately on discovery. Rats that were going to be killed were anesthetized by exposure to CO₂; blood was then collected by cardiac puncture into heparinized tubes. Blood was centrifuged in the dark at 2000 rpm for 10 minutes to separate plasma, which was then stored at 4 °C in the dark for lycopene analysis (see below).

**Histopathology**

The fixed prostate and seminal vesicles were examined, and those found to be normal at a gross level were dissected into seven components for microscopic evaluation: 1) bladder, 2) right ventral lobe, 3) left ventral lobe, 4) right dorsolateral lobe, 5) left dorsolateral lobe, 6) right anterior prostate and seminal vesicle, and 7) left anterior prostate and seminal vesicle. The right and left dorsolateral and ventral lobes were dissected longitudinally and embedded in paraffin. Each anterior prostate and seminal vesicle complex was dissected into four or five sequential pieces and embedded in paraffin. Those tissue specimens with tumors large enough to disrupt normal anatomic structure were dissected into three to five pieces, which were embedded in paraffin. The carcass of each rat was examined, and any tissues showing abnormalities were also fixed in 10% neutral buffered formalin and embedded in paraffin. Step sections (3.5 μm thick) were prepared from all of the blocks and stained with hematoxylin–eosin. Sections were blindly and independently evaluated by two investigators to classify the lesions, and any discrepancies in the interpretation were discussed and resolved. Lesions were classified using previously described histopathologic criteria (32,33). We categorized lesions in the prostate and seminal vesicle complex as carcinoma in situ, as microscopic adenocarcinomas (<0.4 cm), or as macroscopic advanced adenocarcinomas (>0.4 cm). The intraprostatic site of origin was defined for the smaller lesions, whereas the precise origin of larger invasive carcinomas often could not be determined. In agreement with previous reports (32,33) using this rat model of prostate carcinogenesis, we saw no evidence that the ventral lobe was involved in proliferative lesions or a primary source of carcinomas. A total of nine rats developed malignancies in tissues other than the prostate (Zymbal’s gland tumors, leukemia/lymphoma, and sarcoma).

**Extraction and High-Performance Liquid Chromatography Analysis of Lycopene**

Lycopene was extracted from the diet and the plasma and quantitated as previously described (21,22). Briefly, an ethanol–hexane solution was used to extract lycopene from the dietary and biologic samples, and the extracts were then subjected to high-performance liquid chromatography (HPLC) analysis with separations performed on a C30 column (YMC, Wilmington, NC) and detected at 470 nm on a UV/VIS detector (model UV-DII; Rainin Dynamax, Walnut Creek, CA). Standard curves were prepared using crystalline lycopene extracted from a tomato oleoresin (LycoRed Natural Products Industries, Beer-Sheva, Israel) and purified on a C30 column. Lycopene was quantified using an external standard curve. Our laboratory participates quarterly in the National Institute of Standards in Technology micronutrient measurement proficiency testing program. The coefficient of variance for lycopene analysis in our laboratory is less than 12%.

**Statistical Analysis**

The experiment was designed as a survival study, in which rats were monitored carefully and killed at the first sign of morbidity. Thus, the most appropriate outcome events to address the efficacy of the dietary treatments were prostate cancer–specific survival and death from any cause. For survival analysis, time to death was defined as age (in weeks) at the time the rat died with prostate cancer. Kaplan–Meier survival estimates were calculated for each diet composition group (tomato powder, lycopene, and control) and for each food intake group (ad libitum and restricted). The log-rank test was used to test the equality of the survival curves for the treatment groups. This test weights each time point equally in the comparison of survival curves. The Wald test (39) was used to assess statistical signif-
incidence of coefficients in the Cox proportional hazards model. Cox proportional hazards regression was then used to investigate the effects of diet composition, controlling for diet restriction. The proportional hazards assumption was tested for each model. When the proportional hazards assumption was violated, a series of analyses was used, each analysis examining two diet composition variables at a time. A Bonferroni adjustment was used to assess statistical significance (i.e., instead of declaring statistically significant a result whose \( P \) value is less than .05, we use the .017 level as an indicator of statistical significance). In this study, survival time was defined as the age (in weeks) at death or, for those rats that were still alive at the end of the study, 73 weeks of age (i.e., 64 weeks on study). Rats surviving until the end of the study were entered into the statistical model as censored observations (39). All survival analyses were conducted using STATA Statistical Software (release 8.0; Stata Corporation, College Station, TX).

Differences in food intakes, body weights, and plasma lycopene concentration between the rats on the different diets were tested by two-way analysis of variance (ANOVA). Data were log-transformed for analysis if they were found not to be normally distributed but are expressed as original values in the text and tables for ease of interpretation. Differences in mean plasma lycopene concentrations at the time of death were tested by two-way ANOVA, with assessment of main effects of diet composition and dietary intake as well as interactions among diet composition and dietary intake. Statistically significant (i.e., \( P<.05 \)) main effects were further tested by two-way post hoc Fisher’s protected least-square difference test to identify differences in mean plasma lycopene between any two groups. All statistical tests were two-sided.

**RESULTS**

**Food Intake and Growth**

Food intake of rats fed the control beadlet (mean ± standard deviation of 16.6 ± 2.0 g/day), lycopene beadlet (16.1 ± 2.1 g/day), or tomato powder (16.7 ± 2.1 g/day) diets did not differ \((P = .757)\) during the 4 weeks between initial assignment to the diets and the randomization to continued ad libitum feeding or diet restriction. Three days after carcinogen administration, rats in each dietary group were divided into diet restriction and ad libitum subgroups. Diet-restricted rats received 80% of the dietary intake of ad libitum–fed rats for the duration of the study. Food intake of rats having ad libitum access to food gradually increased until they reached 17 weeks of age \((19.6 ± 1.7 \text{ g/day})\) and remained stable \((±2 \text{ g/day})\) until individual rats began to show symptoms of prostate cancer. Body weights (Fig. 1) did not differ statistically significantly among rats fed the control beadlet, lycopene beadlet, or tomato powder–containing diets ad libitum or among the diet-restricted subgroups. Within a week following initiation of dietary restriction, rats in all restricted groups weighed less than the corresponding ad libitum–fed rats and remained smaller (approximately 18%–20% less in body weight) for the duration of the experiment. There was greater variation in rat weights after week 50 following NMU treatment was due to the lower number of surviving rats in each group because of death and/or the presence of tumors.

**Lycopene Content of the Diet**

HPLC analysis showed that the control beadlet diet (Table 1) did not have detectable lycopene or other carotenoids (Table 2).

<table>
<thead>
<tr>
<th>Diet</th>
<th>Total lycopene</th>
<th>All-trans lycopene</th>
<th>5-cis lycopene</th>
<th>Other-cis lycopene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lycopene beadlet, fresh†</td>
<td>161 (143 to 179)</td>
<td>68 (64 to 72)</td>
<td>71 (69 to 73)</td>
<td>21 (17 to 25)</td>
</tr>
<tr>
<td>Lycopene beadlet, exposed‡</td>
<td>106 (104 to 108)</td>
<td>51 (49 to 53)</td>
<td>46 (44 to 48)</td>
<td>13 (7 to 18)</td>
</tr>
<tr>
<td>Tomato powder, fresh§</td>
<td>13 (11 to 15)</td>
<td>5 (5 to 5)</td>
<td>4 (4 to 4)</td>
<td>4 (4 to 4)</td>
</tr>
<tr>
<td>Tomato powder, exposed‡</td>
<td>7 (7 to 7)</td>
<td>3 (3 to 3)</td>
<td>2 (2 to 2)</td>
<td>2 (2 to 2)</td>
</tr>
</tbody>
</table>

*Values were determined by high-performance liquid chromatography. Lycopene was not detectable in the control beadlet diet. Lycopene concentrations in the lycopene and tomato powder diets were compared using one-way analysis of variance followed by post hoc Fisher’s protected least-squares difference test. Values in the same column with different superscripts are statistically significantly \((P<.05)\) different. Data are means and 95% confidence intervals for four diet samples per group. Means and 95% confidence intervals are rounded to the nearest milligram.

†The diet was analyzed immediately after removal from storage at 4°C.
‡The diet was exposed to the atmosphere, temperature, and light of a rat cage for 2 days and then analyzed.
§The tomato powder diet also contains all-trans β-carotene (approximately 0.001 g/kg diet) as well as 9-cis β-carotene and other polar carotenoids eluting before β-carotene (see Fig. 2).
The lycopene concentration in the lycopene beadlet diet was 161 mg lycopene/kg diet, and the tomato powder diet contained 13 mg lycopene/kg diet (Table 1). The tomato powder and lycopene beadlet diets had similar patterns of lycopene isomers and percentages of total lycopene in the cis configuration (Table 2 and Fig. 2). Both lycopene-containing diets showed a decline in lycopene (~40% reduction) after exposure to the atmosphere, temperature, and lighting of the rat housing for 2 days. The tomato powder diet also contained other carotenoids typically found in tomatoes, such as all-trans β-carotene (approximately 1 mg/kg) as well as 9-cis β-carotene and other unidentified polar carotenoids (Fig. 2).

**Plasma Lycopene Isomer Concentrations**

Lycopene was not detected in plasma of rats fed the control beadlet diet. Rats with unrestricted access to food and fed the lycopene beadlet diet had greater plasma concentrations of total lycopene (37% higher, \( P = .017 \)) and all-trans lycopene (61% higher, \( P = .003 \)) than rats fed the tomato powder diet (Table 3). Rats fed under restricted conditions and consuming lycopene beadlet or tomato powder diets accumulated approximately 15% less lycopene in plasma than rats with ad libitum access to the same diet, although the difference was not statistically significant for any of the diets. Although β-carotene was present in the tomato powder diet, it was not detectable in the plasma of rats consuming this diet, which suggests that it was converted completely to retinol (vitamin A).

**Observations From Gross Dissection and Histopathology**

Of the 194 rats that were subjected to the tumor induction protocol, seven died within the first week after NMU administration due to complications of anesthesia or acute toxicity. Of the remaining 187 rats, 165 (88%) died or were killed before reaching 73 weeks of age, when the study was terminated. A total of 134 of the 187 rats (72%) were killed because they displayed symptoms of prostate cancer before 73 weeks of age.

A total of 151 (81%) of the 187 rats developed some form of cancer (adenocarcinoma, sarcoma, or carcinoma *in situ*) in the prostate and seminal vesicle complex. Of the 22 rats that were killed at the end of the study but showed no morbidity, 17 had histologically detected prostate cancer. In addition to using histopathologic criteria, we classified the tumors based on size. Among the 187 rats, microscopic adenocarcinomas (<0.4 cm) were observed in 57 rats (31%), whereas 88 rats (47%) developed locally advanced adenocarcinomas (>0.4 cm). Two of the 187 rats (1.1%) developed prostate sarcomas, and only four (2.1%) were found to have pathology limited to prostatic carcinoma *in situ* at the time of killing. Nine of the 187 rats (4.8%) had extensive metastatic disease from their prostate carcinoma to lymph nodes, liver, or peritoneum. Another nine rats (4.8%) developed cancers at sites other than the prostate (including Zymbal’s gland), leukemia, and lymphoma. The frequency and types of cancer observed are consistent with those previously reported in this model system (30–33).

**Diet Composition and Prostate Cancer—Specific Survival**

The primary outcome evaluated was prostate cancer—specific survival. This outcome was chosen over other outcomes, such as
After controlling for diet restriction, rats fed the control and tomato powder diets, rats fed the tomato powder diet experienced a statistically significantly longer survival than the rats fed the control diet (hazard ratio [HR] = 0.74, 95% confidence interval [CI] = 0.59 to 0.93; P = .009) after controlling for diet restriction. This result remained statistically significant even after applying the Bonferroni adjustment. Finally, the Cox model comparing survival of tomato powder– and lycopene-fed rats suggested that rats fed tomato powder had prolonged survival compared with lycopene-fed rats after controlling for diet restriction, but the difference did not reach statistical significance (P = .07). The percentages of rats dying with some form of prostate cancer (adenocarcinoma, carcinoma in situ, or sarcoma) were 80% (95% CI = 68% to 99%), 72% (95% CI = 60% to 83%), and 62% (95% CI = 48% to 75%) for the control, lycopene, and tomato powder groups, respectively.

**Diet Restriction and Prostate Cancer–Specific Survival**

The percentages of rats that died with prostate cancer were 79% (95% CI = 69% to 86%) and 65% (95% CI = 54% to 74%) for the *ad libitum* and diet-restricted groups, respectively. Kaplan–Meier survival functions for the two groups were statistically significantly different (log-rank test, P = .03), indicating an increase in prostate cancer–free survival in the rats assigned to diet restriction (Fig. 3, B). The two factors (diet composition and level of intake) and their interaction term were entered into the Cox model. The interaction term for the type of diet and the amount of dietary intake was not statistically significant (Wald test, P = .38) and was therefore removed from the model. The model results suggest that diet-restricted rats had a statistically significantly lower risk of dying with prostate cancer over their lifespan than *ad libitum*–fed rats, after controlling for diet type (HR = 0.68, 95% CI = 0.49 to 0.96; P = .029) (Table 4). The precision of this P value is somewhat questionable, however, because a statistically significant violation of the proportional hazards assumption had occurred (P = .02). We believe that this P value is conservative because the crossing of the Kaplan–Meier survival curves may well be the reason for the rejection of the proportional hazards assumption.

**Diet and Risk of Death From Any Cause**

A second Cox model was fitted to the survival data, using death from all causes as the outcome. The final model coefficients, along with hazard ratio estimates and 95% confidence intervals, are shown in Table 4. The interaction term was not statistically significant (Wald test, P = .55) and was removed from the final model. The test for the proportional hazards assumption indicated no statistically significant violations (P = .13). No statistically significant effect of diet composition or intake on all-cause mortality was noted.

**Tumor Histopathology**

This study focused on survival, and thus this design would bias an interpretation of tumor grade and stage because these are time-dependent outcomes of carcinogenesis that are best evaluated in a study with a fixed termination point. Nevertheless, we provide these data for descriptive purposes. Among rats dying...
with prostate cancer, 70%, 71%, and 45% of the total cancers in rats fed the control beadlet diet, lycopene beadlet diet, and tomato powder diet, respectively, were macroscopic, poorly differentiated lesions. We observed that 55% of the rats in the diet-restricted group had macroscopic prostate adenocarcinomas as compared with 69% of the rats with ad libitum access to food.

**Discussion**

The majority of lycopene intake (82%) by American men is from a single food source, tomato products (1,6,20). Thus, it is not possible for epidemiologic studies to differentiate whether intake of lycopene alone, as opposed to intake of one or more of the vast array of phytochemicals found in tomatoes, is related to risk of disease outcomes. In contrast, a laboratory animal model allows investigators to address this critical question. Our study is the first, to our knowledge, that compares the abilities of purified lycopene and tomato powder to alter the risk of prostate cancer in a highly controlled model of prostate carcinogenesis. In addition, we examined the interaction between intake of lycopene or tomato products and modest (i.e., 20%) dietary restriction on prostate cancer–specific survival because a previous study (28) suggested that the response to dietary variables may change with different levels of energy intake.

Our observations support the concept that tomato products contain components in addition to lycopene that may inhibit prostate carcinogenesis. Although we can conclude that lycopene alone, in this model system and at this dose, did not statistically significantly alter the risk of prostate cancer, it remains possible that lycopene, when provided in combination with the other phytochemicals found in whole tomato powder may contribute to the benefits observed. Tomatoes contain an array of phytochemicals, including other carotenoids in addition to lycopene, that could potentially modulate prostate cancer risk (40–46). These substances include all-trans β-carotene and 9-cis β-carotene, polyphenolic compounds such as quercetin (47,48), other phenolic compounds (40), and vitamin C and folate (20). Results of recent in vitro studies suggest that several of the phytochemicals found in tomatoes, such as other carotenoids (13,41), vitamin C (42–44), and vitamin E (45), can influence prostate tumor cell growth and quench reactive oxygen species (46). Additional efforts to characterize bioactive phytochemicals in tomatoes, their mechanisms of action, and, most important, any additive or synergistic effects on prostate carcinogenesis, are necessary.

Lycopene concentrations in the plasma provide some insights into the outcomes of this study. Rats fed the lycopene beadlets had statistically significantly higher plasma lycopene concentrations (means of 99 and 118 nmol of lycopene per liter of plasma for diet-restricted and ad libitum–fed rats, respectively) than rats fed tomato powder (means of 74 and 85 nmol of lycopene per liter of plasma for diet-restricted and ad libitum–fed rats, respectively) (Table 3). It is interesting that the plasma lycopene concentrations were so similar, given that the lycopene beadlets achieved the concentration in the diet increases or that the bioavailability of lycopene from tomato powder is much greater than that from beadlets. In addition, because the lycopene beadlets achieved the greatest plasma lycopene concentrations but did not protect against prostate cancer, these data further support the hypothesis that tomatoes must contain phytochemicals in addition to lycopene that may modulate prostate carcinogenesis.

Rats that experienced a modest total diet restriction demonstrated a statistically significant reduction in prostate cancer risk in the NMU model of prostate carcinogenesis. This observation complements, reinforces, and extends our results previously reported using transplantable prostate cancer models (28). It is important to recognize that a dietary restriction of this extent (i.e., 20%) allows continued growth of the rats and does not result in malnutrition but rather in the prevention of obesity (28). Interestingly, no statistically significant interactions were observed between energy intake and diet composition. Thus, tomato products and diet restriction may have additive independent benefits.

In summary, our results suggest that tomato products may be more effective for the inhibition of prostate carcinogenesis than...
supplementation with pure lycopene. This observation is consistent with epidemiologic findings (1–3) and recent results from small clinical trials (8,9). Our results do not rule out the possibility that lycopene is one of several phytochemicals in the tomato that contributes to an inhibition of prostate carcinogenesis. At the present time, many men are consuming lycopene-containing supplements with the hope that they may prevent prostate cancer or enhance the treatment of their prostate cancer. We suggest that a focus on interventions with whole tomato products and energy balance should be a priority while clinical studies simultaneously investigate the risks and benefits of lycopene supplementation.

REFERENCES


NOTES

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