

Interaction of Tomato Lycopene and Ketosamine against Rat Prostate Tumorigenesis

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Abstract

Prior investigations on the beneficial effect of dietary processed tomato products and lycopene on prostate cancer risk suggested that lycopene may require the presence of other constituents to exert its chemopreventive potential. We investigated whether ketosamines, a group of carbohydrate derivatives present in dehydrated tomato products, may interact with lycopene against prostate tumorigenesis. One ketosamine, FruHis, strongly synergized with lycopene against proliferation of the highly metastatic rat prostate adenocarcinoma MAT-LyLu cell line *in vitro*. The FruHis/lycopene combination significantly inhibited *in vivo* tumor formation by MAT-LyLu cells in syngeneic Copenhagen rats. Energy-balanced diets, supplemented with tomato paste, tomato powder, or tomato paste plus FruHis, were fed to Wistar-Unilever rats ($n = 20$ per group) treated with *N*-nitroso-*N*-methylurea and testosterone to induce prostate carcinogenesis. Survival from carcinogenesis was lowest in the control group (median survival time, 40 weeks) and highest in the group fed the tomato paste/FruHis diet (51 weeks; $P = 0.004$, versus control). The proportions of dying rats with macroscopic prostate tumors in the control, tomato paste, tomato powder, and tomato paste/FruHis groups were 63% (12 of 19), 39% (5 of 13), 43% (6 of 14), and 18% (2 of 11), respectively. FruHis completely blocked DNA oxidative degradation at $>250 \mu\text{mol/L}$ *in vitro*, whereas neither ascorbate nor phenolic antioxidants from tomato were effective protectors in this assay. FruHis, therefore, may exert tumor-preventive effect through its antioxidant activity and interaction with lycopene. [Cancer Res 2008;68(11):4384–91]

Introduction

Prostate cancer incidence differs significantly across geographic regions and, to a large extent, may be influenced by environmental factors such as lifestyle habits, including diet (1, 2). Epidemiologic studies have shown an inverse relation between dietary antioxidants and prostate cancer in populations of western males. Specifically, ingestion of lycopene, a lipid-soluble carotenoid antioxidant, which in the western diet originates mostly from consumed tomato products, was associated with reduced prostate cancer risk (3). Mechanisms of lycopene bioactivity as a cancer-preventive agent may include its antioxidant activity, inhibition of cell cycle progression, induction of apoptosis, increase of gap-junctional cell communication, inhibition of insulin-like growth

factor I signal transduction, inhibition of androgen activation and signaling, etc. (4, 5).

Whereas lycopene has been hypothesized as a major candidate compound responsible for the protective effects of tomato-based foods (6), other constituents may contribute to the cancer prevention. There are several observations that point to tomato processing as a possible source of still unidentified contributors to the cancer chemoprevention. First, during processing, the content of lycopene, ascorbate, and other natural antioxidants decreases as a result of thermal degradation and isomerization (7, 8). However, the total antioxidant activity of heat-processed tomato juice or other products may not decrease and in fact often increases (7–9). Second, consumption of processed tomato products, rather than fresh tomatoes, was associated epidemiologically with a decreased risk of prostate cancer (3). Third, consumption of tomato powder, rather than an equivalent lycopene supplement, inhibited prostate carcinogenesis in rats (10), suggesting that, in addition to lycopene, tomato products contain other compounds that increase survival of the experimental animals. Most of the clinical trials with tomato products also suggest a synergistic action of lycopene with other nutrients (11).

During food processing, multiple chemical reactions occur between food components as a result of thermal and mechanical treatment, dehydration, and other physical conditions. One of the most notable and common chemical transformations is the Maillard reaction, which is mainly responsible for browning and specific flavoring of baked, roasted, and dried foods (12). The Maillard reaction generally starts with the initial condensation reaction between a reducing sugar, such as glucose, and amino groups of amino acids or proteins, and brings about formation of ketosamines, also referred to as Amadori rearrangement products (Fig. 1). Dried fruits and vegetables, including processed tomato powder, contain significant amounts of ketosamines, mostly in the form of nondigestible fructose-amino acid conjugates (13), which are partially absorbed into circulation but excreted unchanged.

Previously, we have established the antitumor potential of synthetic ketosamines in melanoma, breast, and prostate cancer models (14–16). *In vitro* and *ex vivo* mechanistic studies suggested that these compounds may act as inhibitors of homotypic and heterotypic cancer cell adhesion, possibly targeting tumor-associated carbohydrate antigens and lectins (17, 18).

Here we show that ketosamines represent a novel class of potential antioxidants in tomato products, which may interact with lycopene against prostate carcinogenesis and tumor cell proliferation.

Materials and Methods

Materials

Tomato paste. Samples of cold break (37% natural tomato soluble solids) and hot break (30.9% natural tomato soluble solids) tomato pastes were donated by The Morning Star Packing Co. and Rio Bravo Tomato Co. The percentage in the paste names refers to the solids content, with the rest

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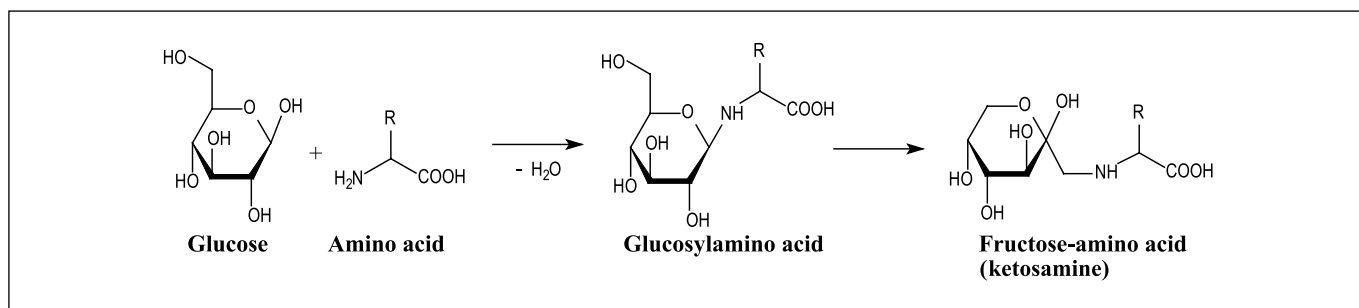


Figure 1. The initial step of the Maillard reaction in processed foods. During heating and dehydration, glucose reacts with amino groups of proteins or free amino acids to form unstable glucosylamines, also referred to as Schiff bases. Weak organic acids catalyze transformation of glucosylamines, through the Amadori rearrangement, into more stable ketosamines.

being water. The cold or hot break term refers to a technological process of initial heating of raw tomatoes at 66°C or 100°C, respectively.

Tomato powder. Samples of cold break and hot break tomato powder (TRANSA) were obtained from Henry Broch & Co.

Antioxidants and other reagents. Antioxidants and other reagents were of analytic purity grade, purchased from reagent companies (Sigma-Aldrich), and used without any further purification. Crystalline lycopene was a gift from LycoRed Natural Products Industries. Lycopene stock solutions were prepared and stabilized with 0.025% butylated hydroxytoluene in anhydrous tetrahydrofuran. To disperse lycopene in cell culture medium, an aliquot of the lycopene stock solution was injected into a sealed vial containing the medium under argon gas and vortexed vigorously. All manipulations with lycopene solutions were done under dim lights.

Fructose-amino acids. Fructose-amino acids were from a collection previously prepared in our laboratory using published general methods (17, 19). Their purity was confirmed chromatographically immediately before the *in vitro* experiments.

Synthesis of FruHis. For this study, FruHis was prepared from an aqueous solution of food-grade L-histidine, glacial acetic acid, and D-glucose, and purified, as previously described (20). It was obtained as nonhygroscopic crystalline powder (21), free of any detectable impurities.

Cell line and cell culture. Rat prostate adenocarcinoma cell line MAT-LyLu was obtained from American Type Culture Collection at passage 68. To ensure that MAT-LyLu cells were *Mycoplasma*-free, the cells were passaged once in a male Copenhagen rat before any *in vitro* and *in vivo* experiments. Cells were cultured in complete RPMI 1640 at 37°C, at 100% humidity, 5% CO₂/95% air, and routinely checked for any bacterial contamination with commercial test kits.

***In vitro* Antioxidant Assays**

Water extracts from tomato products were prepared by centrifugation of reconstituted 8% (percent dry weight) suspensions of tomato powder or paste samples at $2,500 \times g$ for 20 min, followed by ultrafiltration of the supernatants with Biomax-5 centrifugal filters (Millipore). Stock solutions of phenolic antioxidants were prepared in dimethylformamide/water (1:1).

DNA protection. To a 200- μ L solution of 50 μ g polymeric DNA/mL of chelex-treated PBS (pH 7; polymeric DNA was from calf thymus, Sigma) were added, in order, an aqueous tomato extract or an antioxidant, CuSO₄ (final concentration, 50 μ mol/L), H₂O₂ (final concentration, 2 mmol/L), and ascorbic acid (final concentration, 2 mmol/L). The reaction was left to proceed at room temperature for 2 h and then stopped by addition of EDTA to a final concentration of 10 mmol/L. Ethidium bromide was added at 20 μ g/mL, and the fluorescence of the solutions was measured by FLx800 plate reader (BioTek) at 508 nm excitation/590 nm emission wavelengths.

Folin-Ciocalteu assay. Following a standard protocol (22), 10 μ L of Folin-Ciocalteu reagent (Sigma) and 10 μ L of an antioxidant solution were added to 150 μ L of distilled water per well. After 3 min, 30 μ L of 20% Na₂CO₃ were added per well, and after 3 h at room temperature, the absorbance in the wells was read at 760 nm.

Ferric reducing/antioxidant power assay. Solutions of a test antioxidant (10–20 μ L) were added to wells containing 200 μ L of the ferric

reducing/antioxidant power (FRAP) reagent (23) and incubated at 37°C for several hours. The absorbance in the wells was read at 593 nm.

***In vitro* Cytotoxicity**

In vitro cytotoxicity was evaluated as follows: Subconfluent (70–95%) cell monolayers were trypsinized for 5 to 10 min with 0.1% trypsin/EDTA, washed with complete, phenol red-free RPMI 1640, resuspended in the medium to 1.25×10^3 cell/mL, added to inner wells of cell culture-treated 96-well plates at 200 μ L/well, and placed into an incubator. The seeded cells were allowed to attach for 24 h, then fresh medium (150 μ L/well) was added, with or without test compounds. In 48 h, the old media/compounds were replaced with fresh ones and the cells were incubated for the next 48 h, thus totaling cell exposure to the agents to 4 d. Finally, the media/compounds were removed, the cells were washed with PBS, and fresh media were added; then 0.5 mmol/L resazurin (Sigma) was immediately added at 15 μ L/well. After 6 to 9 h in an incubator, the fluorescence in the wells was measured at 530/590 nm.

Animal Studies

All experimental protocols involving animals were initially approved by the University of Missouri Animal Care and Use Committee.

***In vivo* Survival of Treated Cancer Cells**

MAT-LyLu cells harvested from subconfluent monolayers were added to plastic T75 cell culture flasks, at 5×10^4 per flask, and allowed to attach for 24 h in complete phenol red-free RPMI 1640. Fresh media, containing tetrahydrofuran (control), lycopene, FruHis, or lycopene/FruHis combination, were added to the flasks, and the cells were further incubated for 4 h. The cells were then detached by trypsin/EDTA, washed, and examined for viability with trypan blue. Suspensions of the cells deemed >90% viable were adjusted to 5×10^5 /mL in sterile PBS and immediately used for injections into experimental animals. The cell suspensions were injected s.c. (0.1 mL per injection) into hind legs and flanks of fourteen 6-month-old, 350- to 400-g male Copenhagen rats. Each rat received four s.c. inoculations; each of these four inoculations was from a different pool of cells (control, lycopene, FruHis, and lycopene/FruHis pretreated). The inoculation sites (flank, leg, right, and left) were randomized for each of the pretreated cell suspensions. The rats were given a regular rodent diet *ad libitum* throughout the experiment. In 14 d, the experiment was terminated and sizes of growing tumors were measured with a caliper. The tumor volumes were estimated according to the following formula: $V = 0.523 \times L \times W \times W$.

Prostate Carcinogenesis Chemoprevention Experiment

The experiment was done using a well-established rat prostate-specific carcinogenesis model, which was introduced by Bosland (24) and successfully used in the most relevant work to our prostate cancer chemoprevention study of tomato and lycopene (10), as well as in a number of other studies (25, 26).

Animals. Experimental animals, 5-wk-old male Wistar-Unilever rats (HsdCpb:Wu), 150 to 170 g in weight, were obtained from Harlan and housed in polycarbonate cages (two animals per cage) with 12 h light: 12 h dark cycle. The animals were fed Teklad 2014 diet (Harlan) for 1 wk to

adapt. The rats were then randomly assigned to four experimental diets. Rats were weighed weekly.

Diet formulations and consumption. All rats consumed one of the four Teklad 2014 meal-based experimental diets (Table 1). Water was added to the formulations to balance moisture content in all diets, as well as to prevent diets from drying completely within a day in the animal feeders and, therefore, to minimize possible formation of additional amounts of ketosamines in diets. The diets were prepared biweekly as meals and stored frozen. Initially, all animals were allowed to have an unlimited access to food and water. After 2 wk, the *ad libitum* amounts of daily consumed diets per cage were measured. It was determined that, on average, animals in the control group consumed the least amount of food, ~70 g/d/cage. From that point on and for the rest of the experiment, all groups received daily the same amounts of diet meals, 35 g/rat, and tap water *ad libitum*.

Hormone and carcinogen treatment. Starting at 6 wk of age and continuing for the next 3 wk, all rats received 0.1-mL i.p. injections of cyproterone acetate (Sigma Chemical) in peanut oil, daily at 50 mg/kg body weight dose. Cyproterone inhibits androgen secretion from the testis, thereby causing androgen starvation of the prostate epithelial cells and the effect of chemical castration to the animals. Starting on the day after the last injection of cyproterone, the rats received three daily 0.2-mL s.c. injections of testosterone propionate (Sigma) in peanut oil/benzyl alcohol/benzyl benzoate (26:1:4) at 100 mg/kg body weight to maximally stimulate proliferation of prostatic epithelial cells. On the day after the last testosterone injection, the rats were briefly anesthetized with isoflurane and injected i.v. (via the tail vein) with a 10 mg/mL solution of the carcinogen *N*-nitroso-*N*-methylurea (NMU; obtained from Sigma), at a dose of 50 mg/kg body weight. NMU was initially saturated at 20°C in 0.1 mol/L sodium phosphate/0.05 mol/L sodium citrate buffer (pH 5); NMU content was determined spectrophotometrically at 395 nm; and the solution was immediately frozen and stored at -80°C until use. Immediately before injections, the NMU solution was thawed at 37°C and sterile filtered. One week after NMU administration, the rats received testosterone as two 2.5-cm-long implants of Silastic laboratory tubing (1.98 mm ID × 3.18 mm OD; Dow Corning), each filled with ~40 mg of crystalline testosterone (Sigma) that had been packed into the tubing under vacuum before the ends were sealed with silicone adhesive 732 (Dow Corning). The tubes were sterilized in alcohol, dried under UV illumination, and inserted s.c. in the dorsothoracic region of the back using a sterile technique, and then the incisions were stapled.

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 51 wk of age, when the study was terminated. When death happened to healthy-looking rats, the rats were necropsied immediately on discovery. Rats that were going to be euthanized were first anesthetized with ketamine/xylazine Rat Cocktail at 1 mL/kg body weight. Blood was then collected by cardiac puncture. Rats were then killed by bilateral pneumothorax puncture, and the prostate, seminal vesicle, and other organs with suspected macroscopic tumors were removed and preserved in 4% neutral-buffered formalin.

Statistical Analysis

For survival analysis, Kaplan-Meier survival estimates were calculated for each diet composition group. 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. In this study, survival time was defined as the number of weeks from the carcinogen injection until death from cancer or, for those rats that were still alive at the end of the study, 51 wk. Rats surviving until the end of the study and lacking any macroscopic tumors on necropsy were entered into the statistical model as censored observations. All survival analyses were conducted using SigmaStat Software (version 3.01, SSPS, Inc.).

Differences in body weights between the rats on different diets, differences in tumor cell survival and volumes of the MAT-LyLu tumors receiving different *in vitro* pretreatments, and differences in DNA protection *in vitro* were tested by one-way ANOVA. Data were rank transformed for Kruskal-Wallis analysis if they were found to fail the normality of distribution test. Differences between antioxidants in the *in vitro* assays were probed by pairwise multiple comparisons following the Holm-Sidak method, whereas Dunn's method was applied for the pairwise comparisons between MAT-LyLu tumor growth inhibitors. All statistical tests were two sided.

Results

Lycopene synergizes with FruHis against MAT-LyLu cell proliferation *in vitro* and *in vivo*. Assessment of the ability of ketosamines to inhibit cellular growth of the highly metastatic rat prostate adenocarcinoma MAT-LyLu line *in vitro* has been done in a conventional cell proliferation assay, with cancer cell exposure to test compounds during 4 days at standard cell culturing conditions.

Table 1. Diet formulations (parts by weight)

	Control	Tomato paste	Tomato powder	Paste/FruHis
Teklad 2014 meal*	70.6	70.6	70.6	70.6
Cold break tomato paste solids [†]	—	7.8	—	7.8
Cold break tomato powder solids [‡]	—	—	7.8	—
FruHis	—	—	—	1.5
Corn starch	3.5	—	—	—
Glucose	2.1	—	—	—
Fructose	2.2	—	—	—
Water	21.6	21.6	21.6	20.1
Total	100	100	100	100

*This diet, according to the manufacturer, "is a fixed formula, nutritionally balanced, nonautoclavable diet containing 14% protein and 3.5% fat which supports growth and maintenance. 2014 does not contain alfalfa or soybean meal, thus minimizing the occurrence of natural phytoestrogens. Absence of animal protein and fish meal eliminates the presence of nitrosamines." The exact composition of the 2014 diet and its nutritional value are given on the manufacturer's website (<http://www.teklad.com/globaldiet/g2014.asp>).

[†]Main constituents of tomato paste (percent dry weight): carbohydrates (glucose, fructose, etc.), 55%; fat, 0.5%; fiber, 9%; protein, 16%; organic acids, 8%; total ash, 9%.

[‡]In tomato powder, free amino acids partially combine with glucose to form up to 8% fructose-amino acids (13), including 0.04% FruHis (20), during the tomato paste drying process at elevated temperatures.

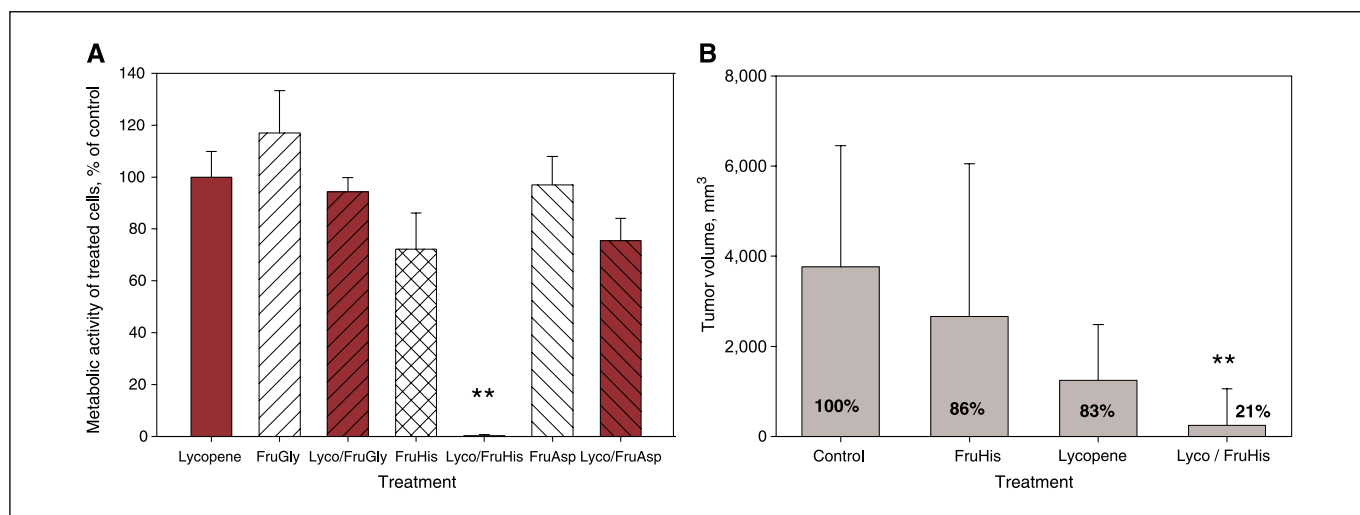


Figure 2. Interaction of FruHis with lycopene against rat prostate adenocarcinoma MAT-LyLu cell line. *A*, cultured MAT-LyLu cells were treated with 1 $\mu\text{mol/L}$ lycopene or tomato ketosamines (2 mmol/L) or their combinations. After 4 d, the surviving cell numbers were evaluated fluorimetrically with resazurin. All experiments were done at least in quadruplicates. The lycopene/FruHis combination produced no detectable survivors in this cytotoxicity assay. *B*, cultured MAT-LyLu cells were pretreated for 4 h with 5.6 mmol/L FruHis, 20 $\mu\text{mol/L}$ lycopene, or their combination. The cells were confirmed as >90% viable before being implanted s.c. into the experimental Copenhagen rats. In 2 wk, the primary growing tumors were measured. Columns, mean tumor volume ($n = 14$); bars, SD. For each treatment, the percent of tumor incidence is also shown. **, $P \leq 0.001$.

Initial profiling of ketosamines did not reveal any significant inhibition of cancer cell proliferation at ketosamine concentrations up to 1 mmol/L (data not shown). At 2 mmol/L, FruHis showed a small but detectable inhibition of MAT-LyLu cell growth (Fig. 2). However, when 2 mmol/L FruHis was combined with 1 $\mu\text{mol/L}$ lycopene, a large synergistic effect of suppression of the cancer cell proliferation was observed, with >98% cell growth inhibition as compared with controls or the single-agent treatments. Other representative ketosamines that we have tested in this assay thus far did not show such synergy with lycopene at similar concentrations of the agents (Fig. 2A).

After identifying FruHis/lycopene combination as growth inhibitory for MAT-LyLu cells *in vitro*, we have investigated whether this combination would affect survival of MAT-LyLu cells in the syngeneic host, male Copenhagen rat. The cells, cultured *in vitro*, were briefly pretreated with lycopene, FruHis, or their combination and, while still >90% viable, implanted s.c. in the animals. As shown in Fig. 2B, there was a significant decrease in average size of the s.c. tumors growing from lycopene/FruHis-pretreated cells (80% decrease; $P < 0.05$, compared with controls).

Intake of the experimental diets. The amount of food given to the animals daily was restricted to 35 g/rat. This amount was based on the control diet consumption by 8-week-old rats having *ad libitum* access to food. This amount of daily ingested diets corresponds to 86 to 87 kcal/d/rat, which is close to the *ad libitum* maintenance energy consumption level for adult rat (27). Body weights (Fig. 3A) did not differ significantly between the diet groups. Rats in the control diet group, however, were consistently heavier than the other groups by 1% to 8% on average.

Survival from prostate tumorigenesis in the NMU/testosterone rat model. Due to a relatively small number of animals in the experimental diet groups, we have chosen survival from dying of nonspecific cancer as the primary outcome. Log-rank test of Kaplan-Meier survival curves for four diet groups (Fig. 3B) showed their inequality ($P = 0.047$). Statistical analysis indicated that rats fed the tomato paste/FruHis diet experienced the longest survival

from macroscopic tumorigenesis (mean survival time, 51 wk), followed by the tomato powder, tomato paste, and control groups, with mean survival times of 50, 45, and 40 weeks, respectively. The pairwise comparison procedures revealed statistically significant differences between survival curves in pairs: control versus tomato powder ($P = 0.026$) and control versus tomato paste/FruHis ($P = 0.004$).

Because the carcinogenesis prevention experiment was designed as a survival study, we did not attempt to interpret tumor grade and stage due to their dependence on duration time, which was not fixed. In addition, the proportion of tumorigenesis at sites other than the prostate was significant and prevailed in all tomato-modified diet groups. Besides in the prostate, macroscopic tumors were found in the jaw, Zymbal's gland, lungs, kidneys, lymph nodes, colon, and seminal vesicles. Therefore, histopathologic examination of the entire body for microscopic tumors was excluded from the statistical analysis because incidence of microfocal malignancies was deemed insignificant to the clinically relevant outcome of "dying of cancer."

The number of macroscopic prostate tumor-bearing rats was largest in the control diet group and smallest in the tomato paste/FruHis group (Fig. 3C), a 6-fold decrease. The numbers of animals with tumors at sites other than the prostate were similar throughout all diet groups. The proportions of dying rats with macroscopic prostate tumors in the control, tomato paste, tomato powder, and tomato paste/FruHis groups were 63% (12 of 19), 39% (5 of 13), 43% (6 of 14), and 18% (2 of 11), respectively.

Ketosamine FruHis shows antioxidant activity *in vitro*. The initial evaluation of the antioxidant potential of water-soluble fractions from tomato products, as well as FruHis, has been done in a polymeric, double-stranded DNA degradation assay. The copper-catalyzed Fenton reaction was used to generate reactive oxygen species, predominantly hydroxyl radicals, in this assay. Fluorescence of DNA-intercalated dye propidium iodide was used as a measure of the DNA fragmentation level. The effects of the aqueous extracts, FruHis, and other antioxidants from tomato on protection

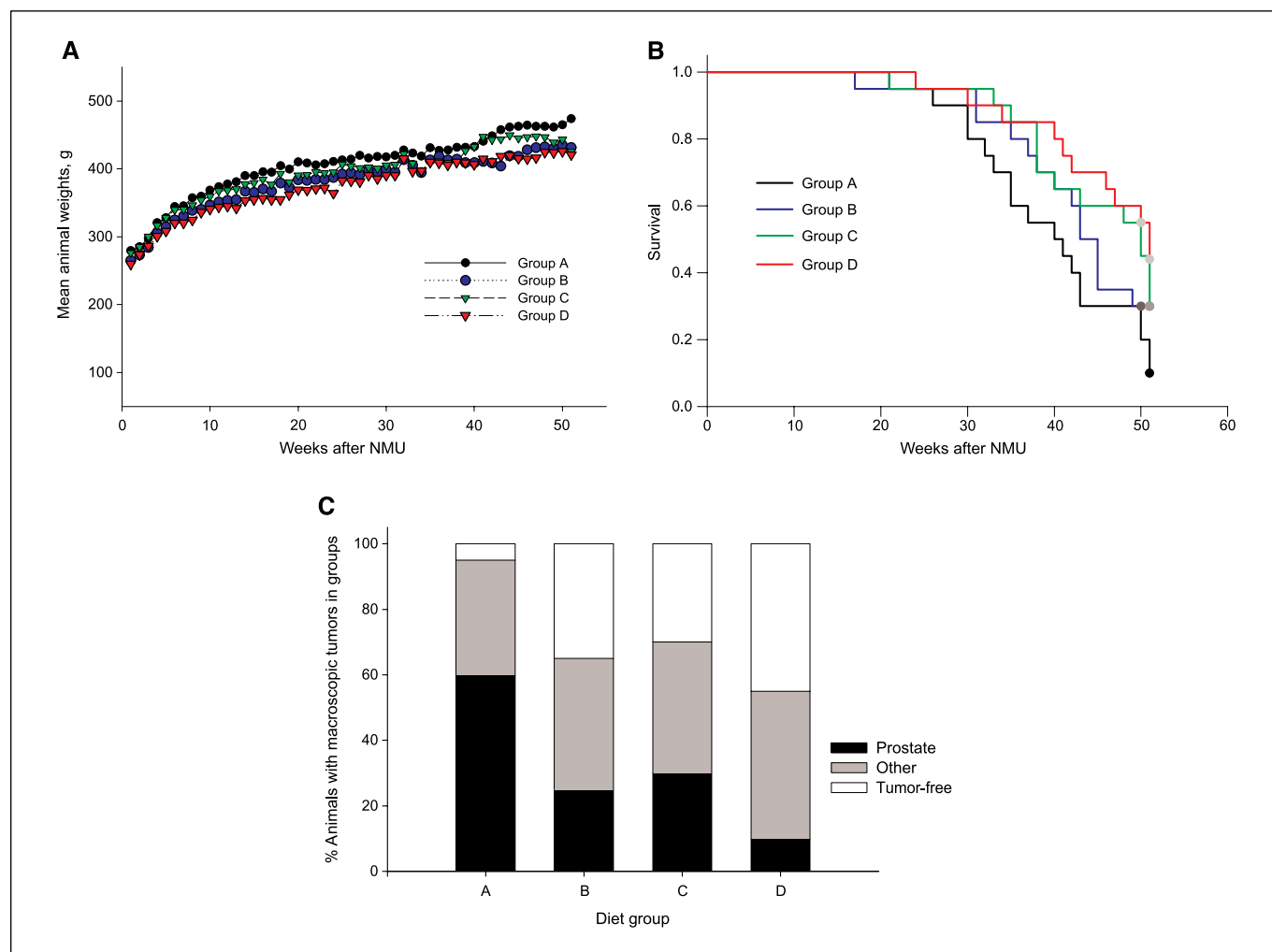


Figure 3. Prevention of NMU/testosterone-induced carcinogenesis in Wistar-Unilever rats receiving energy-balanced experimental diets (Table 1): control (group A); 10% tomato paste (group B); 10% tomato powder (group C); 10% tomato paste + 2% FruHis (group D). A, mean of animal weights in each diet group. No statistically significant differences are detected. B, Kaplan-Meier survival curves. Diets C and D showed statistically significant ($P < 0.05$) improvement of survival from tumorigenesis. C, distribution, in each diet group, of animals with macroscopic tumors in prostate or other organs at the time of animal death or after 51 wk following NMU injection.

of the DNA from the oxidative fragmentation are summarized in Fig. 4A and B. Water-soluble fractions from tomato powder samples showed significantly better protection of DNA from reactive oxygen species (ROS), as compared with those obtained from tomato paste samples. D-Fructose showed no protection. There was a small but evident inhibition of the DNA degradation by chlorogenic, caffeic, and *p*-coumaric acids at the inhibitor concentrations exceeding 1 mmol/L. L-Histidine and quercetin showed significant inhibition activities in this assay in a concentration-dependent manner. We did not observe, however, a total inhibition of DNA oxidative degradation by these compounds, even at concentrations as high as 1 mmol/L. In contrast, FruHis at 500 μ mol/L completely protected DNA from cleavage, whereas at lower concentrations, it exerted better protection against ROS-induced polynucleic acid fragmentation than any of the tested antioxidants within the entire investigated concentration range.

The antioxidant potential of FruHis was also evaluated in the Folin-Ciocalteu and FRAP assays. In both assays, FruHis showed a moderate reducing activity, whereas histidine and fructose were not active, as shown in Fig. 3C and D. In the Folin-Ciocalteu assay,

the color-developing effect of FruHis after 3 hours was ~ 30 times lower than that of chlorogenic acid. FruHis was also ~ 10 times less potent reducing agent in the FRAP reagent as compared with chlorogenic acid after 16 hours of incubation.

Discussion

Processing of many edible plants through heating, grinding, mixing, drying, etc., may dramatically increase their nutritive value to a large extent due to modification of carbohydrates that constitute the main part of plant biomass. There is an increasing evidence that nondigestible oligosaccharides and polysaccharides of pectins, gums, hemicellulose, inulin, etc., are essential for normal functioning of symbiotic microflora in the gut and are responsible for a host of other health-beneficial effects (28), including reduced risk of cancer (29). Ketosamines, such as FruHis, are small non-digestible carbohydrate derivatives that form as a result of dehydration of foods. Ingested ketosamines are partially absorbed but not metabolized (30) and, according to our data, may possess a prevention potential against carcinogenesis in the prostate.

We have recently evaluated FruHis in tomato products using an optimized analysis procedure of gas-liquid chromatography/mass spectrometry technique (20). Thus, FruHis content in samples of cold break and hot break tomato powder was 38.2 ± 0.2 and 41.4 ± 0.4 mg/100 g, respectively, and correlated well with the amounts of other D-fructose-amino acids relative to the content of respective "parent" free amino acids. In contrast, FruHis was not detected in any (cold break or hot break) tomato paste preparations. We hypothesize that FruHis should be present in many other dried fruits and vegetables that contain both histidine and glucose, as well as in foods prepared with tomato powder.

Primary screening of ketosamine/lycopene combinations against prostate cancer cell proliferation was done using the widely used Dunning R3327 rat prostate adenocarcinoma model. The MAT-LyLu cell line is the most aggressive of the Dunning sublines. It is a nondifferentiated, androgen-independent, fast-proliferating cell line, which invariably forms primary tumors in both orthotopic and ectopic sites and readily metastasizes into lymph nodes and lungs from any primary site (31). This cell line is moderately sensitive to lycopene, which accelerated the necrosis rate of growing MAT-LyLu tumors in one study (32), supposedly through reductions in steroid signaling and local insulin-like growth factor I

and interleukin-6 expression. In our hands, any detectable inhibition of MAT-LyLu proliferation rate in culture after 4 days was observed at lycopene concentrations in the medium exceeding $10 \mu\text{mol/L}$. However, we have chosen $1 \mu\text{mol/L}$ lycopene for the screening of ketosamines because this concentration is more relevant to the physiologically achievable concentration in serum (33). Of the 14 tested D-fructose-amino acids, only FruHis showed a noticeable inhibition of cell proliferation at 2 mmol/L concentration, whereas in combination with $1 \mu\text{mol/L}$ lycopene, it produced a remarkably strong synergistic effect against MAT-LyLu proliferation, causing >98% cell growth inhibition, as compared with the controls. The effect of the FruHis/lycopene combination against prostate cancer cell proliferation seems to be unique because the only previously reported synergistic interaction of lycopene with another antioxidant was observed for its combination with 1,25-dihydroxyvitamin D₃ against the HL-60 leukemia cell line (34). Although there are no current pharmacokinetic data available for FruHis, it is known from the studies involving humans, rats, and other mammals that FruLys and other ingested D-fructose-amino acids can penetrate the upper intestine by diffusion at a rate of up to 47% within 90 minutes, and that most of the absorbed D-fructose-amino acids get excreted in urine unchanged (30, 35).

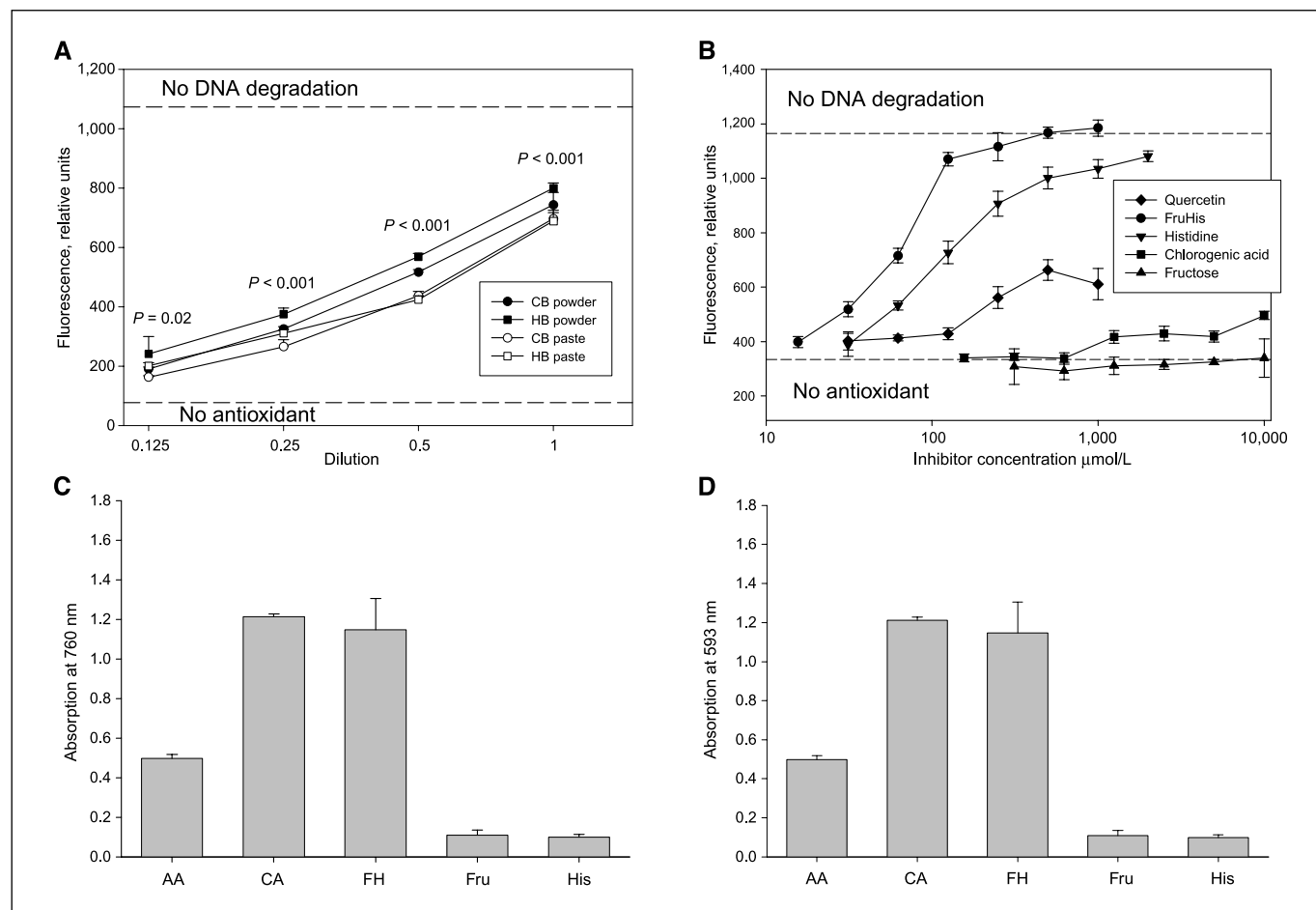


Figure 4. A, degradative oxidation of polymeric DNA by $\text{Cu}^{2+}/\text{H}_2\text{O}_2/\text{ascorbate}$ in the presence of aqueous extracts (5,000-kDa cutoff) from cold break tomato paste, hot break tomato paste, cold break tomato powder, and hot break tomato powder. The highest dilution value of 1 corresponds to $40 \mu\text{L}$ of the extract added per well, as described in Materials and Methods. B, degradative oxidation of polymeric DNA by $\text{Cu}^{2+}/\text{H}_2\text{O}_2/\text{ascorbate}$ in the presence of some antioxidants from tomato powder. C, reducing activity in the FRAP assay: AA, $20 \mu\text{mol/L}$ ascorbic acid; CA, $10 \mu\text{mol/L}$ chlorogenic acid; FH, $100 \mu\text{mol/L}$ FruHis; Fru, 40 mmol/L fructose; His, 40 mmol/L L-histidine. D, color development in the "phenol-specific" Folin-Ciocalteu assay. AA, $100 \mu\text{mol/L}$ ascorbic acid; CA, $100 \mu\text{mol/L}$ chlorogenic acid; FH, 2.5 mmol/L FruHis; Fru, 40 mmol/L fructose; His, 40 mmol/L L-histidine.

At this rate of absorption, 2 mmol/L ketosamine in blood may be achievable, at least theoretically, for the experimental diet containing 1.5% FruHis that we used in the prostate carcinogenesis chemoprevention experiment (Table 1).

We further showed that a short-term pretreatment of MAT-LyLu cells with the FruHis/lycopene combination would affect their survival *in vivo*. We reasoned that pulsing cancer cells with the combination might trigger a programmed cell death and/or make them sensitive to immunosurveillance in the syngeneic animal host. Indeed, the FruHis/lycopene combination caused a significant drop in both tumorigenesis incidence and mean tumor volume, as compared with the control, whereas none of the single-agent pretreatments showed statistically significant tumor growth inhibition. These experiments imply that the FruHis/lycopene combination deserves further investigation as a potential therapeutic antitumor agent.

With the tumor cell proliferation data in hand, we conducted a chemoprevention study using one of the most relevant rodent models of prostate-specific carcinogenesis. Macroscopically detectable tumorigenesis rate in the prostate of NMU/testosterone-treated control rats reached 63% after 51 weeks, which is in good agreement with the literature (10, 26). We did not detect any signs of systemic or organ-specific toxicity in the FruHis/tomato paste diet group, and the animal body weight gain in this group was not statistically significantly different from the others throughout the entire experiment. The results of this experiment clearly showed the chemopreventive effect in the FruHis/tomato paste diet group as compared with the control group, in both survival from any cancer and the incidence of tumorigenesis in the prostate. The survival of the tomato powder diet group was also statistically different from the controls, in agreement with the previously published results of Boileau et al. (10). Interestingly, there was no noticeable effect of the dietary intervention from any of the tested experimental supplements on tumorigenesis in sites other than the prostate. This observation, albeit related to a rat prostate carcinogenesis model, is in agreement with data from the epidemiologic studies that showed the beneficial effect of tomato/lycopene-rich diets on risk of cancer in the prostate more consistently, as compared with other sites (36).

The chemopreventive activity of the diet supplemented with FruHis/tomato paste and, to a lesser effect, of the diet supplemented with tomato powder lends support to the hypothesis that ketosamines, such as D-fructose-amino acids from dried tomato, may contribute to the lowering of tumorigenesis risk in the prostate. The concentration of FruHis in the tomato paste/FruHis diet, however, was about twice as high as the concentration of the total fructose-amino acid fraction in the tomato powder-based diet. Hence, more detailed studies would be necessary to confirm any contribution from this particular compound to the preventive effect of tomato powder. Other ketosamines or compounds that are not yet identified might contribute to the beneficial effects of processed tomato, as well. On the other hand, the present studies show that FruHis deserves an attention as a prospective chemopreventive agent on its own.

A few recently published studies showed an advantage of combining dietary tomato with other dietary chemopreventive agents, such as broccoli powder (37) or vitamin E/selenium (38), for reduction of implanted rat prostate adenocarcinoma growth, or garlic (39, 40), for chemoprevention of other cancers. Interaction of dietary FruHis with tomato paste in this study may be related both to the synergistic effect of lycopene and FruHis against the cancer cell proliferation and to a possible antioxidant activity of FruHis.

Our *in vitro* experiments showed that FruHis is indeed a powerful protector of DNA degradation by ROS in conditions where other water-soluble antioxidants from tomato are not as effective. The protective effect of FruHis against DNA oxidative degradation may be partially related not only to its reducing potential, as shown by the Folin-Ciocalteu and the FRAP assays, but also to its excellent copper-chelating properties (20).

Antioxidant activity is the prevailing suggested mechanism for lycopene, selenium, vitamin E, and other chemoprevention agents that are currently being tested in clinical trials for prostate cancer (41). ROS, generated both exogenously and endogenously, were associated with carcinogenesis and tumor progression in prostate and a number of other tissues. If not trapped efficiently in a tissue, primarily by glutathione, ROS cause oxidative damage to lipids, proteins, and nuclear and mitochondrial DNA. With age, the prostate gradually loses the antioxidant capacity, which is manifested through the loss of glutathione *S*-transferase expression and subsequent generation of glutathione in the reduced form (42), and this is thought as a condition that makes the prostate epithelium sensitive to oxidative stress-induced mutation, leading ultimately to neoplastic transformations (43). Consumption of lycopene-rich, tomato sauce-based diet, however, led to a decrease in DNA oxidative damage in patients with localized prostate adenocarcinoma (33).

A number of ROS-trapping antioxidants have shown a protective potential against experimental prostate carcinogenesis, thus confirming oxidative stress as a valid target for prostate cancer chemoprevention (42, 44). Many of these antioxidants, including lycopene, also act as inhibitors of cancer cell proliferation, thus indicating that they possess the therapeutic potential as well (45, 46). The ability of these agents to inhibit tumorigenesis, both at the initiation stage (as antioxidants) and the progression stages (as cancer cell proliferation inhibitors), may have secured their initial success in the chemoprevention studies. We suggest then that, in a similar way, the observed ability of the FruHis/tomato-enriched diet to decrease rate of prostate tumorigenesis may be related to a combination of both the antioxidant potential of FruHis and the inhibitory activity of FruHis/lycopene against prostate cancer cell proliferation.

Before this study, three main classes of structurally defined antioxidant molecules were usually recognized in tomato products: (a) ascorbic acid (230 mg/100 g in fresh tomato; much less in processed tomato) and other vitamins, such as tocopherol; (b) carotenoids, including lycopene and carotene (up to 60 mg/100 g in fresh tomato; 5% to 25% less in processed tomato); (c) phenolic compounds (up to 550 mg/100 g), including conjugates of caffeic and coumaric acids, flavonoids quercetin and kaempferol, etc. (47). Ketosamines, with their total content of up to 8,000 mg/100 g dry matter (13), may represent a novel type of potential dietary antioxidants, albeit specific for foods prepared from reconstituted dried tomato products.

In conclusion, this study has identified a novel, processing-defined agent in tomato products, the carbohydrate ketosamine FruHis, which showed antitumorigenic potential in two rat prostate cancer models. This result may introduce an additional intrigue into an ongoing dispute over the beneficial effects of dietary lycopene and tomato products in lowering the risk of prostate cancer (48–50) because it suggests the presence of a potential chemopreventive agent(s) in tomato products prepared by rehydration of tomato powder. Whereas it is not possible to retrospectively evaluate ketosamine content in tomato products

from the numerous published intervention and epidemiologic studies, the assessment study of the clinically relevant effects of ketosamines from dietary tomato products in humans is warranted.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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