

TOMATO AND SOY PHYTOCHEMICALS: IN VIVO BIODISTRIBUTION,
BIOAVAILABILITY, ANTIOXIDANT/OXIDATIVE ENVIRONMENT
REGULATION, AND PROSTATE BIOMARKER MODULATION

DISSERTATION

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By

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ABSTRACT

Tomato-based products and soy foods contain compounds that have been shown to beneficially impact health, however, adequate information on phytochemical in vivo biodistribution and bioavailability, as well as the effect of consumption of these foods on antioxidant/oxidative environment regulation and prostate biomarker modulation is not available. First, in a study of healthy individuals, the change in plasma lycopene and resistance of lipoproteins to ex vivo oxidative stress following variation in tomato-based product intake was assessed. Total plasma lycopene concentrations significantly decreased during a 7-day washout period and significantly increased after consumption of tomato-based foods for 15 days. A complex array of plasma lycopene isomers was also detected throughout the study. Additionally, dietary intervention with tomato-based products significantly enhanced the protection of lipoproteins to ex vivo oxidative stress. Secondly, a study was performed to determine if men with prostate cancer would consume tomato-based products or a soy protein supplement daily during the period between their diagnosis and surgery (i.e. a period of 2-4 weeks). In addition, this study was conducted to examine in vivo concentrations of phytochemicals from these foods and their effect on modulating hormone levels. All study participants were extremely compliant. Tomato-based product or soy protein supplement intake significantly increased plasma and prostate tissue carotenoid concentrations or urinary and prostate

tissue isoflavone levels, respectively. Lycopene isomer patterns in plasma and prostate were unique, yet complicated. It also appears that tomato sauce and tomato soup provide a more bioavailable form of lycopene than vegetable juice. In addition, this study showed that dietary intervention with tomato-based products and soy decreases serum PSA concentrations. A significant increase in lycopene and isoflavone concentrations following tomato and/or soy intake suggests a preferential uptake and requirement of these phytochemical-containing foods in biological processes related to reducing both oxidative stress and prostate cancer progression. Finally, using new technologies, an evaluation of stored samples from the first two studies was conducted to assess the influence 2-4 weeks of tomato-based product consumption had on additional markers of oxidative stress in healthy participants and prostate cancer patients. Tomato-based product intake significantly increased plasma lipid-soluble antioxidant capacity in each of the study populations, while decreasing urinary levels of 8-iso-PGF_{2α} in prostate cancer patients.

Dedicated to my wife and parents for their continuous encouragement and support
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TABLE OF CONTENTS

	<u>Page</u>
Abstract.....	ii
Dedication.....	iv
Acknowledgments.....	v
Vita.....	vi
List of Tables.....	x
List of Figures.....	xii
Chapters:	
1. Literature Review.....	1
1.1 Introduction.....	1
1.2 Tomatoes and Tomato-Based Products: Focus on Lycopene.....	2
1.2.1 Nutrient and Phytochemical Content.....	2
1.2.2 Tomato Processing.....	3
1.2.3 The Carotenoids and Carotenoid Chemistry.....	4
1.2.4 Analytical Advances and Carotenoid Isomer Characterization.....	6
1.2.5 Effect of Processing on Carotenoid Stability, Isomerization, and Bioavailability.....	8
1.2.6 Plasma and Tissue Lycopene.....	13
1.2.7 Carotenoid Metabolism.....	15
1.2.8 Oxidative Damage and Antioxidant Activity of Lycopene.....	15
1.3 Prostate Cancer.....	17
1.3.1 Contributing Factors and Pathogenesis.....	17
1.3.2 Impact and Intervention Strategies.....	18
1.4 Tomatoes, Tomato-Based Products, and Prostate Cancer.....	21
1.4.1 Epidemiologic Studies.....	21
1.4.2 In Vitro Studies.....	28
1.4.3 Animal Studies.....	29
1.4.4 Clinical Studies.....	30
1.4.5 Prostate Cancer Causality: Tomato-based Products vs Lycopene.....	32
1.5 Soy Foods: Focus on Isoflavones.....	33

1.5.1	Nutrient and Phytochemical Content.....	33
1.5.2	Isoflavone Chemistry and Biological Activity.....	34
1.6	Soy, Isoflavones, and Prostate Cancer.....	35
1.6.1	Epidemiologic Studies.....	35
1.6.2	In Vitro Studies.....	37
1.6.3	Animal Studies.....	38
1.6.4	Clinical Studies.....	40
1.7	Conclusion.....	40
1A.	Hypotheses.....	47
1B.	Objectives and Specific Aims.....	48
2.	The Consumption of Processed Tomato-based Products Enhances Plasma Lycopene Concentrations in Association With a Reduced Lipoprotein Sensitivity to Oxidative Damage.....	50
2.1	Abstract.....	50
2.2	Introduction.....	51
2.3	Subjects and Methods.....	54
2.4	Results.....	58
2.5	Discussion.....	61
3.	In Vivo Phytochemical Concentrations and Hormone Modulation Following Tomato-based Product or Soy Consumption in Prostate Cancer Patients.....	72
3.1	Abstract.....	72
3.2	Introduction.....	73
3.3	Subjects and Methods.....	77
3.4	Results.....	87
3.5	Discussion.....	93
4.	Evaluation of Additional Markers of Oxidative Stress Using New Technologies.....	113
4.1	Abstract.....	113
4.2	Introduction.....	114
4.3	Methods.....	116
4.4	Results.....	120
4.5	Discussion.....	122
5.	Conclusion.....	131
6.	List of References.....	134

LIST OF TABLES

<u>Table</u>	<u>Page</u>
1.1 Nutrient and carotenoid content of tomatoes and tomato products.....	42
1.2 Common food sources of lycopene.....	43
1.3 Lycopene concentrations in human tissue using HPLC technology as reported in several publications.....	44
2.1 Carotenoid concentrations of tomato products.....	64
2.2 Changes in plasma lycopene concentrations at baseline, after washout, and following intervention at d -7, 0 and 15.....	65
2.3 Consumption of tomato products and changes in plasma lycopene comparing values at the completion of washout to values following intervention for 15 d...	66
2.4 Percentage of plasma lycopene isomers in sixty adults before and after tomato product consumption at d -7, 0 and 15.....	67
2.5 Ratio of individual or groups of cis-lycopene isomers to all-trans-lycopene at d -7, 0 and 15.....	68
2.6 Tomato product consumption protects against the oxidation of isolated LDL + VLDL exposed to Cu ²⁺ -mediated oxidative stress in sixty adults at d 0 and 15...	69
3.1 Plasma carotenoid, tocopherol, retinol, and retinyl palmitate concentrations following tomato-based product or soy protein intervention.....	100
3.2 Changes in the percentage of plasma lycopene isomers following dietary intervention with tomato-based products or soy protein.....	102

3.3	Prostate carotenoid concentrations following tomato-based product or soy protein intervention.....	103
3.4	Percentage of individual lycopene isomers in prostate following consumption of tomato-based products or soy protein.....	104
3.5	Ratio of lycopene isomers to all-trans lycopene in prostate following dietary intervention with tomato-based products or soy protein.....	105
3.6	Urine isoflavone concentrations following tomato-based product or soy protein intervention.....	106
3.7	Prostate isoflavone concentrations following tomato-based product or soy protein intervention.....	107
3.8	Changes in plasma hormone levels following dietary intervention with tomato-based products or soy protein.....	108
4.1	Plasma total lycopene and total tocopherol concentrations following tomato-based product intervention in healthy individuals	125
4.2	Plasma total lycopene and total tocopherol concentrations following tomato-based product intervention in prostate cancer patients.....	126
4.3	Tomato-based product consumption increases plasma lipid-soluble antioxidant capacity in healthy individuals.....	127
4.4	Tomato-based product consumption increases plasma lipid-soluble antioxidant capacity in prostate cancer patients.....	128
4.5	Changes in urinary 8-iso-PGF _{2α} levels following tomato-based product consumption in prostate cancer patients.....	129

LIST OF FIGURES

<u>Figure</u>	<u>Page</u>
1.1 Representative carotenoid and lycopene isomer structures.....	45
1.2 Aglycone structures of daidzein, genistein, and glycitein.....	46
2.1 Representative HPLC chromatogram of tomato product lycopene isomers (A) and plasma lycopene isomers (B) from healthy individuals consuming different lycopene-containing tomato products for 15 d.....	70
2.2 Representative HPLC chromatogram of plasma lycopene isomer profile changes in healthy individuals at enrollment, after the 7-d washout period and after 15 d of consuming different lycopene-containing tomato products.....	71
3.1 Representative chromatogram using a C ₃₀ HPLC system with UV-vis detection of plasma lycopene isomers following consumption of tomato-based products for 2-4 weeks.....	109
3.2 Representative chromatogram using a C ₃₀ HPLC system with electrochemical detection of prostate carotenoids, including multiple lycopene isomers, after tomato-based product intake for 2-4 weeks.....	110
3.3 Representative chromatogram using a C ₁₈ HPLC system with UV-vis detection of urinary isoflavones following 2-4 weeks of soy intake.....	111
3.4 Representative chromatograms using a C ₁₈ HPLC system with UV-vis detection of prostate isoflavones (A) or using a C ₁₈ HPLC system ESI-MS/MS detection of prostate daidzein (B) and prostate genistein (C) after consumption of soy for 2-4 weeks.....	112

4.1	Representative chromatograms using a C ₈ HPLC system with UV-vis detection of urinary 8-iso-PGF _{2α} (A) or using a C ₈ HPLC system with ESI-MS detection of urinary 8-iso-PGF _{2α} (B) in prostate cancer patients prior to the consumption of tomato-based products for 2-4 weeks.....	130
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CHAPTER 1

LITERATURE REVIEW

Introduction

Observational and experimental evidence strongly implicates dietary variables as critical determinants of human health and disease risk. Among the many dietary variables implicated in the promotion of optimal health, a diet rich in a variety of fruits and vegetables appears to be a major contributing factor. Much of the evidence derived from human epidemiological studies suggests that an increased intake of fruits and vegetables is associated with a lower risk of cardiovascular disease (1, 2) and many types of cancer (3-5). Modern epidemiologic techniques employing detailed diet assessment tools have allowed investigators to further define the potential health benefits of specific fruits and vegetables suggested by observational epidemiologic studies and laboratory investigations. Our research program has been particularly interested in the accumulating evidence focusing upon tomato and soy foods in disease prevention. An accumulating body of evidence from human epidemiologic studies has associated an increased consumption of tomato or soy foods with a reduction in the risk for cardiovascular disease (6, 7) and several types of cancer (8-11). There are numerous phytochemicals in these foods that are hypothesized to be responsible for the potential health benefits observed in these studies. This review will cover multiple aspects of tomato and soy

foods as well as phytochemicals from these foods in relation to a number of factors associated with their beneficial health effects.

Tomatoes and Tomato-Based Products: Focus on Lycopene

Nutrient and Phytochemical Content

Tomatoes and tomato-based products are a source of important nutrients and contain numerous phytochemicals, such as carotenoids, that may influence health (Table 1.1). Tomatoes and tomato-based foods provide a small amount of vitamin E and substantial amounts of folate, vitamin C, and potassium. Although not listed in Table 1.1, tomatoes and tomato-based products also contain major minerals, including calcium and magnesium, trace minerals, including copper, iron, manganese, selenium, and zinc, and ultratrace elements, including aluminum, boron, chromium, and molybdenum (14). In addition to the carotenoids listed in Table 1.1, the carotenoids phytofluene, phytoene, neurosporene, γ -carotene, and ζ -carotene are also present in tomatoes and tomato-based foods (14). Lycopene is the predominant carotenoid in tomatoes (31-77 mg/kg of fresh tomatoes) and is the source of the brilliant red color (15). The presence of lycopene in human plasma and tissues primarily results from consumption of tomatoes and a variety of tomato-based products, such as spaghetti sauce, salsa, tomato soup, and ketchup (Table 1.2). It is estimated that greater than 80% of lycopene consumed in the United States is derived from tomato-based products (19), although apricots, guava, watermelon, papaya, and pink grapefruit also provide a dietary source of lycopene (16, 20, 21). The lycopene content of tomatoes can vary considerably with variety and ripening stage of tomatoes.

Lycopene concentrations in the red strains approach 50 mg/kg compared with only 5 mg/kg in yellow varieties (20). With very few exceptions, lycopene from natural plant sources exists primarily in the all-trans form, the most thermodynamically stable configuration (22-24). Tomatoes are also a source of a vast array of phytochemicals including flavonols (i.e., quercetin and kaempferol), phytosterols, and phenylpropanoids (i.e., phenolic acids) (14, 25). In a study performed by Bremner et al. (26), tomato juice was found to contain numerous phenolic compounds, including chlorogenic acid, caffeic acid, p-coumaric acid, naringenin, and rutin. The physiological significance of tomato phenolic compounds and their effects on health and disease at levels provided in the diet by tomatoes and tomato-based products are the subject of much speculation and forms the basis for many ongoing investigations.

Tomato Processing

Tomatoes are processed into many different tomato-based products such as sauce, soup, and juice. The demand for tomato processing typically arises from a need to preserve the product for cooking purposes. Concentration of tomatoes into a paste or puree is a common method employed for production of tomato sauce and tomato soup. Concentration involves removing the water through heating or filtration and preparing a tomato pulp. Tomato pulp can be prepared using certain types of mills or small pulping machines. It is usually necessary to remove seeds and skins, which can be accomplished by sieving through a medium mesh (i.e. 1-2 mm holes). Because these products are high acid foods, heat sterilization in a retort is typically performed at temperatures <100°C for 10-20 minutes. Tomato juice is processed somewhat differently than either tomato sauce

or tomato soup. Tomato juice can be separated from the pulp by filtering, but more commonly the entire pulp is used as juice. Removal of water is minimized when processing tomatoes into juice. The juice can be preserved by hot water pasteurizing in sealed bottles at 90-100°C for at least 10 minutes followed by cooling to room temperature or by hot filling into sterile bottles.

The Carotenoids and Carotenoid Chemistry

Carotenoids are natural pigments synthesized by plants and microorganisms. The most established natural roles of carotenoids are to protect cells against photo-oxidation and to serve as light-absorbing pigments during photosynthesis (27). Some dietary carotenoids, such as β -carotene, provide an important source of vitamin A; however, the majority of carotenoids, including lycopene, do not exhibit provitamin A activity. Lycopene is a carotenoid present in high concentrations in tomatoes and tomato products and is responsible for the characteristic red color of these foods. The associations between tomato products, lycopene, and disease risk have stimulated a greater effort to understand these relationships through cell culture and animal studies, as well as human metabolic studies (28-35).

Approximately 700 carotenoids have been characterized and share common structural features, such as the polyisoprenoid structure and a series of centrally located conjugated double bonds (36-38) (Figure 1.1). The color and photochemical properties of each carotenoid are determined by its structure (37, 38). In addition, the structure also contributes to the chemical reactivity of carotenoids toward free radicals and oxidizing agents, which may be relevant to in vivo biological functions in animals (37, 38).

Lycopene is a forty carbon ($C_{40}H_{56}$) acyclic carotenoid with 11 linearly arranged conjugated double bonds. Lycopene lacks the β -ionone ring structure and is therefore devoid of provitamin A activity. Because of the highly conjugated nature of lycopene, it is particularly subject to oxidative degradation and isomerization. Chemical and physical factors known to degrade other carotenoids, including exposure to light, oxygen, elevated temperature, extremes in pH, and active surfaces, apply to lycopene as well (39-43).

As a polyene, lycopene readily undergoes a cis-trans isomerization. As a result of the 11 conjugated carbon-carbon double bonds in its backbone, lycopene can theoretically be arranged in 2048 different geometrical configurations. Although a large number of geometrical isomers are theoretically possible for all-trans lycopene, Zechmeister et al. (22) and Pauling et al. (44) have found that only certain ethylenic groups of a lycopene molecule can participate in cis-trans isomerization because of steric hinderance. Interconversion of isomers is thought to take place with exposure to thermoenergy, absorption of light, or by involvement in specific chemical reactions. Cis isomers of lycopene have chemical and physical characteristics distinctly different from their all-trans counterparts. Some of the differences observed as a result of a trans-to-cis isomerization reaction include lower melting points, decreased color intensity, a shift in the lambda max, smaller extinction coefficients, and the appearance of a new maximum in the ultraviolet spectrum (45). To avoid underestimating the quantitative measurement of lycopene cis-isomers, the appropriate wavelength maximum and molar absorptivity values should be applied. Because of the difficulty in identifying individual cis forms, quantitative data for isomer content of biological samples are generally estimated values.

Analytical Advances and Carotenoid Isomer Characterization

High performance liquid chromatography (HPLC) is the most commonly used method for the separation, quantitation, and identification of carotenoids found in plasma and biological tissues. Similarities in the structural characteristics of carotenoids causes difficulty when trying to adequately identify individual carotenoids using only fixed wavelength or retention time data. The use of photodiode array detection, allowing for the collection of spectral data across a wide range of wavelengths, has improved our ability to more accurately characterize individual carotenoids. However, measurements of retention time, peak resolution and spectral data for individual absorbing species, and the use of authentic standards for comparison of UV/VIS spectra and retention times, are required (46). Mass spectrometric and tandem mass spectrometric analyses, which provide molecular weight and characteristic fragmentation patterns, provide additional information that increases our confidence in the identification of various carotenoids (46). In addition, both electron impact and fast atom bombardment have been used in mass spectrometric analysis of carotenoids (47-49).

Lycopene is generally separated from other carotenoids using HPLC with reversed-phase C₁₈ columns. Variations in the properties of the silica packing material in terms of carbon load, particle size, porosity, end-capping technique, and polymerization can significantly alter the selectivity and sensitivity of lycopene analysis (50-53). Our ability to detect low levels of carotenoids in biological samples has been somewhat limited by methodology and detection that does not adequately quantify carotenoid concentrations. The development of a C₃₀ reversed-phase gradient HPLC method

coupled with a coulometric electrochemical (EC) array detector provides a much lower detection limit and a unique opportunity to quantify low levels of carotenoids in tissue samples and in the plasma chylomicron fraction (54). The improved sensitivity of this HPLC-EC method (1-10 fmol) allows for detection and quantification of compounds from extremely small sample sizes.

Compared with conventional C₁₈ reversed-phase and silica normal-phase columns, reversed-phase C₃₀ columns are frequently used to achieve superior selectivity of lycopene isomers (53, 55). The polymerically synthesized C₃₀ columns not only provide excellent separation of the all-trans lycopene isomers from the cis counterpart, but they also display extraordinary selectivity among the individual cis-isomers (55, 56). An HPLC method using multiple columns in series has also been shown to similarly resolve cis and trans lycopene isomers (57). Identification and structure elucidation of isomeric carotenoids have been facilitated with the aid of high-resolution NMR spectroscopy. Hengartner et al. (58) reported the use of H- and C-NMR, UV/VIS, mass, and IR spectroscopy to fully characterize 15 (E/Z)-isomeric forms of lycopene. In addition, the use of HPLC-MS with atmospheric pressure chemical ionization, lutein stereoisomers can be distinguished from zeaxanthin stereoisomers during one chromatographic run and detected in the picogram range, whereas HPLC-NMR coupling provides unequivocal identification of each stereoisomer with a concentration in the nanogram range (59). The rapid improvement in analytic technology will significantly impact future investigations designed to elucidate the biological impact of lycopene and its isomers on tissues and organs. Investigators ranging from epidemiologists, clinical scientists, and those involved in rodent studies will be able to more precisely quantitate lycopene isomers in very small biological samples.

Effect of Processing on Carotenoid Stability, Isomerization, and Bioavailability

Consumers use the intensity of the red color as an index of quality for tomato products. Therefore, reducing the loss of lycopene throughout the production process and

during storage has always been an important issue for food processors. Exposure to thermal treatments during food-processing operations causes well-documented changes in the physiochemical stability of carotenoids. Boskovic et al. (60) and Cano et al. (61) observed that processing and extended storage of dehydrated tomato products resulted in a loss of all-trans lycopene content by up to 20%. Food-processing techniques, such as canning and freezing, led to a significant reduction in lycopene and total carotenoid content in papaya slices. In contrast, many studies have found that hydrocarbon carotenoids such as lycopene, α -carotene, and β -carotene in processed fruits and vegetables are fairly heat-resistant (62, 63). According to Khachik et al. (62), most of these carotenoids remain stable after bench-top food preparation. Additionally, no major changes in phytofluene, phytoene, and ζ -carotene were observed during the processing of tomatoes (64). Saini and Singh (65) reported that thermal processing had no effect on the lycopene content in juices made from several high-yield tomato hybrids. Zanori et al. (66) reported that despite the oxidative and thermal severity of the drying process, reflected in the 5-hydroxymethyl-2-furfural and ascorbic acid values, lycopene displayed high stability during drying of tomato halves. Additionally, Nguyen and Schwartz (17) reported that processing does not have a significant effect on the stability of lycopene, independent of product type, moisture content, container type, tomato variety, and severity of heat treatments.

Although lycopene may be fairly stable during standard food processing procedures, less is known about its impact on isomerization. Studies have shown that heating tomato juice and bench-top preparation of a spaghetti sauce from canned tomatoes increases cis-isomer concentrations (57, 67). In contrast, Khachik et al. (63)

observed that common heat treatments during food preparation, such as microwaving, steaming, boiling, and stewing, did not significantly change the distribution of carotenoids in tomatoes and green vegetables. Other studies have also reported low levels of lycopene cis isomers in thermally processed tomato products (17, 68). Nguyen et al. (69) reported that during typical cooking of tomatoes, factors such as genotypic differences in overall carotenoid composition, the presence of oil, and physical changes to tomato tissues did not influence the thermal isomerization of all-trans lycopene, all-trans δ -carotene, all-trans γ -carotene, or prolycopene. In addition, preliminary data from our lab indicates that retort heating had no effect on lycopene stability or isomerization, however, lycopene was not thermally stable following hotplate heating. Additional information needs to be gathered on the thermal behavior of lycopene before definitive answers can be offered regarding its physical state and stability during processing and cooking. Nevertheless, it is evident that lycopene is more stable in native tomato fruit matrices than in isolated or purified form due to the protective effects of cellular constituents such as water (70).

Phytochemicals present in tomatoes and tomato-based products must be readily bioavailable for absorption to mediate their hypothesized beneficial health effects. Bioavailability is defined as the fraction of an ingested nutrient that is accessible to the body through absorption for use in normal physiological functions and for metabolic processes (71). Differences in bioavailability of lycopene may account, in part, for the relatively poor correlations between blood lycopene concentrations and estimated dietary intake. Carotenoids are strongly bound to intracellular macromolecules in many foods, and absorption therefore may be limited unless released from the food matrix (72). Several studies have evaluated the bioavailability of the lipophilic carotenoids found in tomatoes and tomato-based products. Heating tomato juice was shown to improve the uptake of lycopene in humans (67). Gartner et al. reported that lycopene bioavailability from tomato paste, a processed product, was higher than from fresh tomatoes when both were consumed with corn oil (73). These observations seem to be the result of thermal weakening and disruption of lycopene-protein complexes, rupturing of cell walls, and/or dispersion of crystalline carotenoid aggregates. Likewise, various food-processing operations such as chopping and pureeing, which result in a reduction in physical size of the food particle, will also enhance lycopene bioavailability (74, 75). Lycopene bioavailability was studied after a single dose of fresh tomatoes or tomato paste by measuring carotenoid concentrations in the chylomicron fraction of the systemic circulation (73). Each source of lycopene (23 mg) was consumed with 15 g corn oil. Tomato paste was found to yield a 2.5-fold greater total all-trans lycopene peak concentration and a 3.8-fold greater area under the curve than fresh tomatoes. When compared with fresh tomatoes, ingestion of tomato paste resulted in a significantly higher

area under the curve for cis lycopene isomers. In addition, van het Hof and colleagues (76) studied the effect of mechanical homogenization and heating on the bioavailability of carotenoids from canned tomatoes. Homogenization enhanced TRL and plasma lycopene response (TRL fraction: 31% and 62% higher for mildly and severely homogenized tomatoes, respectively, versus no homogenization; Plasma: 16% and 21% higher, respectively; $P < 0.05$). Additional heating also increased lycopene responses in TRL ($P = 0.14$) and plasma ($P = 0.17$). Interestingly, similar effects were found for beta-carotene. These observations support the conclusion that food processing, homogenization, and cooking enhance lycopene bioavailability.

Digestive processes will certainly influence lycopene bioavailability. Several factors affect initial carotenoid release from the physical food matrix and transfer and distribution into lipid droplets within the stomach and proximal duodenum (77). Perhaps of major importance, dietary lipids may serve a critical role in dissolution and subsequent absorption of a very hydrophobic carotenoid such as lycopene. Pancreatic lipases and bile salts act upon the carotenoid-containing lipid droplets entering the duodenum and form multilammellar lipid vesicles containing the carotenoids (78). The transfer of lycopene, like other carotenoids, from the micelle into the mucosal cells appears to occur via passive diffusion (79, 80). Factors such as the structural features of the carotenoid, the dietary fat content, fatty acid patterns, fiber, and other food components may influence the carotenoid content of micelles and subsequent mucosal transfer (78). In addition, drugs that interfere with cholesterol absorption (81) and nonabsorbable fat analogs, such as sucrose polyesters (82), have been reported to reduce carotenoid absorption.

Chylomicrons are responsible for carrying carotenoids from the intestinal mucosa to the blood stream via the lymphatics (78). Little is known about how lycopene in chylomicrons is subsequently accumulated by the liver and other tissues, repackaged in lipoproteins, and returned to the circulation. Lycopene is carried in the plasma entirely by lipoproteins, and no other lycopene-specific binding or carrier proteins have been identified thus far (78, 83). Details of how hepatocytes, the initial source of circulating lipoproteins, transfer lycopene into specific secreted lipoproteins and how this process may be regulated is unclear. However, it is likely that dietary and pharmacologic agents that influence lipoprotein metabolism will influence circulating lycopene concentrations. The physical properties based on the carotenoid structure appear to add to the varying distribution of specific carotenoids among lipoprotein classes. It is hypothesized that very lipophilic carotenoids, such as lycopene, are present within the hydrophobic core of the lipoprotein particle.

Plasma and Tissue Lycopene

It is well known that between 10 and 20 cis isomer peaks are typically observed in human blood and together account for the majority of lycopene in serum (68, 84). Interestingly, we observed that the ratio of cis:trans lycopene isomers change in those on a lycopene free diet. According to Allen et al. (85), plasma lycopene isomer concentrations exhibit a 61:39 ratio for cis:all-trans at the start of a lycopene-free diet whereas after 2 weeks, the ratio shifts to 70:30 which was highly significant. Studies suggest that the all-trans lycopene content of serum is maintained through continuous dietary intake and that mobilization of all-trans lycopene from liver or other tissues, or

reconversion of cis isomers to trans cannot maintain the cis:trans ratio. In addition, it is plausible to hypothesize that there is a biological preference for certain lycopene isomers to be cleared from serum, distributed to tissues, or participate in reactions that cause degradation.

Although data are still limited, it is apparent that carotenoids are not uniformly and equally dispersed in human tissues (30, 67, 86-88). The tissue-specific carotenoid patterns reported thus far suggest a process whereby certain carotenoids may exert unique biologic effects in one tissue but not in another (Table 1.3). Thus far, there is no evidence for a specific receptor or enzymatic process that mediates lycopene uptake by the tissue. We therefore must assume that uptake in the tissue is related to lipoprotein metabolism.

Lycopene has been shown to exist in over 15 different geometrical configurations in human prostate tissue, where the cis isomer content is even greater, at 80 to 90%, than observed in serum (68). The chemical and physiologic processes that account for the high proportion of cis isomers in tissue remains speculative. An intriguing hypothesis is that isomerization reflects the participation of lycopene in antioxidant reactions within the prostate. Isomerization changes the structure of lycopene in a fashion that could alter the intracellular distribution of lycopene within organelles and membrane structures that in turn could influence biological processes. These are hypotheses that will need additional investigation.

Carotenoid Metabolism

Carotenoids can be metabolized via oxidation and/or non-enzymatic dehydration. A number of compounds *in vivo* have been identified as metabolites of various carotenoids. The identification of 2,6-cyclolycopene-1,5-diols A and B in extracts from human serum and milk (90) as well as studies on the chemical oxidation of lycopene with *m*-chloroperbenzoic acid (94, 95) were the basis for the proposed metabolic transformation of lycopene in humans. Oxidation of lycopene with *m*-chloroperbenzoic acid has demonstrated that lycopene is first oxidized at the 1,2- and 5,6-position to form 1,2-epoxide and lycopene 5,6-epoxide, respectively. Relative to the 1,2-epoxide, lycopene 5,6-epoxide is fairly unstable and undergoes cyclization to produce 2,6-cyclolycopene-1,5-epoxide A and B. This cyclization may be non-enzymatic and take place in the human stomach in the presence of acids. Only the corresponding diols, 2,6-cyclolycopene 1,5-diols A and B, are present in human serum (90). These diols may be formed from acidic and/or enzymatic ring opening of their respective epoxides. The concentrations of these lycopene metabolites are present in tomatoes and tomato-based products at much lower levels than in human serum (95). The presence of low concentrations of some of the lycopene metabolites in tomatoes and human serum may also be related to the radical scavenging ability of lycopene (96).

Oxidative Damage and Antioxidant Activity of Lycopene

It has been widely postulated that oxidative damage may contribute to the damage of cellular DNA, proteins, and lipids that initiate or enhance the progression of cancer.

Recent research has focused on the role of reactive oxygen species (ROS) or free radicals that are produced from exogenous and endogenous factors. Mammals have developed multiple defenses against reactive oxygen. Nutritional substances such as vitamin E, vitamin C, selenium, and carotenoids (97) are thought to be important complements to other cellular systems, such as antioxidant enzymes (glutathione peroxidase, catalase, CuZn- and Mn-superoxide dismutase) and antioxidant quenchers (ceruloplasmin, transferrin, ferritin, Cd/Hg/Zn/Cu: metallothioneins), that participate in the free radical defense system and provide protection against oxidative damage. Many of the proposed biological effects and health benefits of tomatoes and tomato-based products are hypothesized to be associated with the ability of certain phytochemicals such as lycopene to enhance the endogenous defense system by protecting against in vivo oxidative damage. However, definitive rodent and human studies have not yet established this relationship. Selection of appropriate markers of oxidation and characterization of their validity presents a problem in assessing the association between antioxidants and disease processes (98).

The ability of lycopene to act as an antioxidant and scavenger of free radicals is considered by most investigators as the most likely mechanism that could account for the hypothesized beneficial effects on human health (31, 99-104). As a result of having an extensive chromophore system of conjugated carbon-carbon double bonds, lycopene can accept energy from various electronically excited species. This is due to its ability to quench singlet oxygen (103), formed by energy transfer from a meta-stable excited photosensitizer (105). Singlet oxygen ($^1\text{O}_2$) is a very reactive high-energy and short-lived oxygen species produced in biologic systems that can react with biomolecules. Lycopene

may also interact with reactive oxygen species such as hydrogen peroxide and nitrogen dioxide (106-108). There is some evidence that lycopene may serve as an antioxidant in biological systems. Lycopene may prevent oxidative damage to lipoproteins and DNA (31, 84). A protective effect against oxidative stress also was illustrated when lycopene was found to be preferentially destroyed relative to β -carotene when human skin was irradiated with UV light (109).

Lycopene is extremely hydrophobic and is most commonly located within cell membranes. Therefore, the interaction of lycopene with reactive oxygen molecules may be most profound in the hydrophobic inner core of the cellular membranes unless the lycopene is associated with specific transmembrane proteins extending to the surface and interacting with the aqueous environment. Isomerization may slightly alter the physiochemical interactions between lycopene and subcellular structures. This in turn allows the lycopene to interact with a greater variety of components within the cell and participate in reactions that may be specific for subcellular compartments.

Prostate Cancer

Contributing Factors and Pathogenesis

Prostate cancer is the most common noncutaneous malignancy in American men, and is the second leading cause of cancer-related deaths (110). The exact causes of prostate cancer are unknown. However, factors such as testosterone, age, heredity, genetics, diet, and others have been identified as contributing components (111).

Testosterone is the major hormonal regulator of prostate growth and function and may

promote the progression of the disease. Men over the age of 50 are at risk for prostate cancer and an increased risk is positively associated with age. African Americans and men with a father or older brother who has had prostate cancer are also at increased risk. In addition, the presence of genes exhibiting common polymorphisms may modulate physiologic processes related to prostate cancer. Finally, multiple dietary factors have been associated with increasing or decreasing the risk for prostate cancer.

Prostate cancer exhibits a long preclinical phase or latency. Multiple features characterize the progression from normal prostatic epithelium, through preneoplasia or dysplasia, and culminating in prostate carcinoma. The continuum begins with progressive loss of markers of secretory differentiation, basal cell layer disruption, increasing nuclear and nucleolar abnormalities, greater proliferation, increased microvessel density, and progressive genetic instability (112). Prostatic intraepithelial neoplasia (PIN) and invasive adenocarcinoma of the prostate (IAP) are putative precancerous stages. In addition, PIN can be divided into two grades, low and high (111). Most pathologists grade prostate cancers according to the Gleason grading system. This system is used to evaluate how effectively the cancer cells are able to structure themselves into glands resembling those of the normal prostate. The ability of a tumor to mimic normal gland architecture is called its differentiation. The range for Gleason grading is from very well differentiated (grade 1) to very poorly differentiated (grade 5).

Impact and Intervention Strategies

The expense of screening programs, diagnostic tests, initial therapies, management of therapeutic complications, and the treatment of metastatic disease add

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

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

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

In summary, prostate cancer is an enormous societal and personal burden due to the lives lost and the morbidity of treatments in those cured as well as those suffering from recurrent disease. Furthermore, the costs of screening, diagnosis, localized therapy, and treatment of metastatic disease adds significantly to our health care expenditures in an aging population. Research from a variety of fronts, ranging from molecular biology to epidemiology, strongly implicates a role for diet and nutrition in prevention. Opportunities for nutritional scientists to contribute significantly to the development of effective interventions should be encouraged through increased research activity and interactions with clinical and basic investigators.

Tomatoes, Tomato-Based Products, and Prostate Cancer

Epidemiologic Studies

In recent decades, we have seen an accumulated body of evidence strongly supporting the conclusion that diets rich in fruits and vegetables are associated with a lower risk of many malignancies. Epidemiologic studies using diet-assessment tools that have the ability to examine the specific role of tomatoes and tomato products in cancer risk is relatively new, with the vast majority of published reports occurring over the last decade. Several studies published in the past ten years have suggested a beneficial relationship between tomato-based product consumption and several types of cancer. A comprehensive review of the epidemiologic evidence regarding tomato products and cancer risk was published by Giovannucci (8). Nearly 80% of the 72 studies reported in the review revealed evidence of a protective association between consumption of tomatoes, tomato products, or carotenoids provided by these foods and the risk of cancer at several sites. In more than 60% of these studies the inverse associations were statistically significant. The observed inverse relationship was strongest for lung, stomach, and prostate cancer and was supportive for cervical, breast, oral cavity, pancreatic, colorectal, and esophageal cancer. In addition, epidemiologic investigations of colon cancer (113), upper aerodigestive cancer (114), prostate cancer (115-118), and lung cancer (119) further support the concept that tomato products have cancer-preventive properties.

Although an association with prostate cancer has not been as strong in comparison with other malignancies, epidemiologic studies have shown that an increase in tomato and tomato-based food or lycopene consumption as well as serum lycopene concentrations are inversely correlated with the risk for prostate cancer. One of the first studies to suggest this relationship was conducted in the late 1970's in the Seventh-day Adventist population (120). A food frequency questionnaire was used to evaluate the diet of approximately 14,000 men. After 6 years of follow-up, 180 men were diagnosed with prostate cancer. The risk of prostate cancer was found to be significantly lower in men consuming five or more servings of tomatoes or tomato products per week compared with men who consumed less than one serving of tomatoes or tomato products per week (RR = 0.60; 95% CI = 0.37–0.97, P for trend = 0.02). In addition, there was a statistically significant inverse relationship with the consumption of beans, lentils and peas (120). This study is in agreement with a smaller (n = 669) case-control study completed at approximately the same time that found a lower risk of prostate cancer in men consuming tomatoes and/or tomato products ≥ 14 times per month compared with those consuming less than three servings per month (OR = 1.41 for < 3 servings/month vs. ≥ 14 servings/month, 95% CI not reported; 121). Conversely, in 1991, Le Marchand et al. (122) published a case-control study of diet and prostate cancer in a multiethnic Hawaiian cohort. They found no association between raw tomato consumption or estimated lycopene intake and prostate cancer risk. It is not clear, however, whether fresh and processed tomato products were included in the analysis.

The study providing the strongest evidence thus far concerning tomatoes and prostate cancer prevention was published in 1995 (123). The dietary habits of over

47,000 men enrolled in the Health Professionals Follow-Up Study (HPFS) initially established in 1986 were examined. Dietary intake was estimated using a 131-item food-frequency questionnaire that was completed twice during the initial 6-year follow-up period. There were 773 cases of prostate cancer (nonstage A1) diagnosed during the follow-up period (1986-1992). The only fruit or vegetables found to be associated with a reduced risk of prostate cancer were raw tomatoes (RR = 0.74 for zero servings vs 2-4 servings/week, 95% CI = 0.58–0.93, P for trend = 0.03), tomato sauce (RR = 0.66 for zero servings vs 2-4 servings/week, 95% CI = 0.49–0.90, P for trend = 0.001), pizza (RR = 0.85 for zero servings vs 2-4 servings/week, 95% CI = 0.57–1.10, P for trend = 0.05) and strawberries (RR = 0.80 for zero servings vs 1 serving/week, 95% CI = 0.57–1.10, P for trend = 0.005; 123). In men who had more advanced prostate cancer (defined as either stage C or D), consuming ten servings of tomato products per week compared with less than 1.5 servings per week was significantly protective (RR = 0.47, 95% CI = 0.22–1.00, P for trend = 0.03). The data derived from this study are considered the most powerful linkage between tomato products and a lower risk of prostate cancer because of the large size of the cohort and the prospective collection of dietary data with a validated assessment tool. Updated data from the HPFS for the period from 1992 through 1998 confirmed the previous findings. Lycopene intake was associated with a decreased risk for prostate cancer (RR for high compared to low quintiles = 0.84; 95% CI = 0.73-0.96; p for trend = 0.003). An even greater reduction in the risk for prostate cancer was correlated with consumption of tomato sauce (RR for 2+ servings/week versus <1 serving/month = 0.77; 95% CI = 0.66-0.90; p for trend = 0.001; 118).

Four case-control studies evaluating the link between tomatoes and prostate cancer incidence have been published with only one demonstrating a protective effect of tomato products. A study, conducted in Greece, compared the dietary habits of 320 men with prostate cancer to 246 controls and found that consumption of cooked tomatoes was inversely associated with prostate cancer risk ($P = 0.005$; 124). The intake of raw tomatoes alone was not significantly protective ($P = 0.12$). From their data, the authors concluded that increasing cooked tomato intake from two times per week to eight times per week reduced the risk of prostate cancer by 15% ($OR = 0.85$, 95% $CI = 0.75-0.97$). In three other case-control studies, no relationship was found between raw tomatoes or cooked tomatoes and prostate cancer risk; however, two of the studies noted a significant protective effect of cruciferous vegetables (125-127). Cohen and colleagues (125) completed a nested case-control study in King County Washington with 628 patients and 602 control patients between the ages of 40 and 64. Food frequency questionnaires were completed and total fruit and vegetable intake was summarized. There were no protective effects of raw tomatoes (<1 serving/week vs ≥ 3 servings/week, $OR = 1.22$, 95% $CI = 0.83-1.80$, P for trend = 0.26), cooked tomatoes (<1 serving/week vs ≥ 3 servings/week, $OR = 0.90$, 95% $CI = 0.57-1.42$, P for trend = 0.68), or estimated lycopene intake (<4900 $\mu\text{g}/\text{day}$ vs ≥ 9900 $\mu\text{g}/\text{day}$, $OR = 0.89$, 95% $CI = 0.60-1.31$, P for trend = 0.96). However, both total vegetable intake (<14 servings/week vs ≥ 28 servings/week, $OR = 0.65$, 95% $CI = 0.45-0.94$, P for trend = 0.01) and cruciferous vegetable intake (<1 serving/week vs ≥ 3 servings/week, $OR = 0.59$, 95% $CI = 0.39-0.90$, P for trend = 0.02) were significantly protective.

Although tomatoes and tomato products have many nutrients and phytochemicals that are proposed to inhibit carcinogenesis, lycopene has received the most intense focus. Giovannucci et al. (123) estimated lycopene intake in the HPFS cohort using the USDA Carotenoid Database. The estimated dietary intake of β -carotene, α -carotene, lutein, and β -cryptoxanthin was not related to prostate cancer risk. However, dietary intake of lycopene (80% of which was derived from tomatoes and tomato products) was inversely related to risk when the highest quartile (>6.4 mg lycopene/day) was compared with the lowest quartile (<2.3 mg lycopene/day, RR = 0.79, 95% CI = 0.64–0.99, P for trend = 0.04). A few years later, a case-control study of 797 men in New Zealand found a weak, nonsignificant trend between lycopene intake and prostate cancer incidence when comparing the lowest quartile (<663 μ g/day) of lycopene intake to the highest quartile ($>1,994$ μ g/day) (OR = 0.76, 95% CI = 0.53–1.26, P for trend = 0.30). Additionally, there was no association between dietary intake of raw tomatoes and prostate cancer (<13 g/day vs >35 g/day, OR = 1.01, 95% CI = 0.66–1.63, P for trend = 0.93) and only a weak, nonsignificant trend between processed tomato products and prostate cancer risk (<18.7 g/day vs >64.2 g/day, OR = 0.83, 95% CI = 0.53–1.26, P for trend = 0.30; 117). Interestingly, in this study the estimated median intake of lycopene was less than half of the median in the HPFS cohort (1.2 mg lycopene/day vs 3.4 to 4.6 mg lycopene/day, respectively).

The dietary intake of lycopene is difficult to precisely quantify for several reasons, thus reducing the sensitivity of an epidemiologic study to detect relationships with cancer risk. Food diaries and food-frequency questionnaires provide an estimate of lycopene-containing foods consumed. The USDA database, or others, can then be

applied to obtain an estimate of lycopene consumed. However, foods do not contain constant concentrations of lycopene. For example, the content of tomato sauce varies significantly between brands. Thus, it is proposed that the measurement of lycopene concentration in blood may provide a useful link between dietary lycopene intake and risk assessment in epidemiologic studies. Interestingly, serum lycopene is not strongly correlated with estimated dietary intake of lycopene with correlation estimates that range from 0.16 to 0.47 (92, 128, 129). Inaccurate estimation of dietary intake, variation in bioavailability among food sources of lycopene, inconsistency in absorption as a result of diet composition, and changes in uptake that may be related to age and genetics will contribute to the low correlations between estimated intake and blood concentrations.

Several reports have investigated the relationship between blood concentrations of various carotenoids found in tomatoes and prostate cancer risk. In a study conducted at Memorial Sloan-Kettering Cancer Center from 1993 to 1997, Lu and co-workers (115) showed that when plasma carotenoid levels from men in the highest and lowest quartiles were compared, inverse associations for prostate cancer risk were statistically significant for plasma lycopene and zeaxanthin concentrations. In addition, plasma lutein and β -cryptoxanthin levels were associated with a significant reduction in PCa risk. Two of three case-control studies have found weak, nonsignificant, inverse relationships between serum lycopene levels and risk of prostate cancer. One study suggested an inverse association between serum lycopene levels and aggressive prostate cancer (29, 130, 131). A nested case-control investigation was undertaken and involved the analysis of carotenoids in blood samples from men enrolled in the Physicians' Health Study, a randomized, placebo-controlled trial of aspirin and β -carotene. In this study, men in the

highest quintile (>580.1 ng/ml) of serum lycopene levels had a significantly lower risk of prostate cancer compared with men in the lowest quintile of serum lycopene (≤ 261.7 ng/ml, OR = 0.56, 95% CI = 0.34–0.92, P = 0.05). The inverse association between serum lycopene and aggressive prostate cancer was particularly significant for men who were not consuming β -carotene supplements (OR for highest quintile versus lowest quintile = 0.40, 95% CI = 0.19–0.84, P for trend = 0.006; 29). Hsing et al. (130) evaluated serum obtained in 1974 from 25,802 persons in Washington County, MD, and reported lower mean serum lycopene concentrations in prostate cancer cases compared to controls. A 50% reduction in the relative risk for PCa was observed when cases in the highest serum lycopene quartile were compared to the lowest quartile. Shortcomings of these and similar case-control studies evaluating serum carotenoids and prostate cancer risk frequently include small sample size, possible lycopene degradation in samples because of factors such as exposure to light or long periods of storage before analysis, and the unknown ability of a single blood sample to represent lycopene concentrations over a longer time span. It is clear that more studies are necessary in order to draw any conclusions regarding serum lycopene and prostate cancer risk.

Giovanucci et al. (118) outlined several possible explanations for the apparent inconsistencies seen in the literature. Lycopene intake may not be high enough to mediate a beneficial effect in some groups. Consumption and bioavailability of lycopene-containing foods may not be adequately accounted for by dietary questionnaires. As prostate cancer develops over many decades, single dietary measurements may therefore not provide an appropriate indication of the influence of diet on the carcinogenic process. In addition, lycopene may be more effective in aggressive

compared to less aggressive stages of prostate cancer. Finally, it is possible that confounding factors contribute to the association between prostate cancer and lycopene. If we take into account some of these issues coupled with the inherent difficulties of precisely defining human intake in epidemiologic studies and the relatively modest changes in risk associated with single dietary components (compared to high risk exposures such as tobacco), we can appreciate that all studies may not provide uniform findings.

Mechanisms by which tomatoes and tomato products may reduce prostate cancer risk have also been investigated in an epidemiologic context. One focus of investigation is the relationship between diet and insulin-like growth factors and binding proteins (132). In a nested case-control study in Greece, Mucci and colleagues (133) collected sera and administered a food-frequency questionnaire to 112 cancer-free men. Consumption of cooked tomatoes was found to be significantly inversely associated with insulin-like growth factor-1 levels with a mean change of -31.5% for each one serving increase per day. Blood lycopene concentrations were not quantitated in this study but would have been a valuable component of the investigation.

In Vitro Studies

The effect of lycopene on prostate cancer cells has been evaluated in a number of in vitro studies. In a study conducted by Pastori et al. (134), the effect of lycopene alone or in combination with other antioxidants was examined on the growth of androgen insensitive DU-145 and PC-3 human prostate carcinoma cell lines. Potent inhibition of prostate carcinoma cell proliferation did not occur with lycopene alone. However, the

combination of 1 μM lycopene and 50 μM α -tocopherol resulted in nearly 90% inhibition of prostate carcinoma cell proliferation, suggesting a synergistic effect. In another study, the effects of 15 kinds of carotenoids on the viability of PC-3, DU-145, and LNCaP human prostate cancer cells, were determined (135). Cells cultured with phytofluene, zeta-carotene, or lycopene at 20 $\mu\text{M/L}$ for 72 h caused a reduction in human prostate cancer cell viability ($P < 0.05$). Kim et al. (136) conducted a study to determine the effect of lycopene on the proliferation of LNCaP human prostate cancer cells in culture. Lycopene at concentrations of 10^{-6} and 10^{-5} resulted in a reduction in the growth of LNCaP cells after 48, 72, and 96 hours of incubation by 24% to 43% ($P < 0.05$). In addition, a dose-dependent decrease in the growth of cells incubated with lycopene for 24, 48, 72, or 96 hours ($F = 3.2, 11.3, 54.5, \text{ and } 297.5$, respectively; $P < 0.05$) was observed. A study by Rossinow et al. (137) evaluated the effect of lycopene and vitamin E on survival and DNA damage in gamma-irradiated DU-145, PC-3, and LNCaP human prostate cancer cells. Only lycopene protected cells against DNA damage as shown by a decrease in micronuclei formation. Additionally, concentrations of lycopene at both 4 μM and 7.2 μM in DU-145 and LNCaP cell lines increased the overall survival (10% and 15% in DU-145 cells, and 26% and 36% in LNCaP cells; $P < 0.05$).

Animal Studies

Several laboratories have conducted studies on tomatoes, lycopene and prostate carcinogenesis in rodents. An investigation using the DMAB and PhIP-induced rat prostate cancer models failed to detect a chemopreventive effect of lycopene provided as an extract of 99.9% purity from LycoRed Ltd (138). In another study, two different

doses of a lycopene-rich tomato oleoresin were fed to lacZ mice to study the effects on short-term benzo[a]pyrene (BaP)- induced and long-term spontaneous in vivo mutagenesis in the colon, prostate, and lungs (34). Spontaneous mutagenesis was inhibited in prostate and colon tissue at the higher dose of tomato oleoresin. In addition, (BaP)-induced mutagenesis in the prostate was also slightly inhibited in mice fed tomato oleoresin (34). Our laboratory has completed a series of studies with a lycopene oleoresin in mice bearing human xenografts of PC-3, DU145, and LNCaP human cell lines and observed no major anti-tumor effects (preliminary data). Our group has also completed a large rat study evaluating the ability of lycopene or freeze-dried tomato powder to inhibit survival in the N-nitrosomethylurea-androgen-induced prostate cancer model. In this system, we observed a very small beneficial trend for lycopene and a significant benefit of tomato powder (139). Thus far it appears that tomatoes may contain components in addition to lycopene that may inhibit prostate tumorigenesis.

Clinical Studies

Carefully designed clinical intervention trials in humans can provide valuable insight into the biological effects induced by the phytochemicals in tomatoes and tomato products. There are few human intervention studies investigating the role of tomatoes and/or lycopene on processes that are related to the development of prostate cancer. A study conducted by Bowen et al. (140) evaluated 32 patients with localized prostate adenocarcinoma. The men consumed tomato sauce-based pasta dishes for 3 weeks (30 mg of lycopene/day) before their scheduled radical prostatectomy. Prostate tissue was obtained as biopsies at baseline and as resected tissue at the time of the prostatectomy.

Serum and prostate lycopene concentrations increased 1.97- and 2.92-fold ($P < 0.001$), respectively. Mean serum prostate-specific antigen (PSA) concentrations decreased by 17.5% ($P < 0.002$) and leukocyte 8-hydroxy-2'-deoxyguanosine, a marker of DNA damage, decreased by 21.3% ($P < 0.005$) after tomato sauce consumption. In addition, apoptotic index was higher in hyperplastic and neoplastic cells in the resected tissue after supplementation. The most provocative observations have been published by Kucuk and colleagues (93). The study involved 26 men diagnosed with presumed localized prostate cancer who were scheduled to undergo a radical prostatectomy. The men were randomized to consume 30 mg of lycopene per day from two tomato oleoresin capsules (Lyc-O-Mato; LycoRed Natural Products Industries, Beer-Sheva, Israel) or to continue their normal diet for 3 weeks prior to surgery. Post-surgical prostate tissue specimens were then compared between the two groups. Men consuming the lycopene supplement had 47% higher prostatic tissue lycopene levels than the control group (0.53 ± 0.03 ng/gm vs 0.36 ± 0.06 ng/gm, $P = 0.02$); however, plasma lycopene levels were not significantly different between the groups nor did they change significantly within each group. Men who consumed the lycopene supplement were less likely to have involvement of surgical margins (73% vs 18% of subjects, $P = 0.02$). Additionally, they were less frequently found to have high-grade prostatic intraepithelial neoplasia (HGPIN) in the prostatectomy specimen (67% vs 100%, $P = 0.05$). HGPIN is considered to be a premalignant lesion predisposing men to prostate cancer. Additionally, the intervention group was found to have smaller tumors, a greater reduction in PSA over the 3-week study period, and a higher expression of connexin 43; however, none of these differences were statistically significant.

Extreme caution should be used when interpreting case reports. One study describes a 62 year old man with hormone refractory prostate cancer who failed multiple treatment regimens, including leuprolide, bicalutamide, ketoconazole and hydrocortisone, doxorubicin, vinorelbine, and prednisone (141). His PSA had increased to 365 ng/ml when he began taking 10 mg of lycopene per day and 300 mg of saw palmetto three times each day. One month after beginning these dietary supplements, his PSA reportedly decreased to 139 ng/dl and remained between 3 and 8 ng/ml for at least 18 months. The authors attributed this dramatic improvement to the lycopene supplements rather than the saw palmetto (141). There are some reports, however, that saw palmetto can influence the prostate and exhibits effects similar to finasteride (a 5- α -reductase inhibitor) so it is difficult to discount the possible effects of saw palmetto (142, 143). Additionally, the source of lycopene was not given and lycopene content and stability within a supplement can vary widely. Although intriguing, this case report should be viewed with great skepticism.

Prostate Cancer Causality: Tomato-based Products vs. Lycopene

The hypothesis that tomato-based products contain phytochemicals, perhaps lycopene and others, that modify prostate carcinogenesis warrants investigation. However, scientists, regulatory agencies, marketers of products, and those defining public health policy have differing opinions regarding the strength of the data when applied to criteria for inference and causality. Criteria such as consistency, strength of association, biological gradient, temporality, specificity, biological mechanisms and coherence, and experimental evidence can be used to better define the current status and future directions

for research. Miller et al. (144) discussed the relevant data regarding tomato-based products, lycopene, and prostate cancer in reference to the above criteria. Many of the studies used to help define this relationship are discussed in this literature review. Although definitive conclusions and recommendations remain controversial, the authors support the recommendation that a minimum of five servings of fruits and vegetables should be consumed from a variety of sources. Miller and colleagues believe that the hypothesized benefits of tomato-based products for prostate cancer prevention may be achieved with approximately five servings of tomato-based products per week and do not currently recommend the consumption of lycopene supplements for prostate cancer prevention or therapy. Future research will allow us to provide more definitive guidelines.

Soy Foods: Focus on Isoflavones

Nutrient and Phytochemical Content

Soybeans contain amino acids, globulins, fiber, and phytochemicals including saponins, lignans, phytates, and isoflavones (145). Although many varieties of grains, vegetables, and legumes contain small amounts of isoflavones, the largest quantities are found in soybeans. The level of isoflavones in soybeans varies with geographic location, environmental conditions of growth, variety, soil type, and year (146). Commonly consumed soy products include tofu, miso, tempeh, and natto, however, soybeans are the only nutritionally relevant natural dietary source of isoflavones. Although there is a large variability of isoflavone composition among soybeans or soy-based food products, most

dietary sources contain a mixture of derivatives based on three isoflavone aglycones with the common names genistein, daidzein, and glycitein (147, 148). In the soybean, these three isoflavones are present as the glycosides, genistin, daidzin, and glycitin. The glycoside can also be esterified with either malonyl or acetic acid. Generally, soy foods contain somewhat more genistein than daidzein and relatively little glycitein (<10% of total isoflavone content) (11). The consumption of 100 mg isoflavones per day, as the glucoside, from soy foods is typical and occurs for a substantial proportion of Asian populations (149).

Isoflavone Chemistry and Biological Activity

Isoflavones are a subclass of the flavonoid nutraceuticals. Isoflavones, of which there are approximately 230 individual types, have a very limited distribution in the plant kingdom (149). Isoflavones play a key role in plant-microbe interactions, serving as the signal molecule for establishing the symbiotic relationship between plants and rhizobial bacteria that results in the formation of nitrogen-fixing root nodules. Isoflavones are also the precursors to the major phenolic phytoalexins, which are used for defense against bacterial, fungal, or viral infection. Some also serve as insecticides because their steroid-mimicking structures could disrupt growth and development in insects (150).

The isoflavones daidzein, genistein, and glycitein contain conjugated double bond systems, hydroxyl groups, and ring structures (Figure 1.2). The chemical structures of isoflavones and estrogen are similar, therefore, it is not surprising that isoflavones bind to estrogen receptors. The binding affinity of isoflavones for the estrogen receptor alpha ($ER\alpha$) and $ER\beta$ is much lower and slightly lower, respectively, than 17β -estradiol.

However, serum isoflavone levels for people who consume soy foods can be detected in the micromolar range, which is nearly 1000-fold higher than endogenous estrogen levels (151), suggesting that isoflavones may potentially exert physiological effects in vivo. Some studies imply that isoflavones and 17 β -estradiol produce similar transcriptional activity when bound to ER β (152). As a result, isoflavones are occasionally referred to as natural selective estrogen receptor modulators. Aside from direct hormonal effects, isoflavones, particularly genistein, modulate signal transduction in vitro and in vivo. The activity of various cellular factors and enzymes that regulate the growth and differentiation of cells are inhibited by genistein (153-155). In addition, some experimental systems have shown isoflavones to exhibit antioxidant activity (156).

Soy, Isoflavones, and Prostate Cancer

Epidemiologic Studies

Intake of soy foods or isoflavones in relation to prostate cancer has been studied by multiple investigators. Among 42 countries for which soy intake data was available, consumption of soy products was inversely related with prostate cancer mortality (157). However, the value of this observation is unclear because only Korea and Japan consumed substantial amounts of soy. In contrast, Negata et al. (158) failed to detect an association between total soy or isoflavone intake and prostate cancer mortality after assessing this relationship in 47 prefectures in Japan.

Although none of the six case-control studies performed have shown a statistically significant association between soy consumption and prostate cancer risk, in

two of these studies only intake of miso (159) or soymilk (160) was reported. In addition, three of these studies, conducted in North America, suggested a protective effect of soy intake on prostate cancer risk (161-163). In the study by Strom et al. (162) of 83 cases and 107 controls, median isoflavone intake of 100 µg/day resulted in an inverse association (odds ratio for control versus cases, 0.71 for genistein and 0.57 for daidzein) with risk for prostate cancer. In the largest of the three North American case-control studies involving 1619 cases and 1618 controls (847 of which had normal PSA levels), Kolonel et al. (163) reported that soy food intake was inversely related (odds ratio for fifth versus first quintile, 0.62; P for trend = 0.06) to prostate cancer risk.

Two prospective studies did not show an inverse association between prostate cancer risk and soy intake (120, 164). However, only miso intake was reported in the study by Hirayama et al. (164) and soy was only one food within the group of vegetarian protein products that was marginally protective (odds ratio for third versus first tertile, 0.67; $P = 0.10$) in the study conducted by Mills et al. (120). Alternatively, Severson and colleagues (165) studied 7,999 Japanese men (follow-up period 1965-1986) residing in Hawaii and found that tofu intake was related with a dose-dependent decrease in risk for prostate cancer (relative risk for first versus third tertile of intake, 0.35; $P = 0.054$). The tertiles for tofu intake in this study were <1 week, 2-4x/week, and ≥ 5 x/week. A second supportive study by Jacobsen et al. (166) of 14,671 (12,395 men, follow-up 1976-1982, 147 cases; 2,276 men, follow-up 1976-1992, 78 cases) Seventh-day Adventists in California, frequent (i.e., more than once/day) intake of soymilk was associated with a marked reduction in the prostate cancer risk (relative risk for fourth versus first quartile of intake, 0.3; P value for linear trend = 0.03). Intake categories for this study were 0, <1 /week, 1x/week, and >1 /day.

In Vitro Studies

Multiple in vitro studies examining the effect of isoflavones on prostate cancer cells have been conducted. In vitro studies involving both androgen-dependent (LNCaP) (167-170) and androgen-independent (DU-145, PSL-10, PC-3, and PC-3-M) (167, 168, 171-173) prostate cancer cells show that genistein inhibits their growth in a dose-dependent manner. Peterson et al. (167) reported that in comparison to unstimulated cells, epidermal growth factor-stimulated DU-145 and LNCaP cell IC_{50} values (i.e. 50%

inhibition of cell growth) were reduced by 85% and 32%, respectively. Kyle and colleagues (172) showed that increased exposure (11 days) to genistein resulted in a two- to threefold lower IC₅₀ value when compared to shorter (3 days) exposure. Interestingly, as demonstrated by Davis et al. (174), the inhibitory effects of daidzein on PC-3 cells were similar to the effects observed with genistein. Although most studies report that a genistein concentration of 25 μM is necessary to inhibit cell growth by 50%, a study by Davis and colleagues (175) suggests that this level of inhibition for LNCaP cells can be achieved at a genistein concentration of 5 μM. However, the serum concentrations of genistein, even in individuals who consume appreciable amounts of soy, are much lower than the IC₅₀ for growth inhibition (176).

In addition to the effects of isoflavones on growth inhibition, genistein has been shown to cause a threefold inhibition of PC-3 cell invasive capacity (168), decrease the growth of human-patient benign prostatic hypertrophy in histoculture (177), increase the ability of radiation to kill prostate cancer cells (178), and reduce PSA secretion in LNCaP cells (169, 175).

Animal Studies

Studies in rodents have provided evidence for a protective effect of soy on prostate cancer. As reported by Pollard and Luckert (179), Lobound-Wistar (L-W) rats fed isoflavone-rich isolated soy protein (ISP+) had a reduction in tumor incidence and increased tumor latency when compared to rats fed casein. The most pronounced effects were noted when the ISP+ was fed prior to methylnitrosourea (MNU) administration. Additionally, in comparison to L-W rats fed a natural-ingredient soymeal diet, L-W rats

consuming an ISP+ diet had an inhibition of tumor growth and decreased tumor incidence (180-182). In a study by Mentor-Marcel and colleagues (183), a significant decrease in the occurrence of advanced prostate lesions was found for transgenic mice fed 0-500 mg genistein/kg for 5-30 weeks.

The effect of soy or isoflavones on prostate tumors induced by inoculating animals with cancer cell lines or cancer tissue has been evaluated in a number of studies (171, 184-189). The null effects reported by Naik et al. (171) and Cohen et al. (189) may have been due to low amounts of genistein in the diet and the type of cell used to induce tumors, respectively. Modest protective effects of soy or isoflavones on prostate cancer were reported in three separate studies (184-186), however, the most convincing results have been reported by Zhou et al. (187, 188). In the first study, severe combined immune-deficient mice inoculated with LNCaP cells had reduced tumor cell proliferation, suppressed tumor formation, reduced microvessel density, increased apoptosis, and lower serum insulin-like growth factor-I levels which was dose-dependent after being fed soy protein and isoflavones (187). In a similar study, mice fed soy phytochemical concentrate (SPC) resulted in tumor development inhibition (188). Interestingly, this study also showed that the combination of SPC and black tea not only inhibited prostate tumorigenicity, but also inhibited metastases and final tumor weight, a phenomenon observed with SPC and green tea as well. In studies where tumors were induced by chemical carcinogen, an isoflavone mixture significantly suppressed the occurrence of prostate and seminal vesicle tumors (190), and ventral carcinoma incidence was reduced following intake of genistin and daidzin (191). Finally, in a study not evaluating prostate tumor development, Dalu et al. (192) reported that L-W rats fed

0.025-1.0 mg genistein/g diet demonstrated a 50% inhibition in the expression of epidermal growth factor, a potent mitogen for the prostate.

Clinical Studies

Prostate cancer is considered a hormone-dependent cancer (193). However, consumption of soy foods, isoflavone-rich soy protein, or isolated isoflavones by men for 3-12 weeks, consuming 40-130 mg isoflavones/day, did not significantly affect testosterone or dihydrotestosterone levels (194-199). In a cross-sectional study of 69 men in Japan, Nagata and colleagues (199) found that soy product intake was inversely correlated with total testosterone ($r = -0.25$, $P = 0.05$) and free testosterone ($r = -0.25$, $P = 0.06$).

Three studies have failed to find an effect of soy or isoflavones on PSA levels (198, 200, 201). Although no effect on PSA levels were noted, Jarred et al. (198) reported a higher level of apoptosis ($P < 0.005$) in treated versus control patients after consumption of 160 mg red clover-derived dietary isoflavones for an average of 20 days. In contrast, Hussain et al. (202) conducted a pilot study in 41 men at the Karmanos Cancer Institute at Wayne State University with treated but uncontrolled prostate cancer, and found that consumption of 120 mg isoflavones from soybeans for 6 months resulted in a decrease in the linear rise in PSA levels in 50-70% of the cases.

Conclusion

Adequate information on in vivo concentrations of tomato and soy phytochemicals, including their isomers and/or metabolites, is lacking. In addition,

research on the effect consumption of tomato-based foods has on increasing antioxidant capacity and reducing oxidative damage in vivo is somewhat conflicting. Studies on the impact of consuming tomato or soy foods in relation to markers of carcinogenesis in properly designed dietary intervention trials involving cancer patients, specifically men with prostate cancer, are also extremely limited. Therefore, the following studies were conducted to provide information that will enable future scientists to conduct larger and more comprehensive studies to obtain a better understanding of the health benefits of phytochemical-containing tomato and soy foods.

	Nutrient Content (per 100g)					Carotenoids (mg/100g edible portion)				
	Vitamin A (IU)	Vitamin C (mg)	Vitamin E (mg, α -tocopherol equivalents)	Folate (μ g)	Potassium (mg)	α -car	β -car	β -cryp	Lut + Zea	Lyc
Tomatoes, crushed, canned	699	9.2	0.532	13	293	NA	NA	NA	NA	NA
Tomatoes, red, ripe, raw, year round average	623	19.1	0.380	15	222	0.112	0.393	0	0.130	3.02
Tomato juice, canned, w/o salt	556	18.3	0.910	20	220	0	0.428	0	0.060	9.32
Catsup	1016	15.1	1.465	15	481	0	0.730	0	0	17.01
Sauce, pasta, spaghetti/marinara, ready-to-serve	375	8	1.252	10	295	0	0.440	0	0.160	15.99
Sauce, ready-to- serve, salsa	602	13.9	0.600	16	213	NA	0.398	NA	NA	NA
Tomato products, canned, paste, w/ salt	2445	42.4	4.300	22	937	0.029	1.242	0	0.170	29.33
Tomato products, canned, puree, w/ salt	1275	10.4	2.520	11	426	0	0.410	0	0.090	16.67
Tomato products, canned sauce	979	13.1	1.400	9	371	0	0.410	0	0.001	15.92
Tomatoes, red, ripe, cooked, boiled, w/o salt	743	22.8	0.380	13	279	0	0.300	0	0.150	4.40
Tomatoes, red, ripe, canned, whole, regular pack	595	14.2	0.320	8	221	0	0.186	0	0.040	9.71
Pizza with pepperoni, cheese and sauce, thin crust, frozen	NA	NA	NA	NA	NA	0	0.264	0	0.015	4.45
Pasta in tomato sauce w/ cheese, canned	NA	NA	NA	NA	NA	0	0.127	0	0	3.16

Table 1.1 Nutrient and carotenoid content of tomatoes and tomato products (12, 13)

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

Table 1.2 Common food sources of lycopene

^aUSDA. 1998. USDA-NCI Carotenoid Database for U.S. Foods. Nutrient Data Lab.,

Agric. Res. Service, U.S. Dept. of Agriculture, Beltsville Human Nutrition Research

Center, Riverdale, MD; ^bFrom Mangels et al. (16); ^cNguyen ML and Schwartz SJ (17);

^dNguyen ML and Schwartz SJ (18).

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

Table 1.3 Lycopene concentrations in human tissue using HPLC technology as reported in several publications

^aCraft NE et al. (89); ^bKhachik F et al. (90); ^cRao AV et al. (91); ^dFreeman VL et al. (92);

^eKucuk O et al. (93); ^fClinton SK et al. (68); ^gClinton SK (84).

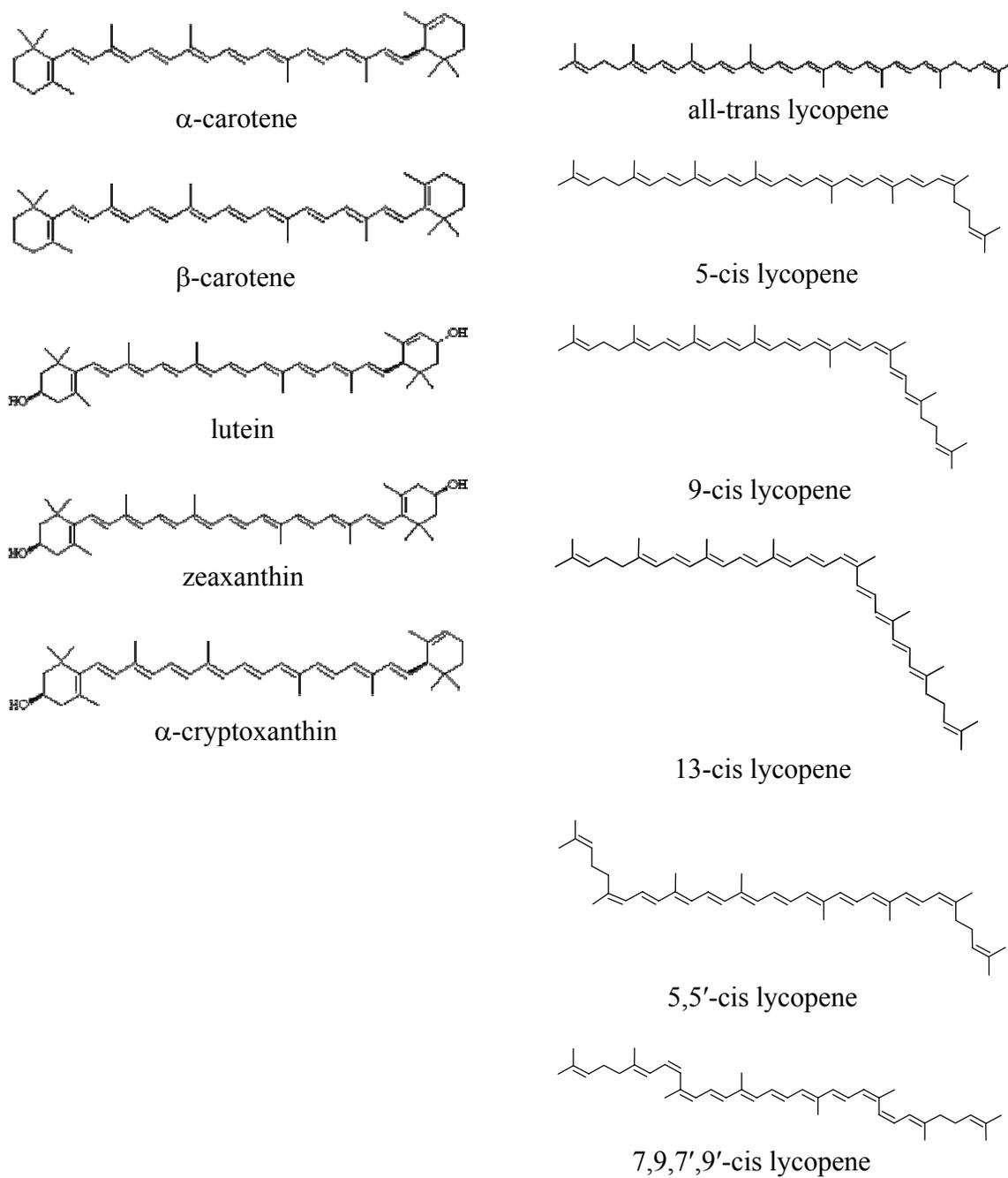


Figure 1.1 Representative carotenoid and lycopene isomer structures.

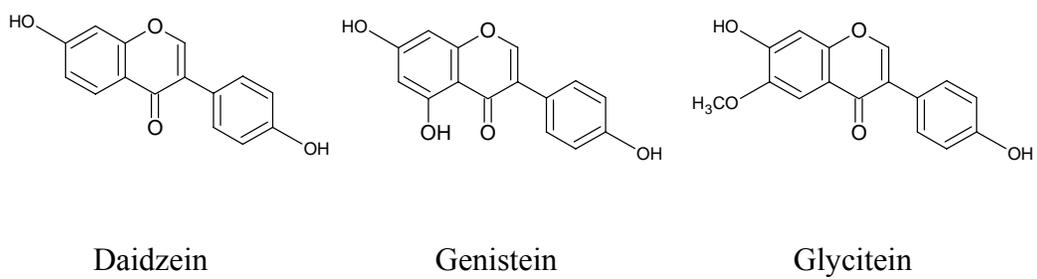


Figure 1.2 Aglycone structures of daidzein, genistein, and glycitein.

HYPOTHESES

1. Changes in tomato-based product and soy protein supplement intake rapidly and significantly modulate in vivo lycopene and isoflavone profiles, respectively, in healthy individuals and/or prostate cancer patients.
2. Consumption of tomato-based products for 2-4 weeks is sufficient to significantly reduce oxidative stress in healthy participants and in men with prostate cancer.
3. Modulation of biomarkers associated with prostate cancer development and/or progression in prostate cancer patients occurs after 2-4 weeks of dietary intervention with tomato-based products or soy.

OBJECTIVES AND SPECIFIC AIMS

1. To define carotenoid patterns, particularly lycopene, in healthy subjects following changes in tomato-based product intake.
 - A. To determine the magnitude of change in plasma carotenoids and plasma lycopene isomers over 7 days of washout and 15 days of tomato soup or V8 vegetable juice consumption.
2. To investigate carotenoid, specifically lycopene, and isoflavone profiles in prostate cancer patients after tomato-based product and soy consumption, respectively.
 - A. To evaluate compliance to the dietary intervention.
 - B. To determine the magnitude of change in plasma and prostate carotenoids, including lycopene, or urinary and prostate isoflavones during 1-2 weeks of washout and 2-4 weeks of tomato sauce, tomato soup, or V8 vegetable juice consumption or soy protein supplement intake, respectively.
3. To assess the effect of tomato-based product intake on biomarkers of oxidative stress in both healthy men and women and in men with prostate cancer.
 - A. To develop methodologies for the measurement of oxidative stress.
 - B. To measure changes in ex vivo lipoprotein oxidation, plasma antioxidant capacity, urinary 8-hydroxy-2'-deoxyguanosine (8-OH-2'-dG)

concentrations, and urinary 8-iso-PGF_{2α} levels after 2-4 weeks of dietary intervention with tomato sauce, tomato soup, or V8 vegetable juice.

4. To evaluate the influence of tomato-based or soy product consumption on prostate biomarkers in prostate cancer patients.
 - A. To measure the impact of dietary intervention with tomato sauce, tomato soup, V8 vegetable juice, or soy protein supplement on serum prostate specific antigen, serum insulin-like growth factor-I, and plasma testosterone levels.

CHAPTER 2

THE CONSUMPTION OF PROCESSED TOMATO-BASED PRODUCTS ENHANCES PLASMA LYCOPENE CONCENTRATIONS IN ASSOCIATION WITH A REDUCED LIPOPROTEIN SENSITIVITY TO OXIDATIVE DAMAGE

Abstract

Lycopene, the predominant carotenoid in tomatoes, is hypothesized to mediate the health benefits of tomato products. We designed a study to examine the change in plasma lycopene and resistance of lipoproteins to ex vivo oxidative stress. Sixty healthy individuals (age > 40; 30M/30F) consumed a lycopene-free diet for 1 wk and were subsequently randomized to receive 35±1, 23±1, or 25±1 mg lycopene/d from Campbell's® Condensed Tomato Soup (CS), Campbell's® Ready To Serve Tomato Soup (RTS), or V8® Vegetable Juice (V8), respectively, for 15 d. Total plasma lycopene concentrations decreased from 0.499±0.044 to 0.322±0.027 (35%, P<0.0001) µmol/L for the 60 participants during the 7-d washout. Following intervention, total lycopene concentrations increased for those consuming CS, RTS, and V8 (compared to washout for each group) to 0.784±0.083 (123%, P<0.0001), 0.545±0.061 (57%, P<0.01), and 0.569±0.061 (112%, P<0.0001) µmol/L, respectively. The concentrations of all lycopene isomers decreased during the washout period. Reported as a percentage of total plasma lycopene isomers for the 60 subjects, all-trans-lycopene decreased from 44.4±1.2 to 39.6±1.2 (P<0.0001) while total cis-lycopene isomers increased from 55.6±1.2 to

60.4±1.2 (P<0.0001) during the washout, a shift that was reversed with consumption of tomato products for 15 d. The ex vivo lipoprotein oxidation lag period, used as a measure of antioxidant capacity, increased significantly from a value of 64.7±2.4 min at washout (all groups) to 70.1±4.0 (P<0.05), 68.3±2.4 (P<0.05), and 71.7±4.0 (P<0.01) min after treatment for CS, RTS, and V8, respectively. This study shows that lycopene concentrations and isomer patterns change rapidly with variation in dietary intake. In addition, 15 d of tomato product consumption significantly enhances the protection of lipoproteins to ex vivo oxidative stress.

Introduction

A number of human epidemiologic and clinical studies have suggested health benefits of tomatoes and processed tomato products (8, 84, 203, 204). The most often cited mechanism underlying these observations is the potential for tomato products to modulate radical mediated oxidative damage that may contribute to the initiation and progression of many chronic disease processes (205). Although tomatoes contain an array of phytochemicals, most of the attention has focused upon lycopene, the predominant carotenoid present in tomatoes that is responsible for the familiar red color. Lycopene exhibits a highly conjugated structure, contributing to its ability to quench singlet oxygen more efficiently than other carotenoids commonly found in the diet (103). The distribution of carotenoids in the lipophilic portion of the cell suggests an improved resistance of lipoproteins to oxidative damage (206, 207).

Tomatoes and an array of processed tomato products account for the majority of lycopene consumed (19) while watermelon, pink grapefruit, guava, and other foods

contribute to intake. Although human epidemiologic studies employ food composition databases to estimate intake from different foods, it is now clear that bioavailability of carotenoids, including lycopene, from various sources is profoundly influenced by food processing, cooking, and other components in the meals such as lipids and fiber, as well as physiologic and genetic factors controlling digestion and absorption (67, 208, 209). A better understanding of lycopene bioavailability will improve our interpretation of human epidemiologic studies and help investigators design intervention trials to assess potential health benefits of lycopene.

Lycopene is a forty carbon acyclic carotenoid and exists in multiple isomeric forms in vivo (68). Interestingly, the all-trans form of lycopene comprises 79-91% of the total lycopene found in tomato-based products (17, 57, 68, 69, 210), whereas > 50% of total lycopene found in serum and 80-90% found in tissues exists as cis-lycopene isomers (68). The processes that influence isomer patterns, the mechanisms of interconversion, and the potential biological endpoints that may be influenced by different isomers remain an essentially unexplored area of research.

The goal of this study was to explore the interrelationships between the intake of different commonly consumed processed tomato products, blood carotenoid and lycopene isomer profiles, and biomarkers of oxidative stress. The following objectives were examined in this study: (1) to determine the rate and magnitude of change in plasma total lycopene and isomer patterns over a period of 1 wk on a lycopene-free diet, (2) to determine the rate and magnitude of change in plasma total lycopene and isomer patterns over a period of 15 d following dietary intervention with Campbell's® Condensed Tomato Soup (CS)¹, Campbell's® Ready To Serve Tomato Soup (RTS), or V8® Vegetable Juice (V8), and (3) to assess the effect of dietary intervention on several biomarkers of oxidative damage (i.e. ex vivo lipoprotein oxidation, urinary 8-hydroxy-2'-deoxyguanosine (8-OH-2'-dG), and urinary F(2)-isoprostane 8-epi-PGF2 α).

Subjects and Methods

Subjects

Participants were healthy, non-pregnant, non-smoking adults (n = 60; 30M/30F). The average age and weight (means \pm SEM) was 52.1 \pm 1.9 y and 88.4 \pm 3.0 kg for men and 53.1 \pm 1.7 y and 70.9 \pm 3.3 kg for women. Exclusions included those with gastrointestinal disease that may have altered food digestibility such as: pancreatic insufficiency, hepatic disease, diabetes, and metabolic enzyme deficiencies. The study was approved by the Biomedical Sciences Institutional Review Board of The Ohio State University, Columbus, OH. The objectives, requirements, and risks/benefits of the study were clearly outlined and informed written consent was obtained for each subject.

Study Design

Baseline blood samples from 12-h fasted subjects were collected at the onset of the study for analysis of plasma carotenoids, vitamin E, and vitamin A profiles. Participants consumed a lycopene-free diet for the next 7 d before being randomized to one of three treatment groups. During the washout phase, subjects were provided with a detailed list of all known lycopene-containing foods and asked to record any consumption of these foods daily. A second 12-h fasted blood sample was drawn following the 7-d washout period. In addition to measuring the changes in plasma carotenoids, vitamin E, and vitamin A on the blood sample obtained at the end of washout, ex vivo lipoprotein oxidation was analyzed. A 12-h overnight urine sample was also collected at the end of the 7-d washout to determine pre-treatment levels of 8-OH-2'-dG and 8-epi-PGF2 α .

Individuals were then randomly assigned to receive CS (300 mL/d, 10 3/4 oz., 2 1/2 servings (as prepared); 35±1 mg lycopene/d), RTS (320 mL/d, 10 2/3 oz., 1 1/3 servings; 23±1 mg lycopene/d), or V8 (340 mL/d, 11 1/2 fl. oz., 1 serving; 25±1 mg lycopene/d), provided by The Campbell Soup Company, Camden, NJ. These products served as their only source of lycopene throughout the 15-d dietary intervention phase. Again, subjects avoided all other lycopene-containing foods outlined on the list. Following the 15-d of tomato product dietary intervention, a third and final 12-h fasted blood sample was collected. Complete plasma carotenoid, vitamin E, and vitamin A profiles along with susceptibility of lipoproteins to oxidation were conducted. Measurements of 8-OH-2'-dG and 8-epi-PGF2 α concentrations in 12-h urine samples were also determined.

Blood Sampling

Venous blood samples were obtained in the General Clinical Research Center at The Ohio State University Medical Center, Columbus, OH. Blood was collected in 10 mL EDTA tubes under dim light. Blood samples were centrifuged at 4°C and 2500 rpm (1500 x g) for 10 min in a Beckman Coulter Allegra 6R Centrifuge (Fullerton, CA). Plasma was allocated for immediate analysis or subsequently stored at -80°C. Total blood and plasma volumes were recorded for each sample.

Tomato Products and Plasma Analysis

Carotenoid profiles of CS, RTS, and V8 as well as plasma total carotenoid, vitamin E, and vitamin A concentrations were performed using the HPLC methodology of Epler et al. (211).

Plasma Lycopene Extraction

An equal volume of ethanol containing 0.1% butylated hydroxytoluene (BHT) was added to 150 μL of plasma. The sample was vortexed for 10 s prior to the addition of 750 μL of Hexane:Acetone (2:1) with 0.1% BHT. Complete mixing was accomplished via a second vortex. The sample was centrifuged at 10,000 rpm (5,585 \times g) in a Corning-Costar 10MVSS Microcentrifuge (New York, NY) for 60 s and the lycopene-containing hexane layer was transferred to a glass vial. The plasma extraction procedure was performed multiple times on each sample to ensure the total removal of lycopene. The combined hexane layers were dried under nitrogen and brought up in the HPLC method conditions used to separate the lycopene isomers.

Plasma Lycopene Isomer Chromatography

A Waters 2690 separation instrument (Milford, MA) equipped with a Waters 996 Photodiode Array Detector was employed as part of the reverse phase HPLC system used to separate plasma lycopene isomers. Separations were carried out using analytical (250 mm \times 4.6 mm id) 3 μm polymeric C_{30} columns prepared at the National Institute of Standards and Technology (Gaithersburg, MD) according to Sander et al. (53). In-line guard columns packed with C_{30} stationary phase were used for all separations. Separation of lycopene isomers was performed at 1.0 mL/min using a linear gradient of 40 to 50% methyl-t-butyl ether in methanol:ammonium acetate (98:2) for 45 min.

Markers of Oxidative Stress

Plasma EDTA-free low-density lipoprotein (LDL) + very low-density lipoprotein (VLDL) was isolated via precipitation with $MgCl_2$ and dextran sulfate (212). The plasma LDL + VLDL cholesterol concentration was analyzed using a cholesterol kit from Sigma Diagnostics, Inc. (St. Louis, MO) and normalized to 75 $\mu g/mL$ using 2% PBS solution. Lipoprotein oxidation was initiated using 8 $\mu mol/L$ $CuSO_4$. The formation of conjugated dienes was monitored at 234 nm and 37°C over a 4-h period (213). All measurements were performed in quartz crystal cuvetts. The lag period was determined by the time interval between the initiation with copper and the time at which the intersection of the line tangent to the slow propagation phase passed through the line tangent to the rapid propagation phase. The coefficient of variance for the assay, including intrassay and interassay factors, was less than 5%. Urine was collected for 12 h overnight and stored at $-80^\circ C$ prior to analysis. Concentrations of urinary 8-epi-PGF 2α and 8-OH-2'-dG were measured using a competitive enzyme-linked immunosorbent assay (Genox, Baltimore, MD).

Statistical Analysis

All statistical calculations were performed using software from Statview (SAS Institute Inc., Cary, NC). Repeated-measures ANOVA or two samples paired Student's t-test was employed for within group comparisons. ANOVA was used for between group comparisons with significance assessed using Fisher's Protected Least Significant Difference test. P values <0.05 were considered to be statistically significant.

Results

The quantitative distribution of various carotenoids was determined for each of the tomato products employed (Table 2.1). Concentrations of all-trans lycopene, total cis-lycopene, zeaxanthin, and cis- β -carotene were higher in CS when compared to RTS or V8. Each tomato product contained $\geq 92\%$ of total lycopene in the all-trans form (Figure 2.1). Increased amounts of β -carotene, α -carotene and lutein were present in V8 compared to other tomato products due to the presence of juice from other vegetables.

The plasma concentrations (means \pm SEM, $\mu\text{mol/L}$) of α -carotene (0.18 ± 0.02), β -carotene (0.72 ± 0.11), cis- β -carotene (0.089 ± 0.009), α -cryptoxanthin (0.059 ± 0.004), β -cryptoxanthin (0.30 ± 0.02), lutein (0.21 ± 0.02), retinol (1.94 ± 0.10), retinyl palmitate (0.046 ± 0.016), α -tocopherol (39.43 ± 3.09), γ -tocopherol (3.32 ± 0.54), and δ -tocopherol (0.17 ± 0.03) did not significantly change based on product consumed or time of sampling. In addition, plasma concentrations (means \pm SEM, mmol/L) of triglycerides (1.53 ± 0.20) were not significantly altered throughout the study. The concentrations of plasma total cholesterol were not different between dietary groups but did show a decrease (means \pm SEM, mmol/L) from 5.23 ± 0.20 at baseline, to 4.98 ± 0.17 after washout, to 4.74 ± 0.17 after intervention ($P < 0.05$, baseline and intervention). Total plasma lycopene concentrations decreased 35% ($P < 0.0001$) for the 60 participants during the 7-d washout (Table 2.2).

Increases in plasma concentrations of α -carotene, β -carotene, and lutein (means \pm SEM, $\mu\text{mol/L}$) from 0.19 ± 0.06 , 0.72 ± 0.17 , and 0.20 ± 0.03 to 0.25 ± 0.04 (32%), 0.83 ± 0.13 (15%), and 0.22 ± 0.02 (10%), respectively, following dietary intervention with V8

reflects the elevated amounts of these carotenoids present in this product (Table 2.1). Of the carotenoids analyzed in plasma following 15 d of tomato product intervention, only plasma concentrations of lycopene and zeaxanthin were significantly increased. Zeaxanthin concentrations were not altered during washout, but increased from 0.10 ± 0.01 $\mu\text{mol/L}$ after washout to 0.13 ± 0.01 $\mu\text{mol/L}$ following intervention (30%, $P < 0.01$). Assessment of each group showed concentrations of plasma lycopene, following dietary intervention, to be significantly higher than washout levels. Total lycopene concentrations increased for those consuming CS, RTS, and V8 (compared to washout for each group) by 123% ($P < 0.0001$), 57% ($P < 0.01$), and 112% ($P < 0.0001$), respectively (Table 2.2).

On the basis of total lycopene consumption (mg/15 d), subjects who consumed CS, RTS or V8 had similar plasma total lycopene responses (Table 2.3). This may indicate that these tomato products provided a similar bioavailable source of lycopene.

We observed a similar array of lycopene isomers in plasma of humans fed CS, RTS, or V8. Based on spectral and NMR-spectroscopic characterization, several of the plasma lycopene isomers have been putatively identified (57, 58, 210, 214). However, our data suggests that in vivo lycopene isomer patterns are more complex than previously reported.

To quantify changes of various cis-lycopene isomers throughout this study, isomer peaks were grouped according to elution order observed during HPLC analysis. Therefore, these lycopene isomers are grouped according to similar physico-chemical characteristics (Figure 2.1). The percentage of all-trans and total cis-lycopene (sum of cis-A, cis-B, cis-C, cis-D, cis-E, and 5-cis-lycopene isomers) was altered following the

washout period, but was not significantly changed with tomato product consumption (Table 2.4). Reported as a percentage of total plasma lycopene isomers, all-trans-lycopene decreased from 44.4 ± 1.2 to 39.6 ± 1.2 ($P < 0.0001$) while total cis-lycopene increased from 55.6 ± 1.2 to 60.4 ± 1.2 ($P < 0.0001$) during the washout. Although the percentage of total cis-lycopene increased significantly throughout the washout period, approximately 66% of these cis-isomers were in the 5-cis form. All-trans-lycopene and 5-cis-lycopene are the most abundant lycopene isomers present in human plasma. Analysis of individual cis-lycopene isomers showed an increase from 36.4 ± 0.8 to 41.6 ± 1.0 ($P < 0.0001$) and subsequent decrease to 33.7 ± 0.5 ($P < 0.0001$) in the percentage of plasma 5-cis-lycopene after washout and treatment phases, respectively. The percentage of 5-cis-lycopene was the only cis-lycopene isomer to significantly change during washout, suggesting a possible trans-lycopene isomer conversion to and/or biological preservation of 5-cis-lycopene. Dietary intervention with tomato products reduced the percentage of 5-cis-lycopene to baseline amounts and concomitantly increased the percentage of cis-B, cis-D, and cis-E lycopene isomers ($P < 0.05$) over baseline and/or washout levels (Table 2.4).

Plasma lycopene isomer ratios changed significantly throughout the trial (Figure 2.2). At the time of enrollment in the study, the major lycopene isomer was in the all-trans configuration. However, 5-cis-lycopene was the predominant plasma lycopene isomer after consumption of a lycopene-free diet for 7 d. For all groups combined, the ratio of plasma 5-cis-lycopene/all-trans-lycopene concentrations increased from 0.85 ± 0.04 to 1.08 ± 0.04 ($P < 0.0001$) during washout. Following intervention with the three tomato products, the ratio returned to that observed at enrollment, 0.86 ± 0.03 ($P < 0.0001$) (Table 2.5). In addition, the ratio of plasma cis-D-lycopene/all-trans-lycopene levels significantly increased after tomato product consumption.

We isolated lipoproteins and examined their resistance to ex vivo oxidative damage. The lag period, used as a measure of antioxidant capacity, increased significantly ($P < 0.05$) after treatment for CS, RTS, and V8 (Table 2.6). No significant changes in urinary 8-OH-2'-dG and 8-epi-PGF 2α following dietary consumption of tomato products were observed.

Discussion

Tomatoes and tomato-based products contain important nutrients (215) and provide an excellent source of many phytochemicals that may mediate a health benefit (14, 25). Although the consumption of certain carotenoids found in tomatoes and tomato products appear to be more highly associated with potential health benefits, synergistic or additive effects by the collective contribution of several tomato phytochemicals may mediate these findings (216, 217). However, the physiological importance of these compounds at levels present in tomatoes and tomato products is not clear.

Changes in plasma lycopene concentrations occur rapidly with variation in dietary intake. The consumption of a lycopene-free diet for a period of one week is sufficient to significantly lower plasma lycopene concentrations, supporting our prior observations of a 10-14 day half-life for blood lycopene (218). Subsequent intervention with tomato products for fifteen days significantly increases plasma lycopene levels.

Tomato and tomato product phytochemicals must be readily bioavailable to ensure maximum absorption. Bioavailability is defined as the fraction of an ingested nutrient that is accessible to the body through absorption for use in normal physiological functions and for metabolic processes (71). A number of studies have evaluated the bioavailability of the carotenoid phytochemicals found in tomatoes and tomato products (67, 73-75, 77, 81, 82, 218, 219). Increases in plasma lycopene and plasma response per mg lycopene intake indicate that CS, RTS and V8 provided a similar bioavailable source of lycopene. Lycopene uptake may be related to processing (209) and/or interactions among other phytochemicals present in these tomato products. However, it is important to note that increases in carotenoid intake do not necessarily produce similar increases in plasma uptake and response given that regulatory mechanisms are in place to limit mucosal cell uptake and/or transport in vivo (77).

Lycopene exists in multiple isomeric forms. The all-trans form of lycopene predominates in various tomato products. However, without consumption of lycopene-containing products for one week, in vivo concentrations of cis-lycopene increases. A significant increase in the percentage of plasma 5-cis-lycopene is accompanied by a significant decrease in plasma all-trans-lycopene following the washout period. In the absence of a lycopene-containing diet, 5-cis-lycopene is the

primary plasma lycopene isomer, suggesting a possible trans-lycopene isomer conversion to and/or biological preservation of 5-cis-lycopene. Additional research is required to identify the mechanism for conversion and to determine the physiological significance of cis isomers of lycopene in vivo.

Research that supports the in vivo oxidation of LDL contributes to the hypothesis that dietary components may be important for LDL oxidative modification. Carotenoids such as lycopene are highly lipophilic and are commonly found within cell membranes and other lipid components (206, 207). It is therefore thought that the ability of carotenoids to scavenge free radicals may be greatest in a lipophilic environment. The reduced susceptibility of lipoproteins to ex vivo oxidative stress from subjects fed a carotenoid-rich tomato product diet may suggest a protective effect against oxidative stress in vivo.

These studies suggest that changes in tomato product intake can significantly modulate carotenoid levels in the bloodstream and alter the unique, yet complex, lycopene isomer patterns that exist in vivo. In addition, these studies imply that consumption of vegetable juice or tomato soup may provide protection from in vivo oxidative damage.

	Condensed tomato soup	Ready to serve tomato soup	V8 vegetable juice
	<i>mg/100g</i>		<i>mg/L</i>
Total lycopene	11.56	7.47	72.90
All- <i>trans</i> -lycopene	10.90	6.90	67.00
Total cis-lycopene	0.66	0.57	5.90
β-carotene	0.22	0.17	4.09
α-carotene	ND	ND	1.78
Lutein	0.06	0.06	1.13
Zeaxanthin	0.11	0.08	0.76
cis-β-carotene	0.10	0.06	0.78
α-cryptoxanthin	ND	ND	ND
β-cryptoxanthin	ND	ND	ND

Table 2.1 Carotenoid concentrations of tomato products¹

¹Tomato products were provided by The Campbell Soup Company, Camden, NJ; ND, not detected.

	Condensed tomato soup ²			Ready to serve tomato soup ²		
	d -7	d 0	d 15	d -7	d 0	d 15
	μmol/L					
Total lycopene	0.554±0.071 a	0.351±0.033 b	0.784±0.083 c	0.570±0.072 a	0.347±0.046 b	0.545±0.061 a
All- <i>trans</i> -lycopene	0.232±0.035 a	0.127±0.011 b	0.289±0.033 a	0.254±0.034 a	0.136±0.019 b	0.223±0.026 a
Total <i>cis</i> -lycopene	0.321±0.040 a	0.224±0.023 b	0.496±0.055 c	0.316±0.041 a	0.212±0.029 b	0.322±0.037 a
	V8 vegetable juice ²			All groups		
	d -7	d 0	d 15	d -7	d 0	d 15
	μmol/L					
Total lycopene	0.372±0.047 a	0.268±0.047 b	0.569±0.061 c	0.499±0.044 a	0.322±0.027 b	0.633±0.044 c
All- <i>trans</i> -lycopene	0.171±0.022 a	0.109±0.018 b	0.239±0.028 c	0.219±0.020 a	0.124±0.010 b	0.250±0.018 a
Total <i>cis</i> -lycopene	0.202±0.030 a	0.159±0.031 a	0.331±0.036 b	0.280±0.025 a	0.198±0.018 b	0.383±0.029 c

Table 2.2 Changes in plasma lycopene concentrations at baseline, after washout, and following intervention at d -7, 0 and 15¹

¹Values are mean ± SEM; n = 20/group or 60 (all groups); Means in a row within groups without a common superscript are significantly different, P < 0.05.

²Tomato products were provided by The Campbell Soup Company, Camden, NJ.

	Lycopene consumption	Change in plasma lycopene	Change in plasma lycopene / lycopene consumption
	mg/d (mg/15 d)	μmol/L	[(μmol/L)/(mg/15 d)] · 10 ⁴
Condensed tomato soup ²	35 ± 1 (525±15) ^a	0.433 ± 0.063 ^a	8.25 ± 1.20
Ready to serve tomato soup ²	23 ± 1 (345±15) ^b	0.198 ± 0.024 ^b	5.73 ± 0.71
V8 vegetable juice ²	25 ± 1 (375±15) ^b	0.302 ± 0.033 ^b	8.04 ± 0.87

Table 2.3 Consumption of tomato products and changes in plasma lycopene comparing values at the completion of washout to values following intervention for 15 days¹

¹Values are mean ± SEM; n = 20/group; Means in a column without a common superscript are significantly different, P < 0.05.

²Tomato products were provided by The Campbell Soup Company, Camden, NJ.

	All-trans-lycopene			Total cis-lycopene			5-cis-lycopene		
	d -7	d 0	d 15	d -7	d 0	d 15	d -7	d 0	d 15
	%								
Condensed tomato soup ³	42.4±2.1 _a	37.7±1.8 _b	36.7±1.4 _b	57.6±2.1 _a	62.3±1.8 _b	63.3±1.4 _b	36.2±1.2 _a	41.0±1.4 _b	33.9±1.0 _c
Ready to serve tomato soup ³	44.6±1.5 _a	39.1±1.4 _b	41.1±1.3 _b	55.4±1.5 _a	60.9±1.4 _b	58.9±1.3 _b	35.9±1.3 _a	42.1±1.9 _b	34.7±0.7 _a
V8 vegetable juice ³	46.3±2.1	42.0±2.4	41.9±1.4	53.7±2.1	58.0±2.4	58.1±1.4	37.0±1.5 _a	41.8±1.6 _b	32.5±1.0 _c
All groups	44.4±1.2 _a	39.6±1.2 _b	39.9±0.9 _b	55.6±1.2 _a	60.4±1.2 _b	60.1±0.9 _b	36.4±0.8 _a	41.6±1.0 _b	33.7±0.5 _c
	cis-B-isomers			cis-D-isomers			cis-E-isomers		
	d -7	d 0	d 15	d -7	d 0	d 15	d -7	d 0	d 15
	%								
Condensed tomato soup ³	1.2±0.3	1.5±0.4	2.0±0.2	13.9±1.2 _a	14.4±1.5 _a	18.5±0.6 _b	6.1±1.0 _a	5.5±1.2 _a	8.1±0.6 _b
Ready to serve tomato soup ³	1.1±0.3	1.4±0.4	1.5±0.3	13.6±1.2 _a	13.0±1.9 _a	18.5±1.0 _b	4.7±0.9	4.4±1.2	4.1±0.6
V8 vegetable juice ³	0.9±0.4	1.1±0.5	1.6±0.4	12.4±1.1 _a	12.0±1.9 _a	18.3±1.1 _b	3.3±1.1	3.1±1.4	5.5±0.9
All groups	1.1±0.2 _a	1.3±0.2	1.7±0.2 _b	13.3±0.7 _a	13.1±1.0 _a	18.4±0.5 _b	4.7±0.6	4.3±0.7 _a	5.9±0.5 _b

Table 2.4 Percentage of plasma lycopene isomers in sixty adults before and after tomato product consumption at d -7, 0 and 15^{1,2}

¹Values are mean ± SEM; n = 20/group or 60 (all groups); Means in a row for a variable without a common superscript are significantly different, P < 0.05.

²The table does not include the cis-A and cis-C lycopene isomers because their low concentrations result in less precise quantitation.

³Tomato products were provided by The Campbell Soup Company, Camden, NJ.

	cis-D / all-trans			5-cis / all-trans		
	d -7	d 0	d 15	d -7	d 0	d 15
Condensed tomato soup ³	0.351±0.046 ^a	0.414±0.051 _a	0.514±0.033 _b	0.887±0.066 _a	1.125±0.058 _b	0.943±0.057 _a
Ready to serve tomato soup ³	0.321±0.035 _a	0.356±0.059 _a	0.460±0.037 _b	0.833±0.048 _a	1.099±0.053 _b	0.854±0.036 _a
V8 vegetable juice ³	0.280±0.035 _a	0.317±0.064 _a	0.448±0.039 _b	0.821±0.062 _a	1.021±0.066 _b	0.786±0.040 _a
All groups	0.317±0.024 _a	0.362±0.034 _a	0.474±0.022 _b	0.847±0.035 _a	1.081±0.036 _b	0.861±0.028 _a

Table 2.5 Ratio of individual or groups of cis-lycopene isomers to all-trans-lycopene at d -7, 0 and 15^{1,2}

¹Values are mean ± SEM; n = 20/group or 60 (combined); n.d., not detected; Means in a row for a variable without a common superscript are significantly different, P < 0.05.

²All other individual cis-lycopene isomer/all-trans-lycopene isomer ratios did not significantly change throughout the study.

³Tomato products were provided by The Campbell Soup Company, Camden, NJ.

	d 0	d 15
	Lag time (min)	
Condensed tomato soup ²	64.0±3.2 ^a	70.1±4.0 ^b
Ready to serve tomato soup ²	64.5±2.9 ^a	68.3±2.4 ^b
V8 vegetable juice ²	65.8±3.6 ^{a*}	71.7±4.0 ^b
All groups	64.7±2.4 ^a	70.0±2.8 ^b

Table 2.6 Tomato product consumption protects against the oxidation of isolated LDL + VLDL exposed to Cu²⁺-mediated oxidative stress in sixty adults at d 0 and 15¹

¹Values are mean ± SEM; n = 20/group or 60 (combined); Means within groups without a common superscript are significantly different, P < 0.05, *P < 0.01, (paired t-test).

²Tomato products were provided by The Campbell Soup Company, Camden, NJ.

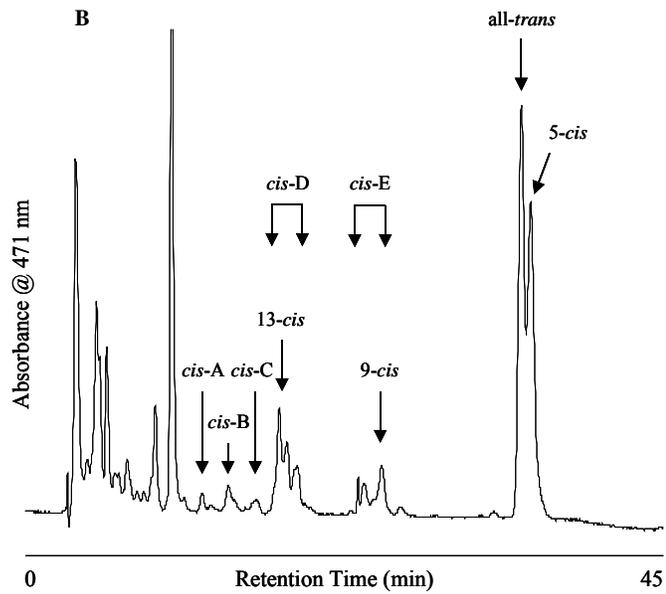
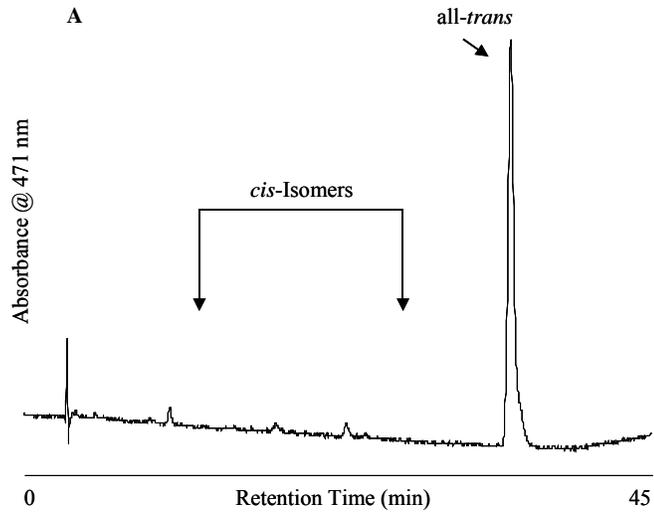


Figure 2.1 Representative HPLC chromatogram of tomato product lycopene isomers (A) and plasma lycopene isomers (B) from healthy individuals consuming different lycopene-containing tomato products for 15 d.

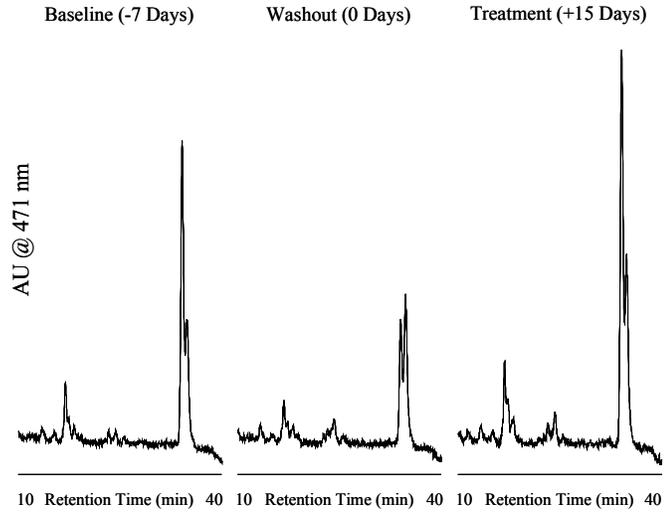


Figure 2.2 Representative HPLC chromatogram of plasma lycopene isomer profile changes in healthy individuals at enrollment, after the 7-d washout period and after 15 d of consuming different lycopene-containing tomato products.

CHAPTER 3

IN VIVO PHYTOCHEMICAL CONCENTRATIONS AND HORMONE MODULATION FOLLOWING TOMATO-BASED PRODUCT OR SOY CONSUMPTION IN PROSTATE CANCER PATIENTS

Abstract

Consumption of tomato-based products and soy has been associated with a reduced risk for prostate cancer. This study was designed to determine if men with prostate cancer would consume tomato-based products or soy daily during the period between their diagnosis and surgery. In addition, this study was conducted to examine in vivo concentrations of phytochemicals from these foods and their effect on modulating hormone levels. Prostate cancer patients consumed a lycopene-free and soy-free diet for 1-2 weeks and were subsequently randomized to receive 25-30 mg lycopene/day from Prego[®] spaghetti sauce, Campbell's[®] tomato soup, or V8[®] vegetable or 80±4 mg isoflavones/day from a soy protein supplement for 2-4 weeks. All study participants were extremely compliant during the washout and treatment phases. Dietary intervention with tomato-based foods showed that the consumption of sauce or soup produced much higher and significantly higher ($P<0.01$) levels of plasma total lycopene ($\mu\text{mol/L}$) when compared to men consuming juice (0.92 ± 0.09 or 1.10 ± 0.08 vs. 0.72 ± 0.07 , respectively). When consumption of any tomato-based product or soy was considered, the percentage of plasma all-trans lycopene increased from 50% to 54% ($P<0.01$), or decreased from

52% to 49% ($P < 0.05$), respectively. For tomato-based product groups, prostate total lycopene concentrations (nmol/g) were significantly lower in men consuming juice (0.37 ± 0.07) versus levels after the consumption of sauce (0.60 ± 0.10 , $P < 0.05$) or soup (0.58 ± 0.06 , $P < 0.05$). Prostate contained a complex array of lycopene isomers. Although isoflavones in urine were not detected after washout, urinary levels ($\mu\text{mol/L}$) of daidzein (2.95 ± 0.94), genistein (2.54 ± 0.58), glycitein (0.43 ± 0.10), O-desmethylangolensin (ODMA) (11.92 ± 3.08), and dihydrodaidzein (2.20 ± 0.73) significantly increased following consumption of soy ($P < 0.0001$ for each). Daidzein and genistein were also detected in prostate after soy consumption (pmol/g; 2.49 ± 0.69 and 5.09 ± 0.81 , respectively). In addition, when intake of any dietary product was considered, PSA concentrations (ng/mL) decreased from 5.35 ± 0.54 to 5.21 ± 0.52 . This study indicates that consumption of tomato-based foods or soy results in unique, yet complex, in vivo profiles of phytochemicals, including carotenoids and isoflavones. Information on these phytochemical patterns may improve our understanding of their role in the risk and/or progression of prostate cancer. It also appears that sauce and soup provide a more bioavailable form of lycopene than juice. Finally, this study shows that dietary intervention with tomato-based products and soy may influence prostate cancer development by modulating serum PSA concentrations.

Introduction

Prostate cancer is the most common noncutaneous malignancy in American men, and is the second leading cause of cancer-related deaths (110). In recent decades, we have seen an accumulated body of evidence strongly supporting the conclusion that diets

rich in fruits and vegetables are associated with a lower risk of many malignancies.

Although an association with prostate cancer has not been as strong in comparison with other malignancies, epidemiologic studies have shown that an increase in tomato and tomato-based food or lycopene consumption (220), as well as tissue and serum lycopene concentrations (29, 35, 91, 130) are inversely correlated with the risk for prostate cancer. In addition, epidemiology supports the relationship between increased soy consumption and reduced prostate cancer risk (11).

Tomato carotenoids, a group of over 700 structurally related compounds manufactured by plants and microorganisms, are important phytochemicals that are thought to potentiate the proposed health benefits of these foods. Lycopene, the predominant carotenoid found in tomatoes, has been the focus of many recent studies because it is found in high concentrations in tomato-based foods, which account for the majority of lycopene consumed in the United States. Interestingly, it has been reported that 79-90% of lycopene in tomato-based products exists in the all-trans configuration, however, more than 50% and 80-90% of lycopene appears as cis isomers in plasma and tissue, respectively (57, 68, 210, 221). The beneficial health effects of lycopene have been hypothesized to be related to its antioxidant activity, antiproliferative and prodifferentiation activities, hypocholesterolemic effects, anti-inflammatory action, and/or immune function improvement (222).

Phytochemicals present in tomatoes and tomato-based products must be readily bioavailable for absorption to mediate their hypothesized beneficial health effects. Bioavailability is defined as the fraction of an ingested nutrient that is accessible to the body through absorption for use in normal physiological functions and for metabolic

processes (71). Factors such as the structural features of the carotenoid, food processing, cooking, dietary fat content, fatty acid patterns, and fiber as well as physiologic and genetic factors controlling digestion and absorption all influence the carotenoid bioavailability (208, 209, 223).

Soy foods provide a unique dietary source of isoflavones, a subclass of nutraceuticals known as flavonoids, and are considered to be responsible for much of the health benefits associated with consuming these foods. In food, naturally occurring isoflavones are present as conjugated glycosides or methylated derivatives. However, in vivo intestinal microflora produces enzymes that cleave the conjugated glycosides or their derivatives into smaller aglycone forms, such as genistein, daidzein, and glycitein, which are then absorbed. Further metabolism of these aglycone units results in a number of isoflavone metabolites that can be detected in vivo. Isoflavones appear to mediate their beneficial health effects via hormonal mechanisms, influence on signal transduction, inhibition of certain enzymes and cellular factors that control the growth and differentiation of cells, and in some experimental systems, antioxidant activity (11).

It has been postulated that dietary factors may exert their beneficial health effects in part by altering hormone metabolism, and that this may play a significant role in the progression of latent lesions into clinically relevant cancer. Both androgen and non-androgenic hormones have been reported to play an important role in the development of prostate cancer (224). In addition to others, serum concentrations of prostate specific antigen (225), insulin-like growth factor (226), and testosterone (227) appear to be associated with prostate cancer incidence and/or progression.

Additional information on the *in vivo* deposition and relative distribution of various carotenoids and carotenoid isomers, such as the *trans* and *cis* forms of lycopene, in men with prostate cancer will contribute to our understanding of the biological relevance of these compounds in relation to this disease. To ensure adequate uptake and obtain the health benefits of these carotenoid phytochemicals, it is necessary to identify foods that deliver a highly bioavailable source of these compounds. Adequate information on prostate isoflavones is not currently available. Knowledge regarding the type and level of isoflavones present in prostate, as well as urinary isoflavone metabolite concentrations, will aid in our interpretation of data supporting a role for these compounds in relation to prostate cancer. In addition, limited studies have been conducted to determine the influence of diet on prostate cancer related hormones. An understanding of the impact tomato-based product or soy consumption have on changes in hormones related to prostate cancer will provide further insight into possible mechanisms of action for these phytochemical-containing foods. This clinical trial was conducted to study compliance to dietary interventions with tomato or soy products, phytochemical profiles *in vivo* after changes in dietary intake of these foods, and influence on prostate biomarkers following tomato or soy product consumption. We hypothesized that men with prostate cancer scheduled for a prostatectomy would consume daily amounts of one of three tomato-based products or a soy protein supplement as part of a low-intensity, cost-effective dietary intervention program. We also hypothesized that *in vivo* phytochemical profiles would rapidly and significantly change with variation in dietary intake of these foods and that consumption of tomato or soy foods would reduce biomarkers associated with prostate cancer progression. In

addition to measuring compliance, the specific objectives of this study were to determine the effect of dietary interventions on (a) plasma carotenoid concentrations, specifically lycopene, and lycopene isomer profiles, (b) prostate carotenoid levels and lycopene isomer patterns, (c) isoflavone concentrations and profiles in prostate and urine, and (d) serum prostate specific antigen (PSA), serum insulin-like growth factor-I (IGF-I), and plasma testosterone levels.

Subjects and Methods

Subjects

Study participants had a biopsy proven as clinically localized adenocarcinoma of the prostate based on standard presurgical staging studies. Subjects chose to undergo a radical prostatectomy for treatment of their disease after the medical team had presented all possible treatment options. In addition, men were not currently taking lycopene, soy dietary supplements, or “alternative” medications such as PC-SPES that can influence hormone profiles. Recruitment exclusions included those with metabolic enzyme deficiencies and history of malabsorptive disorders or other metabolic disorders requiring special diet recommendations. The age and body mass index (BMI) (mean \pm SEM) for men in the study were 59.4 ± 1.1 y and 31.0 ± 1.0 , respectively. The study was approved by the Biomedical Sciences Institutional Review Board and the Clinical Scientific Review Committee, The Ohio State University, Columbus, OH. The background, specific aims, subject responsibilities, and risks/benefits of the study were clearly outlined and informed written consent was obtained for each subject.

Study Design

The study required men ($n = 28$) to undergo 1-2 weeks of washout without consumption of lycopene or soy-containing products prior to randomization to one of four dietary interventions ($n = 7$ /group). During both the washout and treatment phases, subjects were provided with a detailed list of all known lycopene and soy-containing foods and asked to record any consumption of these foods daily. To increase recruitment, men randomized to the tomato groups were allowed to consume 25-35 mg of lycopene each day from Prego[®] spaghetti sauce (between $\frac{1}{2}$ cup plus 2 tablespoons or 5 oz/day and $\frac{3}{4}$ cup plus 2 tablespoons or 7 oz/day; 27 ± 9 mg lycopene/d for 24 ± 8 days), Campbell's[®] tomato soup (between 2 and $2 \frac{3}{4}$ cups as prepared/day; 30 ± 3 mg lycopene/d for 21 ± 2 days), or V8[®] vegetable juice (11-16.5 fl. oz/day; 27 ± 5 mg lycopene/d for 22 ± 4 days) provided by The Campbell Soup Company, Camden, NJ. The fourth group consumed 80 ± 4 mg isoflavones (aglycone units equal to 52 ± 4 mg genistein, 24 mg daidzein, and 4 mg glycitein) per day from a soy protein supplement (40 g soy protein/day) provided by Protein Technologies International (PTI), St. Louis, MO. Because of differences in surgery scheduling, these foods, in specified amounts, were consumed every day for 2-4 weeks. In addition, these products constituted their only source of either lycopene or soy during the dietary intervention period. However, men randomized to receive the soy protein supplement during the intervention period were permitted to consume a small portion of lycopene-containing foods (i.e. not to exceed a lycopene score of 5 mg/day). Compliance to either a lycopene-rich tomato-based product diet or a soy protein supplemented diet was measured by 3-day diet food records, daily dietary records of the

product and amount consumed, and in vivo concentrations of phytochemicals consumed from these foods. Study participants were also asked to complete an abbreviated dietary history questionnaire, in order to establish typical consumption patterns of various soy and tomato-based products, and to adhere to the anti-cancer dietary guidelines provided by the study coordinator for the entire length of the study. These guidelines, recommended by the National Institutes of Health and National Cancer Institute, involve increasing fruit and vegetable intake to at least five servings per day, reducing total fat intake to approximately 30% of energy intake, and increasing consumption of dietary fiber to greater than 20 grams per day. Blood samples were collected after both the washout and treatment periods for carotenoid (including lycopene isomers), tocopherol, retinol, retinyl palmitate, and hormone analysis. Isoflavone levels were assessed in 24-hour urine samples collected after both the washout and dietary intervention phases. In addition, fresh tissue samples were obtained from the prostate at the time of surgery to measure carotenoid (including lycopene isomers) and isoflavone concentrations.

Product Analysis

Analysis and distribution of carotenoids and lycopene isomers in the tomato-based products are as described previously (85). Isoflavone concentrations in the soy protein supplement are as reported by PTI and were confirmed based on the extraction methods of Murphy et al. (228) and HPLC analysis methods of Griffith et al. (229). However, these methods were modified and performed as described by Zhang et al. (230).

Blood Sampling

Venous blood samples were obtained in the urology and medical oncology clinics at The James Cancer Hospital and Solove Research Institute, The Ohio State University, Columbus, OH. In addition to the methods listed below, all study participants had blood drawn for “total chemistry” and “complete blood count (CBC) with differential” after washout and treatment phases. All procedures were conducted in subdued light to minimize carotenoid isomerization.

Plasma Carotenoids, Tocopherols, Retinol, and Retinyl Palmitate

Separation

Venous blood was collected in 10 mL K₃ EDTA tubes (Becton Dickinson, Franklin Lakes, NJ) and centrifuged at 4°C and 1500 x g for 20 min in an Allegra 6R Centrifuge (Beckman, Fullerton, CA). Plasma was removed and stored under nitrogen at -80°C.

Extraction and Analysis

Plasma carotenoid (including lycopene isomers), tocopherol, retinol, and retinyl palmitate extraction and analysis were measured by Craft Technologies, Inc. (Wilson, NC) using the procedures described by Nomura et al. (131) and Craft et al. (231).

Prostate Carotenoids

Extraction

Immediately following surgery, prostate samples were stored in liquid nitrogen. Prostate carotenoid extraction was conducted using modifications to the method of Clinton et al. (68). Approximately 0.05 g of prostate were transferred to a glass vial containing 1.25 mL of C₁₈ Sep-Pak[®] solid phase extraction (SPE)-purified high performance liquid chromatography (HPLC)-grade water and 1.25 mL of ethanol containing 2% butylated hydroxytoluene (BHT) and homogenized thoroughly. Following the addition of 2.5 mL 10% sodium hydroxide in methanol, samples were saponified (w/ stirring) for 30 min at room temperature. Samples were then incubated at 60°C for 30 min with vortexing every 10 min. After cooling in an ice bath, 2.5 mL of C₁₈ Sep-Pak[®] SPE-purified HPLC-grade water was added. Carotenoids were extracted by incorporating 2.5 mL of hexane. After mixing and centrifugation, the hexane epilayer was removed after phase separation. Extractions were repeated two times. The three-hexane layers were combined, dried under nitrogen, and resolubilized in 0.3 mL MTBE:methanol (1:1) for immediate analysis.

Instrumentation, Chromatography, and Analysis

Modifications to the methods of Ferruzzi et al. (54, 232) were used for prostate carotenoid and lycopene isomer analysis. The HPLC system consisted of a Hewlett-Packard 1050 (Santa Clara, CA) solvent delivery system interfaced with a four-channel Coularray[™] electrochemical detector (ESA, Chelmsford, MA) with potentials set

between 380 and 560 mV in 60-mV increments from channels 1 to 4, respectively. Separations were carried out using an analytical (4.6 mm x 150 mm, 5 μ m) polymeric YMCTM C₃₀ column (Waters, Milford, MA). An in-line Vydac C₁₈ guard (4.6 mm x 50 mm, 5 μ m) column (Hisperia, CA) along with a precolumn filter was used for all separations. Ammonium acetate (1.0 M, pH 4.6) was prepared with C₁₈ Sep-Pak[®] SPE-purified HPLC-grade water. Solvent A consisted of methanol:MTBE:water:ammonium acetate (88:5:5:2), whereas solvent B consisted of methanol:MTBE:ammonium acetate (20:78:2). Separation of carotenoids and carotenoid isomers was performed at 1.0 mL/min using the following linear gradient: 0 to 5 min, 100% solvent A; 5 to 45 min, 15% solvent A, 85% solvent B; 45 to 50 min 100% solvent B. Peak confirmation and quantitation were determined with authentic standards for zeaxanthin, β -cryptoxanthin (Indofine, Hillsborough, NJ), α -carotene, β -carotene, and lycopene (Sigma Chemical Co., St. Louis, MO). Data collection and integration were performed using ESA CoullarrayTM version 1.01 software and data management system.

Urinary Isoflavones

Extraction

Urine was stored at 4°C during collection and later stored under nitrogen at -80°C. Extraction of urinary isoflavones was conducted using modifications to the method of Kulling et al. (233). Acetate buffer (1.5 mL, pH 3-5) along with 50 μ L internal standard (0.2 μ mol 2', 4' dihydroxy-2-phenylacetophenone; Indofine, Hillsborough, NJ) was combined with 3 mL of urine and centrifuged at 23°C and 1000 x g for 10 min in an

HN-SII Centrifuge (Damon/IEC, Needham Hts., MA). The supernatant was loaded onto a C₁₈ solid phase extraction (SPE) column (Alltech, Deerfield, IL) preconditioned with 6 mL methanol and 6 mL acetate buffer (pH 3-5). The column was washed with 4 mL acetate buffer (pH 3-5) followed by 1 mL distilled water. Following the wash, 1 mL methanol was added to the column, collected, and discarded. Elution of isoflavones was performed with 7 mL methanol followed by evaporation under nitrogen. The dried eluent was resolubilized in 900 µL acetate buffer (pH 5.5), 100 µL 10% ascorbic acid, and 100 µL β-glucuronidase/arylsulfatase (Roche, Indianapolis, IN). Samples were incubated overnight (>12 h) at 37°C in a shaking water bath. Following incubation, solutions were extracted with 3.5 mL diethylether (2x). Extracts were dried under nitrogen and stored at -20°C. Prior to analysis, samples were brought up in 600 µL methanol:water (4:1).

Instrumentation, Chromatography, and Analysis

Urinary isoflavone analysis was based on the method of Franke et al. (234) with modifications. The HPLC system consisted of a Waters 2996 (Milford, MA) solvent delivery system interfaced with a Waters 996 photodiode array detector monitoring from 210-400 nm. Separations were carried out using an analytical (3 mm x 100 mm, 5 µm) Hydrobond PS C₁₈ column (Mac-Mod Analytical, Inc., Chadds Ford, PA). An in-line Hydrobond PS C₁₈ guard (3.2 mm x 20 mm, 5 µm) column (Mac-Mod Analytical, Inc., Chadds Ford, PA) along with a precolumn filter was used for each analysis. All separations were carried out at 30°C. Solvents A, B, and C consisted of methanol, acetonitrile, and 1% aqueous acetic acid, respectively. Separation of isoflavones was performed at 0.55 mL/min using the following linear gradient: 0 to 5 min, 10% solvent A,

15% solvent B, 75% solvent C; 5 to 10 min, 15% solvent A, 20% solvent B, 65% solvent C; 10 to 13 min, 20% solvent A, 20% solvent B, 60% solvent C; 13 to 14 min, 25% solvent A, 25% solvent B, 50% solvent C; 14 to 18 min, 45% solvent A, 45% solvent B, 10% solvent C; 18 to 20 min, 45% solvent A, 50% solvent B, 5% solvent C; 20 to 25 min, 10% solvent A, 15% solvent B, 75% solvent C. Peak confirmation and quantitation were determined with authentic standards for daidzein, genistein, glycitein (Indofine, Hillsborough, NJ), equol (Sigma Chemical Co., St. Louis, MO), O-desmethyldangolensin (ODMA), and dihydrodaidzein (Plantech, Reading, England). Empower ProTM version 5.0 software was used for data collection and integration.

Prostate Isoflavones

Extraction

Immediately following surgery, prostate samples were stored in liquid nitrogen. Extraction and analysis of prostate isoflavones was carried using the following method developed by our lab. Approximately 0.05 g of prostate were transferred to a glass vial containing 4.5 mL of methanol. Tissue samples were homogenized, vortexed, and centrifuged at 23°C and 1000 x g for 5 min in an HN-SII Centrifuge (Damon/IEC, Needham Hts., MA). The supernatant was removed, followed by the addition of 2.5 mL of methanol to the precipitate. Samples were vortexed and centrifuged under the same conditions mentioned above. The supernatant was combined and dried under nitrogen. Samples were resolubilized in 900 µL acetate buffer (pH 5.5), 100 µL 10% ascorbic acid, and 100 µL β-glucuronidase/arylsulfatase (Roche, Indianapolis, IN) and incubated

overnight (>12 h) at 37°C in a shaking water bath. Following incubation, solutions were extracted with 3.5 mL diethylether (2x). Extracts were dried under nitrogen and stored at -20°C. Prior to analysis, samples were brought up in 350 µL of methanol.

Instrumentation, Chromatography, and Analysis

With assistance from Dr. Qingguo Tian in our laboratory and modifications to the methods of Twaddle et al. (235) and Grace et al. (236), the following methods were used for isoflavone analysis in prostate. The HPLC system consisted of a Waters 2695 (Milford, MA) solvent delivery system interfaced with a Waters 996 photodiode array detector monitoring from 200-600 nm and Quattro Ultima quadrupole mass spectrometer (MS) (Micromass Limited, Manchester, UK) equipped with a Z-spray electrospray ionization (ESI) source. Calibration of the mass spectrometer was performed using sodium iodide and caesium iodide. Separations were carried out using an analytical (3 mm x 100 mm, 5 µm) Hydrobond PS C₁₈ column (Mac-Mod Analytical, Inc., Chadds Ford, PA). An in-line Hydrobond PS C₁₈ guard (3.2 mm x 20 mm, 5 µm) column (Mac-Mod Analytical, Inc., Chadds Ford, PA) along with a precolumn filter was used for each analysis. Solvents A, B, and C consisted of methanol, acetonitrile, and water, respectively. Each of the solvents contained 0.5% formic acid. Separation of isoflavones was performed at 0.55 mL/min using the linear gradient described under urinary isoflavone extraction and analysis. The eluate from the HPLC was directly introduced to the interface of the mass spectrometer without solvent splitting. Both positive and negative ESI-MS/MS were used to confirm the identity of isoflavones. Typical settings for the positive ion ESI-MS were as follows: capillary voltage, 3.35 kV; RF-1, 50 V;

cone voltage, 36V; source temperature, 120°C; desolvation gas temperature, 500°C at a flow of 16 L/min. For negative ion ESI-MS, all parameters were the same except for the following: capillary voltage, -3.35 kV and cone voltage, -36V. During collisionally activated dissociation (CAD) experiments, collision energy was adjusted to attenuate the precursor ions by approximately 50%, typically around 25 eV. Selected reaction monitoring (SRM) was used for isoflavone quantification. Peak confirmation and quantitation were determined with authentic standards for daidzein and genistein (Indofine, Hillsborough, NJ). Instrument control and data analysis were accomplished using MassLynx™ version 3.5 software.

Plasma or Serum Hormone Analysis

Venous blood was collected in 8.5 mL SST gel and clot activator tubes, 8 mL PST gel and lithium heparin tubes, and 3 mL K₃ EDTA tubes (Becton Dickinson, Franklin Lakes, NJ). Plasma or serum was removed and immediately analyzed for PSA, testosterone, and IGF-1 concentrations. PSA and testosterone concentrations were measured using a competitive immunoassay with chemiluminescence (Bayer Diagnostics, Tarrytown, NY). IGF-1 levels were determined using a non-competitive immunoassay with chemiluminescence (Nichols Institute Diagnostics, San Clemente, CA).

Statistical Analysis

All statistical calculations were performed using software from Statview (SAS Institute Inc., Cary, NC). Repeated-measures ANOVA was employed for within group

comparisons. ANOVA was used for between group comparisons with significance assessed using Fisher's Protected Least Significant Difference test. P values < 0.05 were considered to be statistically significant.

Results

General Blood Labs and Patient Compliance

Because a number of study patients were diabetic, blood glucose concentrations after washout were slightly higher than the accepted normal range. Blood "total chemistry" and "CBC with differential" values did not significantly change following the dietary intervention phase. Based on compliance measurements for all study participants, consumption of lycopene or soy-containing foods during the washout phase did not occur. Although men were allowed to consume lycopene within the range mentioned above for variable lengths of time, total lycopene consumption throughout the treatment period between the tomato-based product groups was not significantly different. Total lycopene consumption (i.e. milligrams of lycopene consumed during the treatment phase) for men consuming sauce, soup, or juice was 656.0 ± 210.4 , 630.0 ± 55.9 , or 600.2 ± 116.8 , respectively. Compliance information for men in the tomato groups showed that consumption of lycopene-containing foods throughout the treatment phase other than those provided was minimal and did not significantly affect daily lycopene intake within or between groups or result in significant increases in plasma or prostate lycopene concentrations. In addition, there was no reported consumption of soy-containing foods for the tomato-based product dietary intervention groups. Based on measures of

compliance, variation in treatment days for men consuming the soy protein supplement did not result in significant differences in soy consumption within the group. Men consuming 80 ± 4 mg isoflavone per day from soy did not exceed a daily lycopene score of 5 mg per day throughout the dietary intervention period. Consumption of lycopene-containing foods for men in this group throughout the soy treatment phase was extremely rare and did not produce significant increases in plasma or prostate lycopene levels. In addition, consumption of soy-containing foods other than the soy-protein supplement provided was not reported for the soy dietary intervention group.

Plasma Carotenoids, Tocopherols, Retinol, and Retinyl Palmitate

Differences in plasma concentrations ($\mu\text{mol/L}$) of retinol (2.44 ± 0.10), lutein (0.16 ± 0.01), β -cryptoxanthin (0.19 ± 0.02), cis β -carotene (0.03 ± 0.01), and retinyl palmitate (0.06 ± 0.01) were not significant based on dietary intervention group or time of sampling. Consumption of sauce, soup, or juice did not result in significant changes in plasma zeaxanthin or α -cryptoxanthin levels. However, zeaxanthin and α -cryptoxanthin concentrations in the plasma decreased 21% ($P < 0.01$) and 15% ($P < 0.05$), respectively, in men consuming soy (Table 3.1). Significantly higher levels of plasma zeaxanthin and α -cryptoxanthin after treatment with soy versus concentrations following consumption of sauce, soup, or juice were due to significantly higher plasma levels of these carotenoids in the soy group after washout. For men in the tomato-based product intervention groups, plasma concentrations ($\mu\text{mol/L}$) of α -tocopherol (30.67 ± 1.34) were not significantly different due to product consumed or time of sampling. However, α -tocopherol levels in plasma decreased 14% ($P < 0.05$) in men consuming soy. No significant changes in

concentrations of plasma δ -tocopherol were found following the treatment phase for any group. Following consumption of juice, δ -tocopherol concentrations in plasma were significantly higher when compared to levels found in men consuming soup or soy ($P < 0.01$ for each). Consumption of sauce, soup, or soy did not produce significant changes in plasma α -carotene, β -carotene, and γ -tocopherol concentrations. However, plasma α -carotene, β -carotene, and γ -tocopherol concentrations increased 180% ($P < 0.01$), 50% ($P < 0.05$), and 25% ($P < 0.05$), respectively, following consumption of juice. In comparison to men consuming sauce or juice, γ -tocopherol levels in plasma were significantly lower after consumption of soy ($P < 0.01$ and $P < 0.001$, respectively). In addition, plasma γ -tocopherol concentrations were significantly lower after soup intake when compared to plasma levels after juice consumption ($P < 0.05$).

Plasma Lycopene Isomers

In comparison to the lycopene isomer profiles from tomato-based products, containing 79-91% of lycopene in the all-trans form, a complex array of lycopene isomers are found in plasma (Figure 3.1). Total lycopene and all-trans lycopene levels in plasma increased after consumption of sauce (77% for each; $P < 0.01$ for each), soup (55% and 74%, respectively; $P < 0.05$ for each), or juice (112%, $P < 0.05$ and 141%, $P < 0.01$, respectively). Plasma total cis lycopene increased 77% and 88% ($P < 0.05$ for each) following consumption of sauce and juice, respectively. After comparing the change in plasma total lycopene following sauce (0.40 ± 0.09), soup (0.39 ± 0.15), and juice (0.39 ± 0.10) intake, no significant differences were found. For men consuming soy, plasma total lycopene, all-trans lycopene, and total cis lycopene concentrations decreased

31%, 36%, and 27% ($P < 0.05$ for each), respectively. Plasma total lycopene and all-trans lycopene levels were significantly higher after treatment with sauce ($P < 0.0001$ and $P < 0.001$, respectively), soup ($P < 0.0001$ for each), and juice ($P < 0.01$ for each) when compared to plasma levels found following soy intake. When compared to men consuming juice, consumption of soup produced significantly higher levels of plasma total lycopene and all-trans lycopene ($P < 0.01$ for each). Intake of soup also resulted in significantly higher levels of plasma all-trans lycopene when compared to concentrations present after sauce consumption ($P < 0.05$). Total cis lycopene concentrations in plasma were significantly higher following intake of sauce or soup versus levels found after intake of juice ($P < 0.05$ for sauce; $P < 0.01$ for soup) or soy ($P < 0.0001$ for each).

When consumption of any tomato-based product was considered, the percentage of plasma all-trans lycopene increased from 50% to 54% ($P < 0.01$), whereas total cis lycopene percentages in plasma decreased from 50% to 46% ($P < 0.01$) (Table 3.2). Following consumption of soy, the percentage of plasma all-trans lycopene decreased from 52% to 49% ($P < 0.05$), whereas total cis lycopene percentages in plasma increased from 48% to 51% ($P < 0.05$). Consumption of soup or juice resulted in significantly higher percentages of plasma all-trans lycopene and total cis lycopene when compared to percentages found after sauce ($P < 0.05$ for each vs. soup; $P < 0.01$ for each vs. juice) or soy ($P < 0.01$ for each vs. soup or juice) intake.

Prostate Carotenoids and Lycopene Isomers

A number of carotenoids and complex lycopene isomer patterns exist in prostate (Figure 3.2). Differences in prostate concentrations (nmol/g) of zeaxanthin (0.10 ± 0.02),

β -cryptoxanthin (0.11 ± 0.01), α -carotene (0.06 ± 0.01), β -carotene (0.16 ± 0.02), and 9-cis β -carotene (0.04 ± 0.01) were not significant based on dietary intervention group. When compared to men consuming soy, consumption of sauce or soup resulted in significantly higher prostate concentrations of total lycopene ($P<0.001$ for each), total cis lycopene ($P<0.001$ for each), 5-cis lycopene ($P<0.001$ for each), and all-trans lycopene ($P<0.01$ for each) (Table 3.3). Consumption of sauce, soup, or juice produced significantly higher concentrations of “other cis lycopene” isomers in prostate versus levels found after soy intake ($P<0.001$ for sauce or soup; $P<0.05$ for juice). In comparison to men consuming juice, consumption of sauce or soup resulted in significantly higher prostate concentrations of total lycopene ($P<0.05$ for each), total cis lycopene ($P<0.05$ for each), 5-cis lycopene ($P<0.05$ for each), and all-trans lycopene ($P<0.05$ for each). In addition, prostate levels of total lycopene and total cis lycopene were significantly lower after consuming soy versus concentrations found following consumption of juice ($P<0.05$ for each).

Following dietary intervention, the percentage of prostate total cis lycopene and all-trans lycopene was not significantly different between groups (Table 3.4). However, significantly lower percentages of prostate 5-cis lycopene were found in men consuming soup or juice when compared to percentages present after intake of soy ($P<0.05$ for soup; $P<0.01$ for juice). In comparison to men consuming soy, consumption of sauce, soup, or juice resulted in significantly higher percentages of “other cis lycopene” isomers in the prostate ($P<0.05$ for sauce or soup; $P<0.01$ for juice). In addition, the percentage distribution of lycopene isomers in prostate was much different than what was found in plasma.

Ratios of individual or groups of cis lycopene isomers to all-trans lycopene in prostate following sauce, soup, juice, or soy consumption were not significantly different between groups (Table 3.5). However, after evaluation of these ratios in prostate, the predominant cis lycopene isomer was in the 5-cis configuration.

Urinary Isoflavones

Urinary isoflavones were not detected in study patients following the washout phase. In addition, levels of isoflavones in urine after consuming sauce, soup, or juice were not detected (Table 3.6). Urinary levels of equol were only detected in one individual and were not significantly different from zero. Concentrations of daidzein, genistein, glycitein, O-desmethylangolensin (ODMA), and dihydrodaidzein in urine (Figure 3.3) significantly increased following consumption of soy ($P < 0.0001$ for each). In addition, urinary levels of these isoflavones following intake of soy were significantly higher when compared to the undetected concentrations after sauce, soup, or juice consumption ($P < 0.0001$ for each vs. each tomato product).

Prostate Isoflavones

Prostate isoflavones were not detected in men consuming sauce, soup, or juice. Prostate isoflavones were not detected using UV-vis detection, however, the increased sensitivity and lower detection limit of the ESI-MS/MS detector allowed for the identification and detection of daidzein and genistein in prostate after soy consumption (Figure 3.4). Prostate daidzein and genistein concentrations were significantly higher following soy consumption versus undetected levels after intake of sauce, soup, or juice ($P < 0.0001$ for each vs. each tomato product) (Table 3.7).

Plasma or Serum Hormones

Differences in plasma concentrations (ng/mL) of PSA (5.28 ± 0.37), IGF-1 (158.52 ± 10.37), and testosterone (3.66 ± 0.24) were not significant based on dietary intervention group or time of sampling (Table 3.8). However, when intake of any dietary product was considered, PSA concentrations decreased.

Discussion

This study provides information on the *in vivo* deposition of dietary phytochemicals, identifies dietary sources of carotenoids that provide highly bioavailable forms of lycopene, and gives insight into the relationship between hormones and prostate cancer. Compliance measurements suggest that men with prostate cancer are willing to consume daily servings of tomato-based products or a soy protein supplement for several weeks. A dietary intervention program including the consumption of tomato and/or soy

foods can function as a low-intensity and cost effective method to serve as an adjunct to current therapy of prostate cancer.

As reported by our lab, one week of washout and a minimum of two weeks of treatment with tomato-based products are sufficient to significantly modulate lycopene concentrations in plasma (221). Consumption of sauce, soup, or juice rapidly and significantly increased plasma lycopene concentrations, whereas dietary intervention with soy and a diet devoid of lycopene-containing foods resulted in a rapid and significant decrease in zeaxanthin, α -cryptoxanthin, and lycopene levels in plasma. Levels of α -carotene, β -carotene, lutein, zeaxanthin, and cis- β -carotene are much higher in juice than in sauce or soup. Consumption of juice, a tomato-based product containing tomatoes, carrots, celery, beets, parsley, lettuce, watercress, and spinach, significantly increased plasma α -carotene and β -carotene concentrations. The significant decrease in plasma α -tocopherol concentrations in men consuming soy does not appear to be related to the dietary intervention program provided in this study.

Although Schierle and colleagues (57) report that 10-20% of the lycopene isomers present in tomato-based products exist in the cis configuration, our laboratory has found that at least 90% of these isomers in tomato foods are in the trans form (15). Interestingly, a much higher percentage of cis isomers and complex lycopene isomer patterns can be found in vivo. Significant increases in plasma all-trans and total cis lycopene isomers occurred after intake of tomato-based products, whereas these lycopene isomers in plasma decreased significantly for men consuming soy. A significant increase in the percentage of plasma all-trans lycopene was accompanied by a significant decrease in the percentage of plasma total-cis lycopene after consumption of tomato-based

products. However, the opposite was true for men consuming soy. As a result, following tomato-based product intake, plasma lycopene isomers were predominantly in the all-trans form (~54%). In addition, in the absence of tomato-based food dietary intervention, the majority of lycopene isomers in plasma were in the cis configuration (~46%). It is plausible that the higher percentage of all-trans isomers in plasma following treatment is simply due to the constant influx of these compounds from the tomato-based products, whereas the higher percentage of cis isomers following a diet without lycopene-containing foods suggests a possible all-trans lycopene isomer conversion to or biological preservation of these cis compounds. Consumption of different tomato-based foods produced plasma lycopene isomer profiles that were unique to the product consumed, suggesting that the source of lycopene contributes to the variation in plasma lycopene isomer patterns.

Carotenoids in prostate exist in variable concentrations and isomer forms (68, 210). Although a number of prostate carotenoids were detected, only lycopene levels, including individual isomers and/or groups of isomers measured, were significantly higher in men consuming tomato-based products when compared to concentrations in men after soy intake. Interestingly, the percentage of prostate all-trans lycopene (~20%) and total cis lycopene (~80%) was not different between the tomato-based product and soy groups after the treatment phase. This suggests that all-trans and total cis percentages of lycopene isomers do not change as rapidly in the presence or absence of lycopene-containing foods in prostate as they do in plasma. However, upon evaluation of the cis lycopene isomers, the percentages of “other cis” and 5-cis lycopene isomers were significantly higher and lower, respectively, in prostate from men consuming

tomato-based foods versus levels in men after soy consumption. In addition, when the ratios of individual cis lycopene isomers to all-trans lycopene isomers were considered, the 5-cis to all-trans lycopene isomer ratio was the highest. Although the percentage of total cis and all-trans lycopene isomers don't appear to change as quickly in prostate as they do in plasma, the relative amounts of individual cis isomers in prostate seem to rapidly fluctuate when men consuming a lycopene-free diet are compared to men consuming tomato-based foods. In a study from our lab assessing the percentage of individual cis lycopene isomers in plasma following washout and treatment phases, significant variations were also observed (221). Further study on percent changes of individual cis lycopene isomers in plasma and prostate may help us better understand the biological significance of these configurations of lycopene in relation to prostate cancer. The presence of carotenoids, including the complex array of lycopene isomers, in prostate may provide insight into the proposed health benefits of these compounds as they relate to prostate cancer. In addition, as was seen in plasma, unique prostate lycopene isomer profiles resulted from consumption of different tomato-based foods.

As mentioned earlier, lycopene bioavailability is affected by multiple factors (208, 209, 223). Identification of a bioavailable source of lycopene is useful for delivering the proposed beneficial health effects associated with this compound. No significant differences in plasma lycopene concentrations were observed between the tomato-based product groups after washout, however, plasma lycopene levels in men consuming sauce and soup were much higher and significantly higher, respectively, versus concentrations after the consumption of juice. Due to randomization and because differences in plasma lycopene levels after washout were not significant among the

groups, it is assumed that each group had similar prostate lycopene concentrations after the washout period. Interestingly, prostate tissue concentrations of lycopene were significantly higher in men after consumption of sauce or soup when compared to concentrations after juice intake. In comparison to men consuming juice, increased deposition of lycopene in prostate of men consuming sauce or soup may have been due to higher lycopene uptake signified by elevated plasma lycopene concentrations after consumption of sauce or soup. The non-significant differences in the changes for plasma lycopene between the tomato-based product groups after several weeks of dietary intervention may be explained by lycopene clearance from the plasma by the prostate shortly after consumption. Therefore, increased prostate lycopene levels for men consuming sauce or soup, versus men consuming juice, supports the idea that lycopene may be more bioavailable from sauce or soup than from juice. After assessment of both plasma and prostate total lycopene levels after treatment, it appears that sauce and soup, when compared to juice, may provide a more bioavailable source of lycopene. As mentioned in the articles above, lycopene uptake is improved when consumed with lipid. The increased lipid content of sauce and soup versus juice may partly explain the differences in lycopene bioavailability. The fat content in each ½ cup serving of sauce, soup, and juice is 3, <0.5, and 0 grams, respectively. These variations as well as other dietary constituents consumed along with these foods contribute to the increased bioavailability of lycopene from sauce and soup versus juice.

Concentrations of isoflavones in vivo provide insight into the absorption and metabolism of these compounds (237, 238). Urinary levels of the major isoflavone compounds and a number of isoflavone metabolites significantly increased after dietary

intervention with a soy protein supplement. The urinary excretion of isoflavones and their metabolites contributes to the understanding of the absorption, retention and necessity of genistein, daidzein, and glycitein. When compared to the amounts of daidzein and glycitein consumed, genistein was consumed in relatively higher amounts. However, the relatively equal presence of genistein and daidzein in urine, yet high presence of daidzein metabolites, suggests that daidzein may be preferred for excretion or preferentially used in biological reactions involving isoflavones.

Adequate information on prostate isoflavones is currently not available. Analytical advances in mass spectrometry and tandem mass spectrometry allow for improved sensitivity and lower detection limit in the determination of isoflavones from biological samples (235, 236). When compared to men consuming a diet devoid of soy products, consumption of soy resulted in significantly higher prostate concentrations of genistein and daidzein. Accumulation of isoflavones in prostate may be associated with the beneficial health effects these compounds are thought to impart in relation to prostate cancer.

Although dietary factors are thought to mediate their health benefits in part by influencing concentrations of hormones related to cancer biology (224), no significant changes in serum PSA, IGF-1, or testosterone were observed following consumption of tomato or soy foods. Interestingly, however, when all men consuming either soy or tomato-based foods were considered, serum PSA concentrations decreased. In addition to other mechanisms through which these foods may provide beneficial health effects, reduction in serum PSA levels may aid in slowing the development of prostate cancer.

In conclusion, because this study was successful in showing high compliance rates, definitive larger studies can be conducted to determine if dietary interventions with tomato-based products or soy enhance the efficacy of prostate cancer therapy. This study also helped to characterize the unique, yet complex, in vivo profiles of phytochemicals, including carotenoids and isoflavones, from these foods. Information on these phytochemical patterns may improve our understanding of their role in the risk and/or progression of prostate cancer. In addition, sauce and soup seem to provide a more bioavailable form of lycopene than juice. Finally, although dietary intervention with tomato-based products and soy may influence prostate cancer development by modulating serum PSA concentrations, studies with larger populations and longer intervention periods are required to confirm this role for these foods.

	Sauce		Soup	
	Washout	Treatment	Washout	Treatment
	μmol/L			
Zeaxanthin	0.040±0.008 ¹	0.039±0.006 ¹	0.041±0.007 ¹	0.032±0.003 ¹
δ-tocopherol	0.47±0.15	0.32±0.07 ^{1,1*,2}	0.22±0.05	0.17±0.06 ^{1*,2}
α-cryptoxanthin	0.026±0.003 ¹	0.027±0.003 ¹	0.026±0.003 ¹	0.023±0.003 ¹
γ-tocopherol	5.67±0.97	5.39±0.58 ^{1,1*,2}	3.33±0.51	3.70±0.56 ^{1,3}
α-tocopherol	33.20±5.76	31.29±3.44	30.70±2.81	28.14±1.51
Total lycopene	0.52±0.09 ^a	0.92±0.09 ^{b,1,2}	0.71±0.12 ^a	1.10±0.08 ^{b,2}
All-trans lycopene	0.26±0.05 ^a	0.46±0.04 ^{b,1}	0.35±0.06 ^a	0.61±0.06 ^{b,2}
Total cis lycopene	0.26±0.05 ^a	0.46±0.05 ^{b,1}	0.36±0.06	0.49±0.04 ¹
α-carotene	0.034±0.006 ¹	0.032±0.007	0.143±0.048 ²	0.116±0.046
β-carotene	0.16±0.02	0.15±0.02	0.33±0.09	0.43±0.09

A

Table 3.1 Plasma carotenoid, tocopherol, retinol, and retinyl palmitate concentrations following tomato-based product or soy protein intervention[†]

[†]Values are means ± SEM; n = 7/group; Means in a row within groups without a common letter superscript are significantly different, P<0.05; Means in a row for a particular time point across groups without a common number superscript are significantly different, P<0.05.

(CONTINUED)

	Juice		Soy	
	Washout	Treatment	Washout	Treatment
	μmol/L			
Zeaxanthin	0.054±0.009 ¹	0.048±0.007 ^{1, 2}	0.084±0.008 ^{a, 2}	0.066±0.011 ^{b, 2}
δ-tocopherol	0.42±0.13	0.55±0.16 ^{1, 3}	0.16±0.05	0.09±0.03 ^{1*}
α-cryptoxanthin	0.032±0.005 ¹	0.028±0.004 ¹	0.053±0.010 ^{a, 2}	0.045±0.008 ^{b, 2}
γ-tocopherol	4.78±0.86 ^a	5.96±0.76 ^{b, 1*}	3.02±0.67	2.47±0.52 ³
α-tocopherol	29.73±2.73	30.85±2.26	38.21±5.36 ^a	32.89±4.22 ^b
Total lycopene	0.34±0.10 ^a	0.72±0.07 ^{b, 1}	0.62±0.06 ^a	0.43±0.04 ^{b, 3}
All-trans lycopene	0.17±0.05 ^a	0.41±0.04 ^{b, 1}	0.33±0.03 ^a	0.21±0.02 ^{b, 3}
Total cis lycopene	0.17±0.06 ^a	0.32±0.04 ^{b, 2, 3}	0.30±0.02 ^a	0.22±0.02 ^{b, 3}
α-carotene	0.05±0.02 ^{a, 1, 3}	0.14±0.02 ^b	0.07±0.01 ^{1, 2, 3}	0.08±0.02
β-carotene	0.24±0.07 ^a	0.36±0.06 ^b	0.29±0.05	0.31±0.08

B

Table 3.1: CONTINUED

†Values are means ± SEM; n = 7/group; Means in a row within groups without a common letter superscript are significantly different, P<0.05; Means in a row for a particular time point across groups without a common number superscript are significantly different, P<0.05.

	All-trans lycopene		Total cis lycopene	
	Washout	Treatment	Washout	Treatment
	%			
Sauce	50.4±0.6	50.5±1.0 ¹	49.6±0.6	49.5±1.0 ¹
Soup	49.4±1.1 ^a	54.8±1.5 ^{b, 2}	50.6±1.1 ^a	45.2±1.5 ^{b, 2}
Juice	52.0±1.4	56.0±1.3 ²	48.0±1.4	44.0±1.3 ²
Tomato-based products	50.5±0.6 ^a	53.6±0.9 ^b	49.5±0.6 ^a	46.4±0.9 ^b
Soy	52.0±1.2 ^a	49.4±1.0 ^{b, 3}	48.0±1.2 ^a	50.6±1.0 ^{b, 3}

Table 3.2 Changes in the percentage of plasma lycopene isomers following dietary intervention with tomato-based products or soy protein[†]

[†]Values are means ± SEM; n = 7/group; Means in a row for all-trans lycopene or total cis lycopene without a common letter superscript are significantly different, P<0.05; Means in a column without a common number superscript are significantly different, P<0.05.

	Sauce	Soup	Juice	Soy
	nmol/g			
Total lycopene	0.60±0.10 ^a	0.58±0.06 ^a	0.37±0.07 ^b	0.16±0.02 ^c
Total cis lycopene	0.48±0.08 ^a	0.46±0.05 ^a	0.30±0.06 ^b	0.13±0.02 ^c
Other cis lycopene	0.30±0.05 ^a	0.28±0.02 ^a	0.20±0.04 ^a	0.06±0.02 ^b
5-cis lycopene	0.18±0.03 ^a	0.18±0.02 ^a	0.10±0.02 ^b	0.07±0.01 ^b
All-trans lycopene	0.12±0.02 ^a	0.11±0.01 ^a	0.07±0.01 ^b	0.04±0.01 ^b

Table 3.3 Prostate carotenoid concentrations following tomato-based product or soy protein intervention¹

¹Values are means ± SEM; n = 7/group; ND, not detected; Means in a row without a common superscript are significantly different, P<0.05.

	Sauce	Soup	Juice	Soy
			%	
Total cis lycopene	80.9±1.4	80.3±1.5	82.0±1.6	76.0±4.1
Other cis lycopene	49.6±1.1 ^a	50.0±1.2 ^a	53.0±2.4 ^a	40.5±5.1 ^b
5-cis lycopene	31.3±1.4 ^{a, b}	30.3±1.4 ^a	29.0±1.6 ^a	35.5±1.6 ^b
All-trans lycopene	19.1±1.4	19.7±1.5	18.0±1.6	24.0±4.1

Table 3.4 Percentage of individual lycopene isomers in prostate following consumption of tomato-based products or soy protein¹

¹Values are means ± SEM; n = 7/group; ND, not detected; Means in a row without a common superscript are significantly different, P<0.05.

	Sauce	Soup	Juice	Soy
Total cis/all-trans	4.58±0.62	4.27±0.45	4.74±0.46	3.67±0.78
Other cis/all-trans	2.82±0.34	2.65±0.27	3.08±0.35	1.89±0.61
5-cis/all-trans	1.76±0.28	1.62±0.19	1.66±0.15	1.78±0.24

Table 3.5 Ratio of lycopene isomers to all-trans lycopene in prostate following dietary intervention with tomato-based products or soy protein¹

¹Values are means ± SEM; n = 7/group; ND, not detected; Means in a row without a common superscript are significantly different, P<0.05.

	Daidzein	Dihydrodaidzein	Equol	Genistein	Glycitein	ODMA
			$\mu\text{mol/L}$			
Tomato-based products	ND ^a	ND ^a	ND	ND ^a	ND ^a	ND ^a
Soy	2.95±0.94 ^b	2.20±0.73 ^b	0.18±0.18	2.54±0.58 ^b	0.43±0.10 ^b	11.92±3.08 ^b

Table 3.6 Urine isoflavone concentrations following tomato-based product or soy protein intervention^{1, 2}

Values are means \pm SEM; n = 7/group or 21 (tomato-based products); ND, not detected; Means in a column without a common superscript are significantly different, P<0.0001.

¹Urine isoflavone concentrations were not detected prior to dietary intervention.

²Urine isoflavone concentrations following consumption of soy protein were significantly higher (P<0.0001) than the undetected levels after washout.

	Daidzein	Genistein
	pmol/g	
Tomato-based products	ND ^a	ND ^a
Soy	2.49±0.69 ^b	5.09±0.81 ^b

Table 3.7 Prostate isoflavone concentrations following tomato-based product or soy protein intervention¹

¹Values are means ± SEM; n = 7/group or 21 (tomato-based products); ND, not detected;

Means in a column without a common superscript are significantly different, P<0.0001.

	Prostate Specific Antigen		Insulin-Like Growth Factor-1		Testosterone	
	Washout	Treatment	Washout	Treatment	Washout	Treatment
	ng/mL					
Sauce	5.85±1.18	5.98±1.04	175.6±11.4	186.2±15.1	3.10±0.46	3.08±0.53 ^b
Soup	6.43±0.75	6.40±0.82	138.4±19.2	149.6±24.2	3.80±0.65	4.11±0.35 ^b
Juice	3.14±0.77	3.02±0.86	155.2±57.9	150.0±55.1	2.82±0.67	2.47±0.67 ^a
Soy	4.97±1.16	4.40±0.96	162.2±25.3	162.6±17.8	4.11±0.74	5.23±0.95 ^b

Table 3.8 Changes in plasma hormone levels following dietary intervention with tomato-based products or soy protein¹

¹Values are means ± SEM; n = 7/group; Means in a column without a common superscript are significantly different, P<0.05; No within group statistical differences were observed.

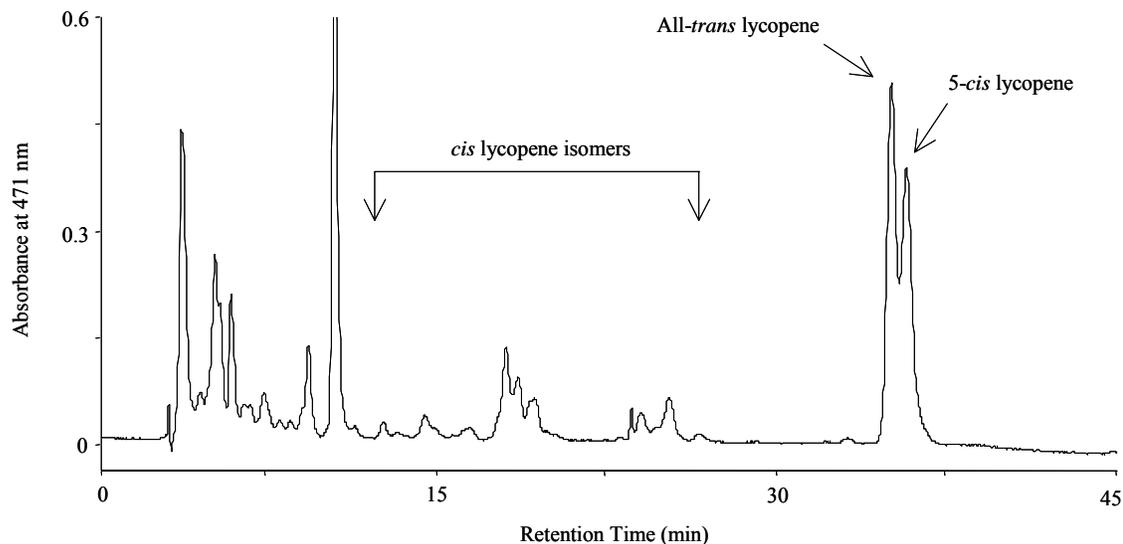


Figure 3.1 Representative chromatogram using a C₃₀ HPLC system with UV-vis detection of plasma lycopene isomers following consumption of tomato-based products for 2-4 weeks. A Spherisorb ODS2 column was used to separate plasma carotenoids for this study. Due to differences in mobile phase conditions and in the ability of the columns to separate lycopene isomers, concentrations of 5-cis lycopene in plasma were combined with the other cis lycopene isomers and reported as total cis lycopene isomers.

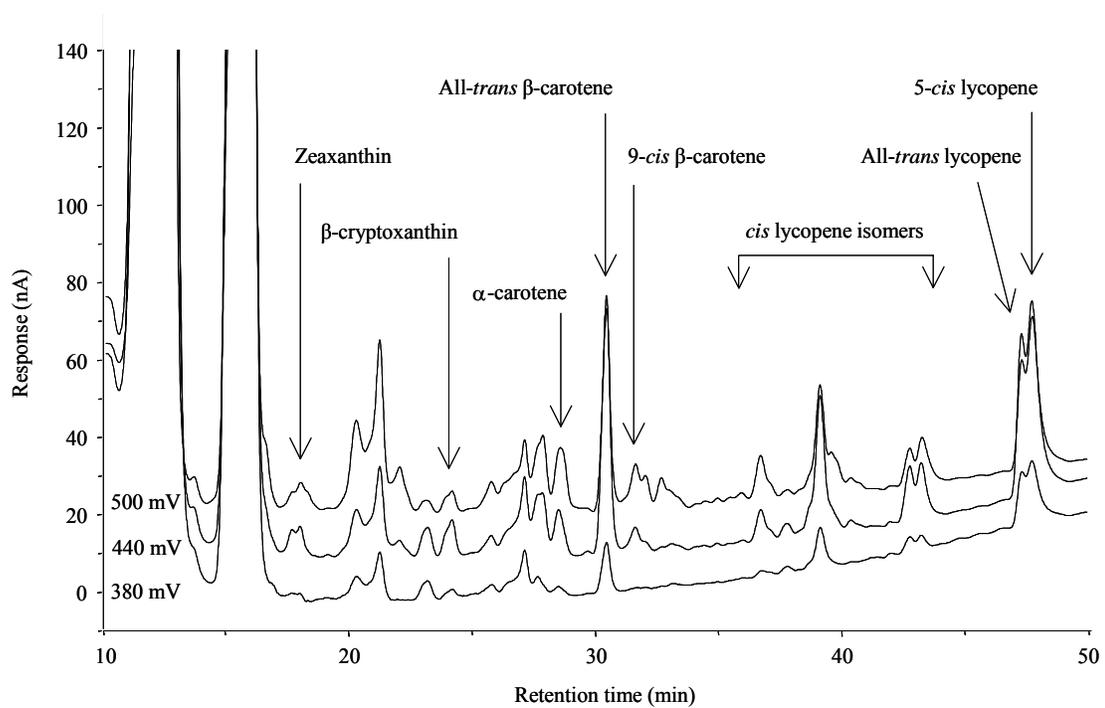


Figure 3.2 Representative chromatogram using a C₃₀ HPLC system with electrochemical detection of prostate carotenoids, including multiple lycopene isomers, after tomato-based product intake for 2-4 weeks. No response for carotenoids or lycopene isomers was noted for the applied potential of 560 mV and is therefore not displayed. Co-elution of compounds with lutein, which has a retention time between 16 and 17 minutes, made it impossible to quantify this carotenoid.

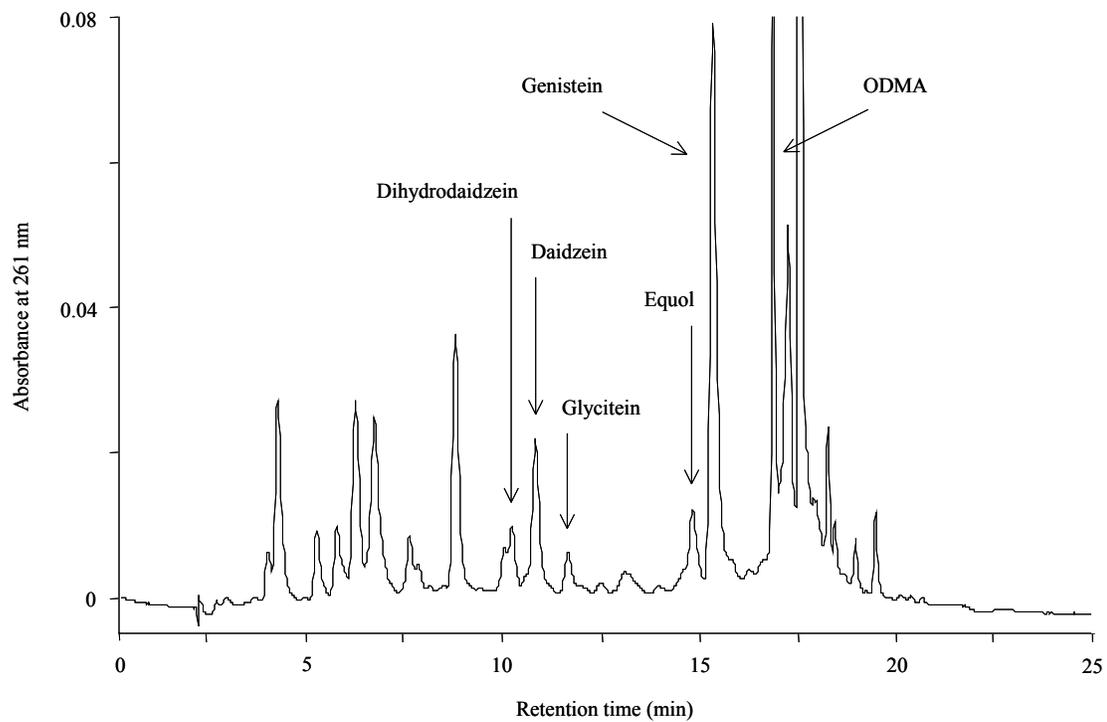


Figure 3.3 Representative chromatogram using a C₁₈ HPLC system with UV-vis detection of urinary isoflavones following 2-4 weeks of soy intake.

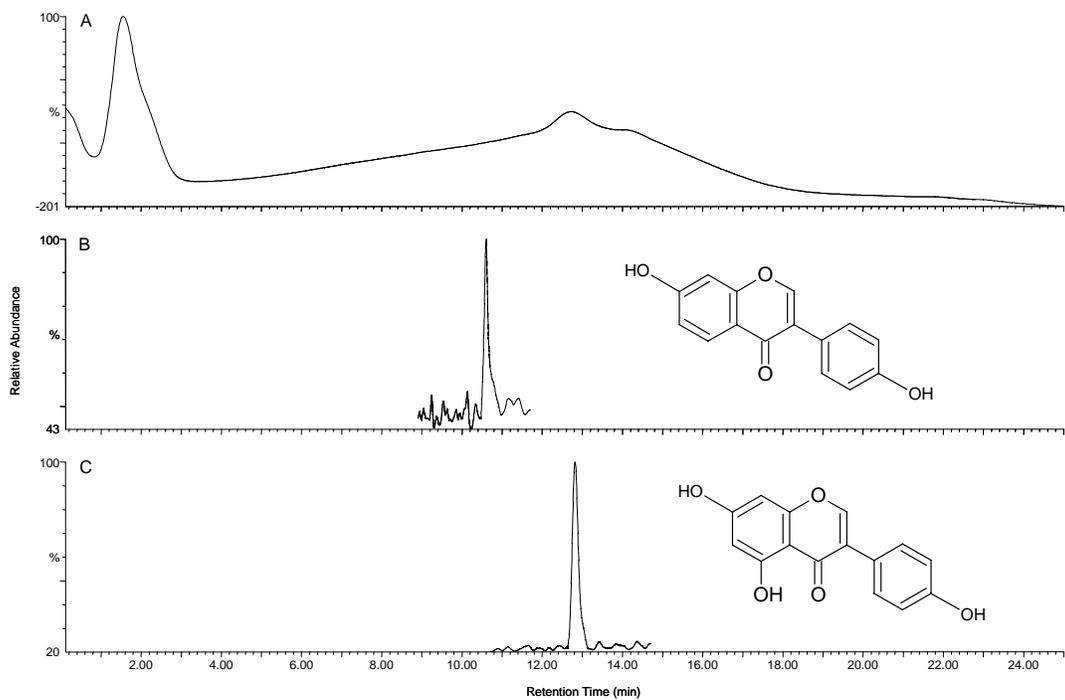


Figure 3.4 Representative chromatograms using a C₁₈ HPLC system with UV-vis detection of prostate isoflavones (A) or using a C₁₈ HPLC system ESI-MS/MS detection of prostate daidzein (B) and prostate genistein (C) after consumption of soy for 2-4 weeks.

CHAPTER 4

EVALUATION OF ADDITIONAL MARKERS OF OXIDATIVE STRESS USING NEW TECHNOLOGIES

Abstract

Consumption of tomato-based products is thought to modulate the antioxidant/oxidative environment. This evaluation was conducted to assess the effect of tomato-based product consumption on changes in antioxidant capacity as well as urinary F₂-isoprostane 8-iso-PGF_{2α} levels in healthy individuals and prostate cancer patients from the first two clinical trials using new technologies. For stored samples from selected healthy participants in the first clinical trial, plasma total lycopene concentrations increased 144%, 87%, and 152% (P<0.0001 for each) while plasma lipid-soluble antioxidant capacity (LS-AC) increased 21% (P<0.01), 16% (P<0.001), and 15% (P<0.0001) following consumption of soup, RTS soup, and juice, respectively. As described in the second clinical trial, total lycopene levels in plasma for prostate cancer patients increased after consumption of sauce (77%, P<0.01), soup (55%, P<0.05), or juice (112%, P<0.05). Upon evaluation of stored plasma samples from these men, LS-AC increased 56% (P<0.05), 50% (P<0.01), or 27% (P<0.05), respectively. Although no statistical differences in urinary concentrations of 8-iso-PGF_{2α} were noted in samples obtained from either clinical trial, levels for prostate cancer patients decreased 70%, 65%,

and 37% after soup, juice, and tomato-based product intake, respectively. Evaluation of new technologies used to measure additional markers of oxidative stress shows that consumption of tomato-based products rapidly increases plasma lipid-soluble antioxidant capacity while decreasing lipid peroxidation in healthy individuals and/or prostate cancer patients.

Introduction

Free radical-mediated oxidative damage is thought to play a role in the initiation and/or progression of chronic diseases, such as cancer and cardiovascular disease (205). Oxidative stress results from a disruption of the prooxidant/antioxidant cellular balance. In addition to environmental components that contribute to increased oxidative stress, changes in certain in vivo factors associated with chronic disease can increase oxidative stress exposure. Elevated susceptibility to oxidizing agents due to a disruption in the oxidation/reduction balance in individuals with chronic diseases may influence the progression of these diseases.

Numerous epidemiological studies have shown that the consumption of tomatoes and tomato-based foods or lycopene is inversely associated with the risk for certain cancers (8) and cardiovascular disease (6). Intake of tomatoes and tomato-based products contributes to the uptake of numerous vitamins, minerals, and phytochemicals, such as carotenoids and polyphenols, in human serum and tissue. Accumulating evidence suggests that phytochemicals consumed from these foods play a role in oxidative stress reduction, and disease prevention and/or progression.

Although a number of other potential mechanisms through which tomato-based product intake may reduce the risk for various chronic diseases has been suggested, the antioxidant activity or free-radical quenching ability of tomato carotenoids has been hypothesized as a primary mechanism for the beneficial health effects of these compounds (239). Lycopene, the most abundant carotenoid present in tomatoes and tomato-based foods, has received the most attention. The extensive chromophore system of conjugated carbon-carbon double bonds of lycopene contributes to its chemical reactivity toward free radicals and oxidizing agents. In addition to scavenging peroxy radicals (240), lycopene is the most efficient singlet oxygen quencher when compared to other dietary carotenoids (103).

Because oxidative damage may contribute to the initiation and/or progression of prostate cancer, this evaluation was conducted to better understand how consumption of lycopene-containing tomato-based products influences the antioxidant/oxidative status in prostate cancer patients. This evaluation was also performed to provide additional support for this relationship in healthy individuals. We hypothesized that consumption of lycopene-containing tomato-based products rapidly and significantly reduce oxidative stress. Specifically, the objective of this evaluation was to do an analysis of stored plasma and/or urine samples from clinical trials one and two using new technologies to determine the effect of tomato-based product intake on plasma antioxidant capacity and urinary F₂-isoprostane 8-iso-PGF_{2α} levels in healthy subjects and in patients with prostate cancer.

Methods

Sample Retrieval

All analysis was performed on stored samples from the first two clinical trials. Only post-washout and post-intervention samples were retrieved. Plasma samples from the first clinical trial were a sub-sample of healthy individuals (n = 30; 15 men/15 women). Plasma and urine samples from prostate cancer patients in the second clinical trial were only from those men who consumed tomato-based products (n = 21). The study design, product analysis, and plasma carotenoid separation, extraction, and analysis were performed as previously described in clinical trials one and two.

Plasma Antioxidant Capacity

Water-soluble Phase Extraction

Blood collection methods were identical to those described under plasma carotenoid, tocopherol, retinol, and retinyl palmitate extraction and analysis in the first two clinical studies. The addition of 60 μL of methanol or water to 15 μL of plasma, followed by vortexing and filtration, was performed to remove all water-soluble compounds. Water-soluble phase extraction was performed immediately prior to analysis.

Lipid-soluble Phase Extraction

Blood collection methods were identical to those described under plasma carotenoid, tocopherol, retinol, and retinyl palmitate extraction and analysis in the first two clinical studies. An equal volume of ethanol was added to 150 μL of plasma. Samples were vortexed prior to the addition of 750 μL of hexane:acetone (2:1). Following a second vortex, samples were centrifuged at 5585 x g in a 10MVSS Microcentrifuge (Corning-Costar, New York, NY) for 1 min and the lycopene-containing hexane layer was transferred to a glass vial. Extraction with hexane:acetone (2:1) was repeated (2x). The hexane layers were combined, dried under nitrogen and stored at -80°C. Prior to measurement of plasma lipid-soluble antioxidant capacity (LS-AC), samples were brought up in 200 μL of hexane.

Instrumentation and Analysis

A Photochem® instrument (Analytik Jena AG, Jena, Germany), using the principle of photochemiluminescence, was used to assess the antioxidant capacity of the plasma (241, 242). Radicals are generated photochemically by UV irradiation of a photosensitizer compound. These radicals are partially eliminated from the reaction with antioxidants present in the sample extract. In the detection unit, the remaining radicals are quantified by luminescence generation by a chemical reaction. Antioxidants are quantified due to their inhibitory effect on luminescence generation by comparison with a standardization compound (i.e. trolox for LS-AC and ascorbic acid for water-soluble antioxidant capacity (WS-AC)). For analysis of LS-AC, the integral of the curve for a

defined time is calculated. The antioxidant capacity of a sample results in a characteristic inhibition (i.e. reduction of the integral) relative to the blank. For analysis of WS-AC, the duration of the lag phase (i.e. intersection of the s-shaped curve slope and x-axis) is calculated. The antioxidant capacity of a sample results in an increased lag phase relative to the blank. Antioxidant capacity is calculated as equivalent units of trolox (TE) or ascorbic acid (AA) expressed in $\mu\text{mol TE/L plasma}$ or $\mu\text{mol AA/L plasma}$. The coefficient of variance for the assay, including intrassay and interassay factors was less than 8%.

Urinary F₂-Isoprostanes

Extraction

Urine was stored at 4°C during collection and later stored under nitrogen at -80°C. Concentrations of urinary 8-iso-PGF_{2 α} for healthy individuals were measured using a competitive enzyme-linked immunosorbent assay (ELISA) (Genox, Baltimore, MD). However, for prostate cancer patients, urinary isoprostane extraction and analysis was carried out using modifications to the methods of Ohashi et al. (243). Ten nanograms of internal standard (²H₄-8-iso-PGF_{2 α} , Cayman Chemical, Ann Arbor, MI) was added to 2 mL urine and centrifuged at 23°C and 1000 x g for 10 min in an HN-SII Centrifuge (Damon/IEC, Needham Hts., MA). Hydrochloric acid (40 μL , 1M) was added to 1 mL urine supernatant to adjust the pH to 3. Following acidification, solutions were extracted with 3 mL ethyl acetate and evaporated under nitrogen. Samples were then resolubilized in 2 mL 5% (v/v) ethanol (pH 3) and added to an EmporeTM high performance solid

phase extraction (SPE) SDB-XC (standard density bonded copolymer of poly(styrene-divinylbenzene) with cross linking, 7mm/3mL) disk cartridge (3M, St. Paul, MN) preconditioned with 0.5 mL methanol followed by 0.5 mL 1 mM HCl. The cartridge was washed with 0.5 mL 1 mM HCl followed by 0.5 mL hexane. Isoprostanes were eluted with 1 mL ethyl acetate containing 1% (v/v) methanol, dried under nitrogen and stored at -20°C. Prior to analysis, samples were resolubilized in 2.5 mL water.

Instrumentation, Chromatography, and Analysis

The high performance liquid chromatography (HPLC) system consisted of a Waters 2695 (Milford, MA) solvent delivery system interfaced with a Waters 996 photodiode array detector monitoring from 200-400 nm and Quattro Ultima quadrupole mass spectrometer (MS) (Micromass Limited, Manchester, UK) equipped with a Z-spray electrospray ionization (ESI) source. Calibration of the mass spectrometer was performed using sodium iodide and caesium iodide. Separations were carried out using an analytical (3.9 mm x 150 mm, 5 µm) Symmetry C₈ column (Waters, Milford, MA). An in-line C₁₈ guard (7.5 mm x 4.6 mm, 5 µm) column (Alltech, Deerfield, IL) along with a precolumn filter was used for each analysis. Solvents A and B consisted of 0.1% acetic acid and acetonitrile, respectively. Separation of 8-iso-PGF_{2α} was performed at 0.7 mL/min using an isocratic elution with an A/B ratio of 7/3. The eluate from the HPLC was directly introduced to the interface of the mass spectrometer without solvent splitting. Both positive and negative ESI-MS/MS were used to confirm the identity of 8-iso-PGF_{2α}. Typical settings for the positive ion ESI-MS were as follows: capillary voltage, 3.35 kV; RF-1, 50 V; cone voltage, 36V; source temperature, 120°C; desolvation

gas temperature, 500°C at a flow of 16 L/min. For negative ion ESI-MS, all parameters were the same except for the following: capillary voltage, -3.35 kV and cone voltage, -36V. During collisionally activated dissociation (CAD) experiments, collision energy was adjusted to attenuate the precursor ions by approximately 50%, typically around 25 eV. Selective ion recording (SIR) was used for 8-iso-PGF_{2α} quantification. Peak confirmation and quantitation were determined using an authentic standard for 8-iso-PGF_{2α} (Cayman Chemical, Ann Arbor, MI). Instrument control and data analysis were accomplished using MassLynx™ version 3.5 software.

Statistical Analysis

All statistical calculations were performed using software from Statview (SAS Institute Inc., Cary, NC). Repeated-measures ANOVA was employed for within group comparisons. ANOVA was used for between group comparisons with significance assessed using Fisher's Protected Least Significant Difference test. P values < 0.05 were considered to be statistically significant.

Results

Plasma Carotenoids, Tocopherols, Retinol, and Retinyl Palmitate

For stored samples retrieved from the first clinical trial of healthy individuals, plasma total lycopene concentrations increased 144%, 87%, or 152% (P<0.0001 for each) for those consuming soup, RTS soup, or juice, respectively (Table 4.1). When compared to individuals consuming RTS soup or juice, consumption of soup produced significantly

higher levels of plasma total lycopene ($P<0.01$). Lycopene intake from soup, however, was higher when compared to RTS soup or juice ($P<0.05$). Plasma total tocopherol levels in individuals consuming RTS soup or juice decreased 6% and 7%, respectively ($P<0.05$ for each). In addition, after consumption of tomato-based products, plasma total tocopherol concentrations decreased 5% ($P<0.05$). However, other carotenoid, retinol, and retinyl palmitate concentrations did not change significantly based on dietary intervention group or time of sampling.

As described in the second clinical trial, plasma α -carotene and β -carotene concentrations ($\mu\text{mol/L}$) increased from 0.05 ± 0.02 to 0.14 ± 0.02 (180%, $P<0.01$) and from 0.24 ± 0.07 to 0.36 ± 0.06 (50%, $P<0.05$), respectively, following consumption of juice for prostate cancer patients. Total lycopene levels in plasma increased after consumption of sauce (77%, $P<0.01$), soup (55%, $P<0.05$), or juice (112%, $P<0.05$) (Table 4.2). In addition, when compared to men consuming juice, consumption of soup produced significantly higher levels of plasma total lycopene ($P<0.01$). However, concentrations of other carotenoids, tocopherols, retinol, and retinyl palmitate did not change significantly based on dietary intervention group or time of sampling.

Plasma Antioxidant Capacity

Plasma water-soluble antioxidant capacity did not significantly change after the treatment phase for any group. For stored samples obtained from healthy men and women in the first clinical trial, plasma lipid-soluble antioxidant capacity (LS-AC) increased 21% ($P<0.01$), 16% ($P<0.001$), and 15% ($P<0.0001$) following consumption of soup, RTS soup, and juice, respectively (Table 4.3). When all groups were considered,

LS-AC in the plasma increased 17% ($P < 0.0001$) following consumption of tomato-based products. Plasma LS-AC increased following intake of sauce (56%, $P < 0.05$), soup (50%, $P < 0.01$), or juice (27%, $P < 0.05$) for stored prostate cancer patient samples (Table 4.4). A 45% increase in plasma LS-AC occurred after tomato product intake for all groups ($P < 0.001$).

Urinary 8-iso-PGF_{2α}

As mentioned in the first clinical trial, no significant changes in urinary 8-iso-PGF_{2α} were found for individual groups of healthy participants following consumption of soup, RTS soup, or juice using ELISA techniques. In stored samples from the second clinical trial involving prostate cancer patients, urinary concentrations of 8-iso-PGF_{2α} were not detected using UV-vis detection, but the increased sensitivity of the ESI-MS detector allowed for identification and detection of 8-iso-PGF_{2α} levels in urine before and after tomato-based product consumption (Figure 4.1). Although not statistically significant, urinary levels of 8-iso-PGF_{2α} decreased 70%, 65%, and 37% after soup, juice, and tomato-based product intake, respectively, for men with prostate cancer (Table 4.5).

Discussion

Observations from this study provide a better understanding of the effects tomato product intake have on markers of oxidative stress in healthy men and women and prostate cancer patients. Two studies have suggested that tomato-based product intake does not increase plasma antioxidant capacity (244, 245). However, using a photochem instrument, based on principles of photochemiluminescence, plasma lipid-soluble

antioxidant capacity significantly increased for every group of study participants after consumption of sauce, soup, RTS soup, or juice. Because lycopene is extremely lipophilic and was the only carotenoid to significantly increase in plasma following intake of any tomato-based product, it can be suggested that the elevated levels of plasma lycopene significantly contribute to the increased antioxidant capacity of the plasma lipid-soluble phase. For prostate cancer patients consuming juice, significant increases in plasma α -carotene and β -carotene may also be responsible for the increased lipid-soluble antioxidant capacity of the plasma.

Extreme variability in the urinary isoprostane concentrations detected for the study populations in this evaluation contributes to the statistically non-significant results obtained. In addition to differences in urinary isoprostane levels among subjects, variation in the data may in part be due to cross reactivity concerns and slight error in instrument reproducibility for levels detected by ELISA and HPLC with ESI-MS, respectively. Preliminary data from detection of isoprostanes in urine using HPLC with ESI-MS, potentially providing more selective determination of isoprostane isomers without fear of cross reactivity, showed that following tomato-based product consumption, prostate cancer patients had a decrease in urinary levels of the 8-iso-PGF_{2 α} F₂-isoprostane. Because F₂-isoprostanes are produced in vivo from free-radical catalyzed lipid peroxidation (246) and because lycopene is carried in the plasma entirely by lipoproteins (78, 83), a reduction in F₂-isoprostane 8-iso-PGF_{2 α} levels along with an increase in plasma lycopene concentrations implies an additional antioxidant role for this compound.

The beneficial health effects of tomato and tomato-based product consumption are related to the important nutrients and phytochemical carotenoids present within these foods. This evaluation shows that consumption of tomato-based products rapidly increases plasma lipid-soluble antioxidant capacity and decreases lipid peroxidation. It also suggests that lycopene may influence the antioxidant/oxidative balance more so than other carotenoids present in tomato-based foods. Furthermore, tomato-based product intake may aid in preventing the initiation and/or progression of chronic diseases by helping to reduce free-radical mediated oxidative damage.

	Total lycopene		Total tocopherols	
	Washout	Treatment	Washout	Treatment
	$\mu\text{mol/L}$			
Soup	0.34±0.04 ^a	0.83±0.07 ^{b, 1}	43.26±6.51	42.15±5.88
RTS soup	0.30±0.04 ^a	0.56±0.04 ^{b, 2}	37.59±3.41 ^a	35.32±3.35 ^b
Juice	0.23±0.03 ^a	0.58±0.06 ^{b, 2}	40.64±5.60 ^a	37.88±5.61 ^b
Tomato-based products	0.29±0.02 ^a	0.66±0.04 ^b	40.50±3.00 ^a	38.45±2.88 ^b

Table 4.1 Plasma total lycopene and total tocopherol concentrations following tomato-based product intervention in healthy individuals[†]

[†]Healthy individuals; Values are means \pm SEM; n = 10/group or 30 (tomato-based products); Means in a row for total lycopene or total tocopherols without a common letter superscript are significantly different, P<0.05; Means in a column without a common number superscript are significantly different, P<0.05.

	Total lycopene		Total tocopherols	
	Washout	Treatment	Washout	Treatment
	$\mu\text{mol/L}$			
Sauce	0.52±0.09 ^a	0.92±0.09 ^{b, 1, 2}	39.34±6.72	37.00±3.52
Soup	0.71±0.12 ^a	1.10±0.08 ^{b, 2}	34.25±2.44	32.01±1.58
Juice	0.34±0.10 ^a	0.72±0.07 ^{b, 1}	34.94±2.05	37.36±2.00
Tomato-based products	0.53±0.07 ^a	0.92±0.06 ^b	36.24±2.50	35.36±1.51

Table 4.2 Plasma total lycopene and total tocopherol concentrations following tomato-based product intervention in prostate cancer patients[†]

[†]Prostate cancer patients; Values are means \pm SEM; n = 7/group or 21 (tomato-based products); Means in a row for total lycopene or total tocopherols without a common letter superscript are significantly different, P<0.05; Means in a column without a common number superscript are significantly different, P<0.05.

	Plasma lipid-soluble antioxidant capacity	
	Washout	Treatment
	$\mu\text{mol TE/L}$	
Soup	83.58 \pm 8.78 ^a	100.98 \pm 11.30 ^b
RTS soup	66.73 \pm 6.28 ^a	77.49 \pm 6.89 ^b
Juice	83.20 \pm 12.67 ^a	95.35 \pm 12.80 ^b
Tomato-based products	77.83 \pm 5.55 ^a	91.27 \pm 6.21 ^b

Table 4.3 Tomato-based product consumption increases plasma lipid-soluble antioxidant capacity in healthy individuals[†]

[†]Healthy individuals; Values are means \pm SEM; n = 10/group or 30 (tomato-based products); TE = trolox equivalent; Means in a row without a common superscript are significantly different, P<0.01; No between group statistical differences for washout or treatment for individual groups were observed.

	Plasma lipid-soluble antioxidant capacity	
	Washout	Treatment
	$\mu\text{mol TE/L}$	
Sauce	57.71 \pm 6.18 ^a	90.10 \pm 17.77 ^b
Soup	38.60 \pm 8.88 ^a	58.07 \pm 13.10 ^b
Juice	56.76 \pm 10.26 ^a	72.00 \pm 9.90 ^b
Tomato-based products	50.74 \pm 5.04 ^a	73.46 \pm 8.43 ^b

Table 4.4 Tomato-based product consumption increases plasma lipid-soluble antioxidant capacity in prostate cancer patients[†]

[†]Prostate cancer patients; Values are means \pm SEM; n = 7/group or 21 (tomato-based products); TE = trolox equivalent; Means in a row without a common superscript are significantly different, P<0.05; No between group statistical differences for washout or treatment for individual groups were observed.

	Urinary 8-iso-PGF _{2α}	
	Washout	Treatment
	ng/mg creatinine	
Sauce	1.15±0.59	1.64±1.40
Soup	1.47±0.54*	0.44±0.19*
Juice	0.88±0.37	0.31±0.15
Tomato-based products	1.17±0.27	0.74±0.40

Table 4.5 Changes in urinary 8-iso-PGF_{2α} levels following tomato-based product consumption in prostate cancer patients[†]

[†]Prostate cancer patients; Values are means ± SEM; n = 7/group or 21 (tomato-based products); No within or between group statistical differences were observed, *P = 0.063; Measured using LC-ESI-MS.

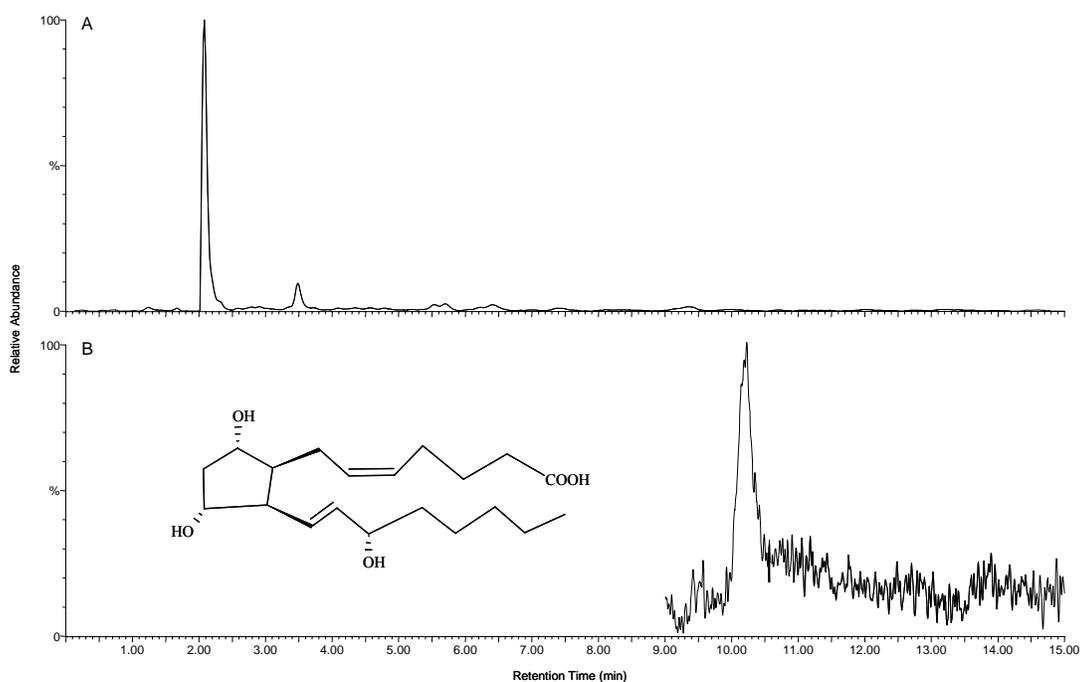


Figure 4.1 Representative chromatograms using a C₈ HPLC system with UV-vis detection of urinary 8-iso-PGF_{2α} (A) or using a C₈ HPLC system with ESI-MS detection of urinary 8-iso-PGF_{2α} (B) in prostate cancer patients prior to the consumption of tomato-based products for 2-4 weeks.

CHAPTER 5

CONCLUSION

As indicated by an overwhelming number of epidemiologic studies, consumption of fruits and vegetables reduces the risk of many chronic diseases. These foods appear to contain nutrients and phytochemicals that protect against or delay the onset of diseases such as cancer through a number of mechanisms, including modulation of oxidative stress. In vitro, animal, and clinical studies have begun to identify definitive compounds that modulate these and other processes related to disease promotion and/or progression. Additional information on the in vivo concentrations and physiological role of individual compounds from these foods will aid in the determination of specific health promoting foods. Our lab has been particularly interested in the beneficial health effects of lycopene-containing tomato-based products and isoflavone-rich soy foods. More specifically, the goals of this research were to obtain a better understanding of tomato and soy phytochemicals in relation to in vivo biodistribution, bioavailability, antioxidant/oxidative environment regulation, and prostate biomarker modulation.

Assessment of healthy individuals and prostate cancer patients in two separate clinical trials allowed for determination of the objectives set forth in this research. These studies show that changes in tomato-based product or soy protein supplement intake can rapidly and significantly modulate plasma and prostate tissue carotenoid concentrations

or urinary and prostate tissue isoflavone levels, respectively. In addition, evaluation of in vivo lycopene isomer profiles demonstrates that unique, yet extremely complex, patterns exist. It also appears that tomato sauce and tomato soup provide a more bioavailable form of lycopene than vegetable juice. Additionally, it is evident that as little as 2-4 weeks of dietary intervention with tomato sauce, tomato soup, or vegetable juice significantly reduces the susceptibility of plasma lipoproteins to oxidation, significantly increases plasma lipid-soluble antioxidant capacity, and decreases urinary F₂-isoprostane levels in healthy participants and in men with prostate cancer. Finally, serum PSA levels in prostate cancer patients can be lowered after a similar 2-4 week period of tomato-based product and soy protein supplement consumption.

Lycopene from tomato-based products and isoflavones from soy appear to be important phytochemicals that play significant roles in reducing oxidative damage and/or slowing the development and progression of prostate cancer. Identification of these compounds in vivo, in addition to their significant increase following intake of tomato-based products or a soy protein supplement, suggest a preferential uptake and requirement of these phytochemicals in biological processes related to the modulation of both oxidative stress and prostate cancer biomarkers. Although lycopene, including the complex array of isomers, and isoflavones may contribute considerably to their health promoting effects, it is the consumption of tomato-based products or soy protein supplement that resulted in the beneficial health effects observed in these studies.

Although this research increases our knowledge on the physiological significance of phytochemical-containing tomato and soy foods, additional studies are required to improve our understanding of these foods, and compounds within, in relation to health

and disease. Future studies on the importance of cis-lycopene isomers, identification of additional or improved oxidative stress biomarkers that contribute to the antioxidant theory of carotenoid phytochemicals, further examination of isoflavones and their metabolites in vivo, and the effect of prolonged consumption of tomato-based products or soy on reducing oxidative damage and prostate carcinogenesis are needed. In addition, because tomato and soy food intake seem to provide unique protective effects, development and consumption of a combined tomato-soy product may increase the beneficial health impacts received from consumption of these foods individually.

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