

Tangerine tomatoes increase total and tetra-cis-lycopene isomer concentrations more than red tomatoes in healthy adult humans

BETTY JANE BURRI^{1,2}, MARY H. CHAPMAN³,
TERRY R. NEIDLINGER¹, JUNG S. SEO⁴ & BETTY K. ISHIDA³

¹Western Human Nutrition Research Center, USDA, ARS, PWA, Davis, California, USA,

²Nutrition Department, University of California, Davis, California, USA, ³Western Regional Research Center, USDA, ARS, PWA, Albany, California, USA, and ⁴Department of Nutrition, Yeungnam University, Gyeongsan, Korea

Abstract

Lycopene, or the foods that contain it, may prevent prostate cancer. Studies suggest that some *cis*-lycopene isomers are more bioavailable than the *trans*-lycopene isomer. We hypothesized that tangerine tomatoes, which predominantly contain the *tetra-cis* isomer, should be a good source of bioavailable lycopene. We fed lunches containing 300 g tangerine or red tomato sauce per day to 21 healthy adults in a double-blind crossover design. We collected blood at baseline and after each treatment and washout period. We measured *tetra-cis*, other *cis*, and *trans* lycopene, as well as other carotenoids, by reversed-phase high-performance liquid chromatography. Both tomato sauces increased lycopene concentrations in blood, but the tangerine tomato sauce caused a greater increase of total and *tetra-cis*-lycopene. The *cis* isomer(s) may also have facilitated absorption of the *trans*-lycopene isomer. Indices of oxidative damage decreased as serum lycopene concentrations increased. Our results suggest that total lycopene concentrations can be increased by substituting *tetra-cis*-lycopene-rich tangerine tomatoes for common red tomatoes in the diet.

Keywords: Lycopene, isomer, tomato, bioavailability, antioxidants

Introduction

People who eat greater than average amounts of carotenoid-rich foods have lower risks of cancer (Ziegler 1991; Pomerleau et al. 2003). However, clinical trials using β-carotene supplements to decrease cancer rates were disappointing (Patrick 2000; Lee and Park 2003). Research now suggests that maximum benefit from β-carotene is provided by lower dosages than those provided in these clinical trials (Lin et al. 1998). A probable reason for this miscalculation was that carotenoids from supplements are more bioavailable than carotenoids from foods (Cohn et al. 2004; Frohlich et al. 2006). Because of the difficulty in translating supplement studies to dietary recommendations, studies using foods are preferable. We report a study where we exchanged tangerine tomatoes for red tomatoes.

Dr Ishida and Dr Burri contributed equally to this study. Correspondence: Betty Jane Burri, PhD, Western Human Nutrition Research Center, United States Department of Agriculture, 430 West Health Sciences Drive, Davis, CA 95616, USA. Tel/fax: 1 530 752 4748. E-mail: betty.burri@ars.usda.gov

ISSN 0963-7486 print/ISSN 1465-3478 online © 2009 Informa UK Ltd
DOI: 10.1080/09637480701782084



Lycopene is the most abundant carotenoid in the blood of people in the United States (Ford 2000; Ford et al. 2002). It is associated with antioxidant status and gap-junction formation (Di Mascio et al. 1989; Hadley et al. 2003; Bertram et al. 1991; Heber and Lu 2002). High lycopene intakes are associated with decreased risks for heart disease and cancer, especially prostate cancer (Rao 2002; Levy et al. 1995; Giovannucci et al. 1995; Etminan et al. 2004). Lycopene is an acyclic carotenoid having 11 conjugated double bonds in all-*trans* isomer and various *cis* configurations. About 90% of total lycopene is the all-*trans* isomer in common red tomatoes, but 90% of the lycopene found in tangerine tomatoes (*Lycoperison esculentum* var. Tangella) is the *tetra-cis* isomer (7Z, 9Z, 7'Z, 9'Z)-lycopene (Zechmeister et al. 1941; Johjima 1993) due to loss of the *tangerine* gene CRTISO (Isaacson et al. 2002).

The isomeric form of lycopene probably does not influence its antioxidant properties. However, it may influence the rate of uptake and maximal amount of lycopene absorbed by the human body. In the ferret, *cis*-lycopene absorption is significantly greater than that of *trans* lycopene (Boileau et al. 1999, 2002). Moreover, although virtually all lycopene in plants is in the *trans* form, a higher percentage of *cis* isomers occurs in human blood (Heber and Lu 2002). Furthermore, humans absorb lycopene from processed tomato products (which contain more *cis* lycopene) more efficiently than from fresh tomato products (Gartner et al. 1997; Cohn et al. 2004; Frohlich et al. 2006; Unlu et al. 2007). Finally, postprandial concentrations of *tetra-cis* lycopene in humans fed a single dose of tangerine tomato sauce increased rapidly in the triacylglycerol-rich lipoprotein fraction of plasma (Unlu et al. 2007). Such evidence suggests that at least some *cis* isomers are more easily absorbed than *trans* lycopene. It then follows that tomatoes rich in these forms of *cis* lycopene would be better food sources of lycopene than *trans*-lycopene-rich tomatoes.

Although the indirect evidence that some *cis*-lycopene isomers are more bioavailable than the *trans* isomer is strong, it is not conclusive because *cis* and *trans* isomers can be inter-converted in the body (Re et al. 2001; Moraru and Lee 2005). The *trans*-isomer might be well absorbed, but converted into *cis* isomers in the body. Furthermore, we know little about the absorption and metabolism of individual *cis* isomers. Thus, there is a need to investigate the absorption and metabolism of specific *cis*-lycopene isomers, and their food sources. We fed controlled diets to healthy human subjects in a randomized, crossover design to determine whether *tetra-cis*-rich tangerine tomatoes had more bioavailable lycopene than *trans*-lycopene-rich red tomatoes.

Materials and methods

Apparatus, reagents and supplies

All solvents used were high-performance liquid chromatography (HPLC) or reagent grade. Reagent-grade chemicals were purchased from Sigma-Aldrich Chemical Company (St Louis, MO, USA), Fisher Scientific (Pittsburgh, PA, USA), Burdick and Jackson Laboratories (Muskegon, MI, USA) and J. T. Baker Chemical Company (Phillipsburg, NJ, USA). β -Carotene (>97% purity) standards were from Sigma-Aldrich. Lycopene for standard solutions was extracted and purified from berries of autumn olive (*Elaeagnus umbellata* Thunberg) plants.

Red tomato sauce was purchased in a local supermarket (Safeway Select tomato sauce lots 3S0-031-1109; Safeway Inc., Pleasanton, CA, USA). Tangerine tomato sauce was prepared in-house at the University of California Pilot Plant under the

supervision of Robert Marquardt by kitchen-batch processing. Details of the method are described elsewhere (Ishida et al. 2007).

Subjects

We enrolled 24 non-smoking healthy adults (12 men and 12 women) from the greater Sacramento area of California for this study. Informed written consent was obtained from each subject using a protocol approved by the Internal Review Board of the University of California Davis. Each subject was given a health questionnaire, a physical examination by a physician, and standard health screening, which included measurements of body composition, blood pressure, cholesterol, triacylglycerols, thyroid function, and serum enzymes associated with health status such as C-reactive protein (CRP) and blood urea nitrogen. No evidence of chronic fat malabsorption, diarrhea, recent significant weight gain or loss, or gastrointestinal surgery was found in any subject. Subjects were not taking any tobacco or prescription drugs during the study. We obtained complete data for 21 subjects (10 men and 11 women). We eliminated the data from one person because of an illness unrelated to the study, and from two people because they ate red tomatoes one day during the tangerine tomato treatment phase of the study. Subject characteristics are presented in Table I.

Diet

We fed controlled lunches containing tomato sauce rich in either *tetra-cis* or *trans* lycopene to these volunteers in a double-blind, randomized, crossover design. Tomato sauces were made into chili in-house in the dietary kitchen of the Western Human

Table I. Demographic and physiologic characteristics of volunteers at baseline ($n=21$).

Characteristic	Mean	Standard error of the mean
Gender ratio (male:female)	1.0:0.91	
Age (years)	35.7	1.5
Height (cm)	171	2.2
Weight (kg)	72.3	3.2
Body mass index	24.3	0.78
Blood pressure—systolic (mmHg)	112	2.2
Blood pressure—diastolic (mmHg)	68	1.6
Heart rate (beats/min)	67	2.4
Total cholesterol (mg/l)	1904	74
HDL cholesterol (mg/l)	485	29
LDL cholesterol (mg/l)	1244	57
Triacylglycerols (mg/l)	874	86
Protein (g/l)	69	0.81
Glucose (g/l)	840	16
Hemoglobin (g/l)	141	3.3
Hematocrit (%)	40.5	0.83
Thyroid-stimulating hormone (μIU)	2.2	0.22
C-reactive protein (mg/l)	2.7	0.69
Retinol (μmol/l)	2.3	0.10
α-Tocopherol (μmol/l)	36	6.9
β-Carotene (μmol/l)	0.21	0.03
Lutein (μmol/l)	0.43	0.06
Lycopene (total, μmol/l)	0.53	0.06

4 B. J. Burri et al.

Nutrition Research Center. The chili contained tomato sauce, kidney beans, oil, and spices. Chili meals were cooked each morning on a stovetop for 45 min (General Electric Profile Dual Oven Electric Range, setting 3; GE Appliances, Louisville, KY, USA) in amber glass-lidded saucepans (Vision, Corning Inc., Corning, NY). Prior to serving, meals were reheated for 3 min in a standard household microwave oven, having a maximum power of 1.58 KW (General Electric Spacemaker). Chili was fed with lettuce, bread, and butter for lunch (served from 11:00 a.m. to 1:00 p.m.). Lunches were eaten under the observation of our dietary staff. Each person ate 300 g sauce per day. The chili lunches contained approximately 42% carbohydrate, 19% protein, and 38% fat with a P:S ratio of 0.6. Total dietary fiber was relatively high, at 10 g per serving.

The major carotenoid in the lunches was lycopene, as expected. The red tomato sauce contained approximately 5 µg β-carotene and 10 µg α-carotene per gram of tomato sauce while the tangerine tomato sauce contained undetectable amounts of these carotenoids. The tangerine tomato sauce lost an unexpected amount of lycopene during processing, and so it contained 35% as much total lycopene as the commercial red sauce (32 µg/g versus 92 µg/g weight). The chili meals were rich sources of lycopene, moderate sources of β-carotene and contained relatively small amounts of other carotenoids (Table II).

Study design

Subjects served as their own controls during this study, each subject consuming both types of tomato sauce (tangerine and common red) in equal amounts but at different times. The study ran for 4 weeks, with 7-day treatments and 7-day washouts. The study design is presented in Table III.

Subjects were randomly assigned to eat the tangerine or red tomato-based chili first. Each subject ate tomato sauce-based chili for 1 week, followed by a low-lycopene

Table II. Carotenoid composition in chili meals.

Sauce	Carotenoid	Concentration (mg/day)	Concentration (mg/treatment)
Tangerine tomato	Tetra-cis lycopene	4.95	34.65
	'Other cis' lycopene	2.46	17.22
	Trans lycopene	2.22	15.54
	Total lycopene	9.63	67.41
	β-Carotene	1.35	9.45
	α-Carotene	0.31	2.17
	β-Cryptoxanthin	0.16	1.12
	Lutein	0.28	1.96
	Total carotenoids	11.72	81.97
Red tomato	Tetra-cis lycopene	0	0
	'Other cis' lycopene	10.35	72.45
	Trans lycopene	17.37	121.6
	Total lycopene	27.72	194.0
	β-Carotene	1.42	9.94
	α-Carotene	0.29	2.03
	β-Cryptoxanthin	0.15	1.05
	Lutein	0.27	1.89
	Total carotenoids	29.85	209.0

Table III. Outline of the experiment.

Week	Treatment (Group 1)	Treatment (Group 2)	Product
1	Tangerine	Common red	Tomato chili (300 g/day)
2	Washout	Washout	No tomato products
3	Common red	Tangerine	Tomato chili (300 g/day)
4	Washout	Washout	No tomato products

40 ml blood was collected on the first day of the study and at the end of weeks 1, 2, 3, and 4.

washout period. Then they ate their second tomato sauce-based chili treatment daily for 1 week, followed by a second washout period. Subjects were given a list of lycopene-rich foods and asked to refrain from eating them for 1 week before the beginning of the study, during the washout periods, and when eating food away from our facility during the study.

Blood collections

Forty milliliters of overnight-fasting blood was collected on study days 1, 8, 15, 22, and 29 for fat-soluble vitamin, carotenoid isomer, oxidative damage, and health status analysis. Blood was collected from the antecubital vein into 10 ml serum tubes via standard vacutainer. The tubes were immediately placed into a covered ice bucket to protect samples from heat and ambient light. Serum was harvested within 3 h by centrifuging for 15 min at $3,000 \times g$ at 4°C and then stored in tightly capped 2-ml plastic cryovials at -70°C until analysis. Samples were thawed at room temperature under plastic sleeve covered fluorescent lights to minimize sample degradation from exposure to ultraviolet light.

Blood chemistries

Complete blood counts, cholesterols, triacylglycerols, CRP, and thyroid-stimulating hormone were measured three times (on study days 1, 8, and 29). Body weights, heights, and blood pressure were measured on standard medical scales and apparatus. Complete blood counts were determined using the CellDyn 3200 (Abbott Laboratories, Abbott Park, IL, USA). Total, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterols, and triacylglycerols were analyzed on a Hitachi 902 Clinical Chemistry Analyzer (Roche Diagnostics, F. Hoffman-La Roche Ltd, Basel, Switzerland). Thyroid-stimulating hormone and CRP were analyzed on an Immulite 2000 (Diagnostics Products Corp., Los Angeles, CA, USA). Analyses were conducted according to manufacturers' recommendations, using their standards and reagents, which were provided by respective manufacturers.

Chromatography

Liquid chromatography of vitamins A and E was run on an Agilent 1100 gradient chromatograph with a binary pump, degasser, refrigerated autosampler, column heater, and diode array detection, as described elsewhere (Burri et al. 2003). Chromatographic analysis was run and interpreted using a Chemstation for LC 3D revision A.08.03 (847) for Agilent Technologies, running on a HP Kayak XM600 computer with Windows NT (Hewlett-Packard GmbH, Chemical Analysis Group,

6 B. J. Burri et al.

Waldbonn, Germany). Chromatography was run on Prodigy 5 mm C18 ODS 3 100 angstrom pore 250×4.6 mm reversed-phase column (Phenomenex, Torrance, CA, USA).

Chromatography of lycopene isomers was run by the methods described by Ishida et al. (2001a,b). Plasma samples were diluted, using an equivalent amount of 2 mM phosphate buffer, pH 7.2, and carotenoids were analyzed by HPLC.

Oxidative damage

Oxidative damage was estimated using two established tests, superoxide dismutase and malondialdehyde as estimated by the thiobarbituric acid reactive substances (TBARS) test (Yagi 1976). Samples were analyzed in the laboratory of Dr Seo in Gyeongsan, Korea. Samples were flown from San Francisco, CA, USA to Seoul, Korea on dry ice, and then kept at -70°C until analysis.

Data analyses

Blood plasma volumes from each subject were estimated from their 'ideal' and 'excess' body weights (Diwadkar-Navsariwala et al. 2003). *Trans*-lycopene HPLC peaks were identified and quantified by comparison with authentic standards. No such standards are available for *cis* isomers. We were able to conclusively identify the *tetra-cis* isomer of lycopene, which has a distinctive absorption spectrum. Several other peaks appeared to be *cis*-lycopene isomers based on their absorption spectra and retention times (Ishida et al. 2001a), but these could not be conclusively identified. We list these peaks as 'other *cis*' lycopene. *Cis* isomers of lycopene were quantified using the all-*trans*-lycopene calibration, as has been done in other studies (Ishida and Chapman 2004; Moraru and Lee 2005; Fröhlich et al. 2006). *Cis*-lycopene isomers, other than *tetra-cis* lycopene, were grouped together for quantification. The molar extinction coefficients for *cis* isomers differ from that of the all-*trans* isomer and are not known for all lycopene isomers. Therefore, total HPLC peak areas are only approximations for the mass of the individual isomers. 'Total' lycopene concentrations were estimated by adding the areas of *trans*-lycopene, *tetra-cis*-lycopene and 'other'-*cis*-lycopene peaks and comparing with the all-*trans*-lycopene standard. Results are presented separately by treatment group.

Simple statistics were calculated for independent and dependent variables with SigmaPlot version 9 (Systat Software Inc., Point Richmond, CA, USA). More complex statistics, such as two-tailed *t*-tests and analysis of variance, were estimated using the Statistical Analysis System (SAS, Cary, NC, USA). Differences between means were tested by Student's *t*-test, with *P*=0.05 as significant. Differences between carotenoid isomers were tested, with or without total, LDL, and HDL cholesterol as covariants, using a mixed procedure with the REML residual variance and Prasad Rao Jeske Kakar Harville fixed effects methods.

Results

Although tangerine tomatoes and common red tomatoes are easy to distinguish by their color, chili made from these tomatoes had very similar taste, texture, and appearance. When compared side by side most volunteers could distinguish between them, but not when they were served separately. The tangerine tomato sauce

produced in-house by kitchen-batch processing had higher moisture content than the purchased common red tomato sauce. Moreover, substantial amounts of lycopene were degraded in the tangerine tomato sauce during processing. Therefore, the red sauce contained approximately three times as much total lycopene as the tangerine sauce.

All subjects remained healthy throughout the study and had normal body weights, body composition indices, plasma cholesterol, triacylglycerols, blood pressure, and hematocrit levels typical of healthy adults in the United States (Table I). Thyroid-stimulating hormone and CRP, an indicator of acute illness and inflammation, were also in the normal range and did not change with time. No baseline differences were noted between subjects who ate red or tangerine tomato sauce first.

The chromatographic conditions described gave good separation for retinol, α -tocopherol, lycopene, lutein, and β -carotene in all samples with good reproducibility; coefficients of variation for standard aliquots ($n = 14$) ranged from 1.7 to 5.1%, being 2.8% for lycopene (data not shown). Vitamin and carotenoid concentrations (Table I) were normal for healthy well-fed adults in the United States (Ford 2000; Ford et al. 2002) and Europe (al-Delaimy et al. 2004). No significant changes in serum vitamin A and vitamin E concentrations were seen between groups or by day.

Serum β -carotene, α -carotene, and lutein concentrations were highly variable in these subjects, but concentrations were consistent with other results reported for well-fed adults, as expected (Ford 2000; Ford et al. 2002; al-Delaimy et al. 2004). No differences were seen between groups or by day for β -carotene, α -carotene, or lutein. Carotenoid concentrations were not different in the men and women who participated in this study at any time, although non-significant trends were seen for men to have lower α -carotene and β -carotene concentrations and women to have higher *tetra-cis*-lycopene concentrations ($P = 0.06$). Changes in lycopene concentrations did not appear to influence other carotenoids.

All subjects had measurable lycopene concentrations in their serum at baseline. Baseline concentrations ranged from 0.24 to 1.59 $\mu\text{mol/l}$ lycopene, with a mean and standard deviation of $0.53 \pm 0.29 \mu\text{mol/l}$, which is consistent with, but somewhat higher than, current data on serum lycopene concentrations in the US population (Ford 2000; Ford et al. 2002; Ganji and Kafai 2005). This is a typical result for our laboratory: our study populations tend to have moderately high serum concentrations and dietary intakes of lycopene. We believe this may reflect a real difference in dietary intake since the Sacramento, CA, USA region is a major producer of tomatoes and tomato products and good quality tomatoes are plentiful. Lycopene concentrations in blood were highly variable in our subjects, with several subjects showing very low lycopene concentrations throughout the study. Feeding either red or tangerine tomato sauce based-chilis increased serum concentrations of lycopene in 20 out of 21 subjects. Changes in lycopene concentrations, corrected for differences in baseline concentrations, are shown in Figures 1–3. *Trans*-lycopene concentrations (Figure 1) increased with common red tomato-based chili ($P = 0.0031$).

More surprisingly, the *trans*-lycopene concentration also increased with the tangerine tomato sauce treatment ($P = 0.012$). Despite the much greater *trans*-lycopene concentration in the red sauce, the red tomato sauce-based chili diet increased serum *trans* lycopene only to a slightly greater extent than the tangerine tomato sauce-based chili diet.

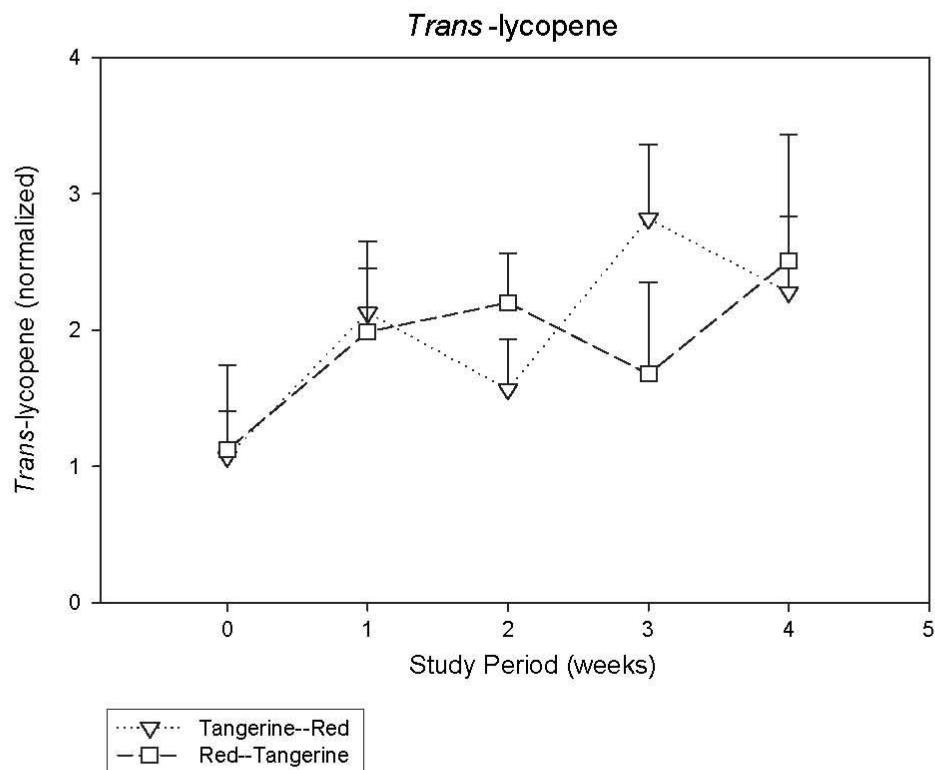


Figure 1. *Trans*-lycopene isomer concentrations in blood after tangerine tomato and common red tomato feedings. Data presented as the mean \pm standard error of the mean, with data normalized to baseline concentrations. (a) ∇ = Group 1, \square = Group 2.

Tetra-cis-lycopene concentrations (Figure 2) increased with the tangerine tomato sauce chili treatment ($P=0.00022$) and to a much lesser extent with the red tomato sauce chili ($P=0.0097$). Not surprisingly, *tetra-cis*-lycopene concentrations were increased more by the tangerine tomato sauce treatment compared with the red sauce treatment ($P=0.049$).

'Other'-*cis*-lycopene concentrations were significantly increased by the red tomato chili treatment ($P=0.0088$). 'Other'-*cis*-lycopene concentrations also appeared to be increased by the tangerine tomato chili treatment, but the trend was not quite significant ($P=0.056$) because of the large variation in serum concentrations caused by all treatments (Figure 3).

Using total cholesterol, LDL cholesterol, or HDL cholesterol as covariants did not change these results. On average, *trans*-lycopene and *tetra-cis*-lycopene concentrations appeared to increase rapidly in the blood and were highest in the blood collection taken on the last day of the treatments (Figures 1 and 2). However, peak concentrations of 'other' *cis*-lycopene isomers were delayed frequently, with *cis*-lycopene concentrations that were higher in blood after the washout period than the treatment period. This was especially common after the red tomato sauce treatment, with 11 subjects showing this delayed effect (Figure 3).

The commercial red tomato sauce was more concentrated and had more total lycopene than the kitchen-batch processed tangerine tomato sauce. When changes in serum lycopene were normalized to the amounts of lycopene contained in the sauces,

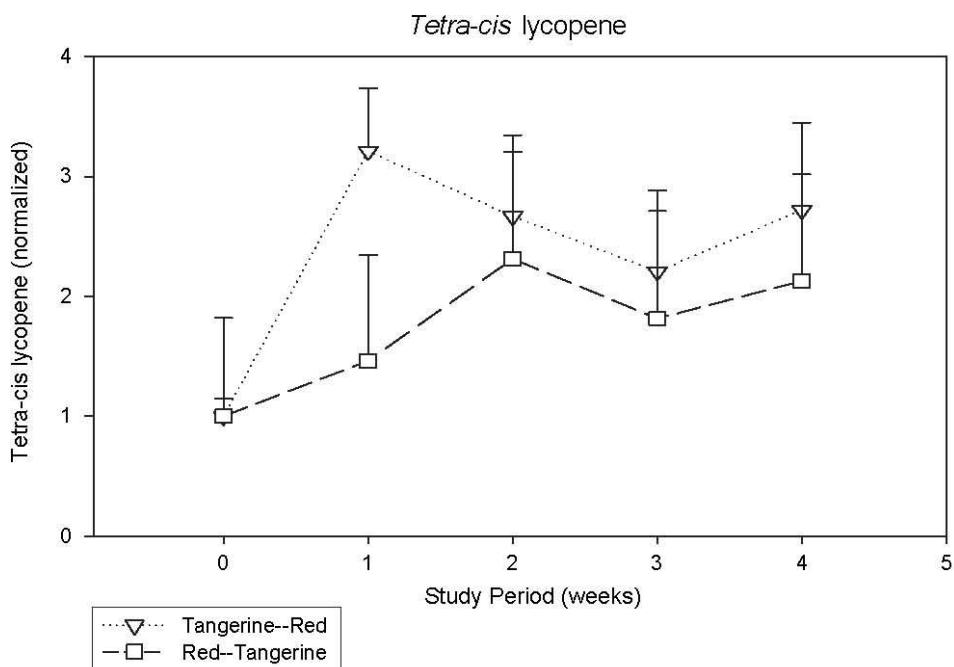


Figure 2. *Tetra-cis*-lycopene concentrations in blood after tangerine tomato and common red tomato feedings. Data presented as the mean \pm standard error of the mean, with data normalized to baseline concentrations. (a) ∇ = Group 1, \square = Group 2.

it became obvious that the *tetra-cis*-lycopene isomer from the tangerine tomato sauce resulted in a greater increase in total serum lycopene than equivalent amounts of the all-*trans* lycopene (Figure 4).

We estimated the total body lycopene in these subjects by multiplying the blood plasma volume by plasma total lycopene concentration. The maximum change in total body lycopene (calculated by subtracting the maximum total body lycopene caused by our treatments from baseline values) varied widely in these subjects, from 0 to 37 mg/treatment. The mean change in total lycopene concentration was 26.5 ± 15.5 mg/treatment when subjects were fed the tangerine-tomato based chili, and was 21.0 ± 15.0 mg/treatment when subjects were fed the red chili.

Superoxide dismutase concentrations were not significantly influenced by changes in lycopene. However, oxidative damage, as estimated by TBARS, decreased after both tomato sauce treatments. TBARS decreased after each tomato intervention, and then reverted to baseline after each washout period (Table IV).

When results from all of the data on red and tangerine tomato sauce treatments were combined into one data-set, the results showed that tangerine tomato sauce treatment was more effective at improving oxidative damage than the red tomato sauce (Figure 5).

Discussion

Although the common red tomato sauce used in our study contained about three times more lycopene as the tangerine tomato sauce, chili prepared from

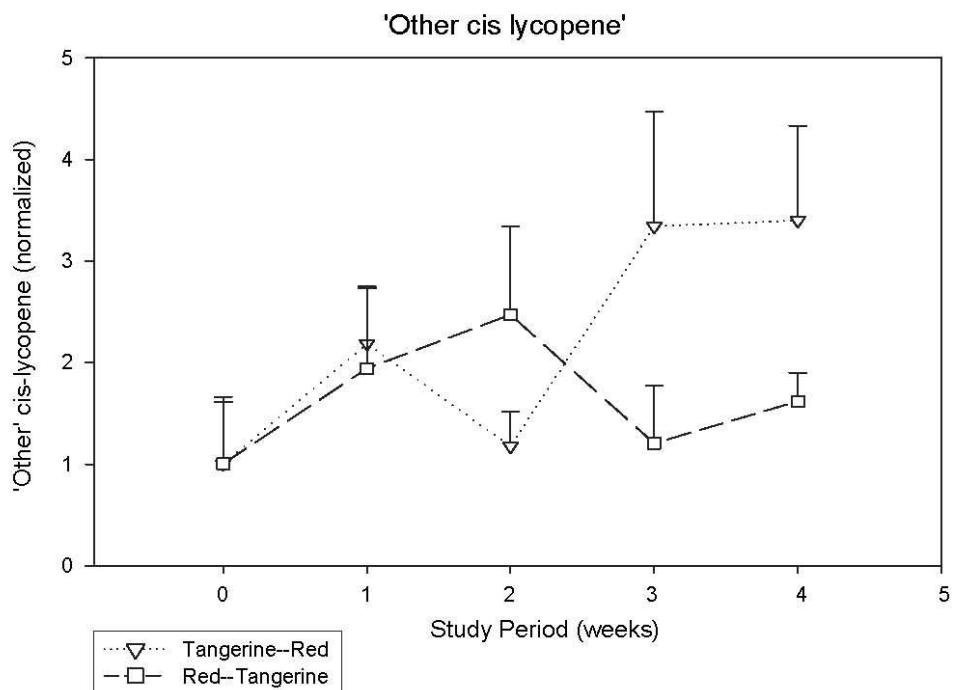


Figure 3. 'Other'-*cis*-lycopene concentrations in blood after tangerine tomato and common red tomato feedings. Data presented as the mean \pm standard error of the mean, with data normalized to baseline concentrations. (a) ∇ = Group 1, \square = Group 2.

the latter increased blood lycopene concentrations more than the red tomato-based chili (Figures 1–4). Therefore, our results show that *tetra-cis* lycopene from tangerine tomatoes is absorbed more efficiently than *trans* lycopene from common red tomatoes.

These data show clearly that feeding *tetra-cis* lycopene increased concentrations of *trans* lycopene in the blood (Figure 1). Since isomerization of *tetra-cis* lycopene to *trans* lycopene appears complex and difficult, our results suggest that *tetra-cis* lycopene in the chili facilitated the absorption of *trans* lycopene to increase the total lycopene absorbed. Thus, eating high *cis*-lycopene-isomer-containing food increased the efficiency of total lycopene absorption and therefore increased the health benefits of this meal. Feeding *trans*-lycopene-rich red tomatoes did not result in an increase in *tetra-cis*-lycopene concentrations—a complex and unlikely isomerization (Figure 2) but did increase the 'other'-*cis*-lycopene concentrations (Figure 3). Interestingly, 'other'-*cis*-lycopene concentrations in 11 of our subjects peaked in the blood during the washout period following the *trans* lycopene intervention, so that the highest mean 'other'-*cis*-lycopene concentrations were measured after the washout periods (Figure 4). Thus, *trans* lycopene appears to isomerize to *cis*-lycopene isomers in the blood after being absorbed. These results suggest that there are two reasons that *cis* isomers are more common in human blood than they are in the diet. First, some *cis* isomers, in particular the *tetra-cis* isomer, are better absorbed from food. Second, *trans* lycopene from food is isomerized to *cis* isomers after being absorbed.

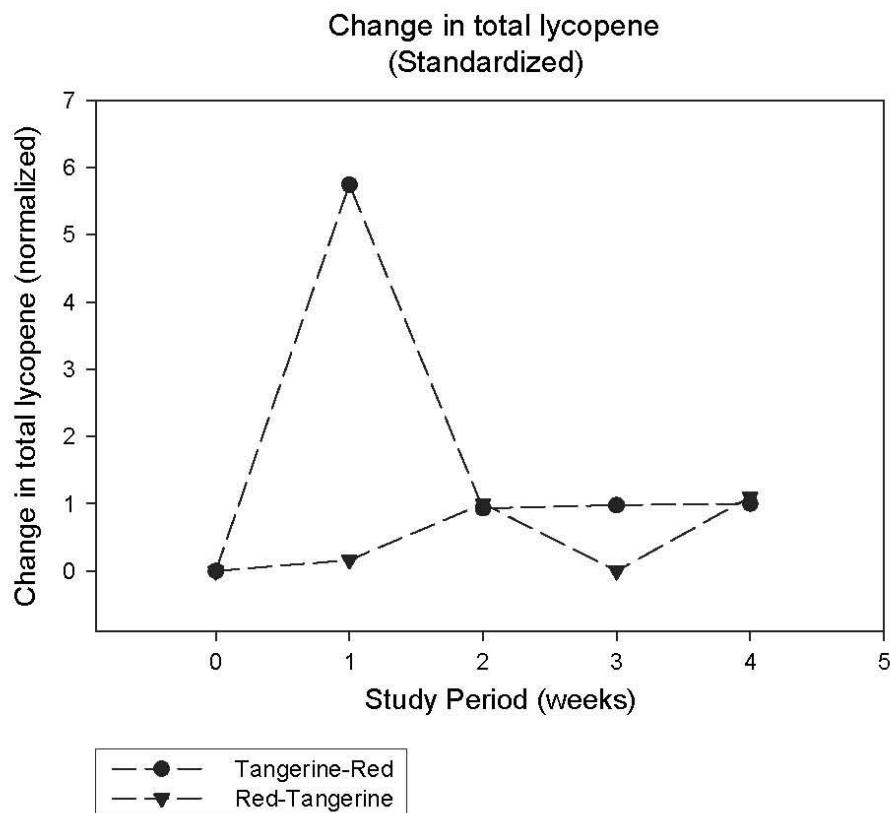


Figure 4. Change in the percentage of total serum lycopene concentration standardized to the amount of total lycopene in the chili diets. Total serum lycopene concentrations were estimated by adding areas of *trans*-lycopene, *tetra-cis*-lycopene and ‘other’-*cis*-lycopene HPLC peaks and then comparing with authentic *trans*-lycopene standards. (a) ▼ = Group 1, □ = Group 2.

Classic research suggested that carotenoids were absorbed passively (Hollander and Ruble 1978), but more recent work suggests that they are also absorbed by active transport mechanisms that are saturated at relatively low dosages (Clifford and Burri 2004; During et al. 2005). Results with graded doses of lycopene-rich foods suggest that greater percentages of lycopene are absorbed at lower dosages (Edwards et al.

Table IV. Effect of tangerine tomato and red tomato based-chili treatments on TBARS concentrations ($n=21$).

Study period	Treatment TBARS ($\mu\text{mol/l}$)	
	Red-tangerine	Tangerine-red
Baseline	$3.10 \pm 0.26^{\text{a}}$	$3.12 \pm 0.14^{\text{a,b}}$
First treatment	$2.82 \pm 0.25^{\text{a,b}}$	$3.04 \pm 0.11^{\text{a,b}}$
Washout	$2.57 \pm 0.33^{\text{a,b}}$	$3.31 \pm 0.13^{\text{a}}$
Second treatment	$2.12 \pm 0.25^{\text{b}}$	$2.82 \pm 0.12^{\text{b}}$
Washout	$2.40 \pm 0.25^{\text{a,b}}$	$3.17 \pm 0.12^{\text{a,b}}$

Data presented as the mean \pm standard error of the mean. Different superscript letters in the same row denote statistically significant differences, $P=0.5$.

12 B. J. Burri et al.

2003; Cohn et al. 2004; Novotny 2005). A study of lycopene absorption in healthy young men (Diwadkar-Navsariwala et al. 2003) reported that only 20% of their subjects absorbed more than 6 mg lycopene/day and that the mean lycopene absorbed was 4.7 ± 0.6 mg/day irrespective of the dose given. Therefore, it was possible that we observed higher absorption for *tetra-cis* lycopene because our preparation of tangerine tomatoes contained less lycopene than our preparation of red tomato sauce. Our results are difficult to compare because that study (Diwadkar-Navsariwala et al. 2003) measured total lycopene concentrations derived from single lycopene doses fed to men, while we measured the increase in total lycopene caused by feeding five lycopene-rich meals to men and women. However, it is noteworthy that the apparent change in total lycopene caused by red tomatoes in our study was 21 mg, while the change in total lycopene caused by tangerine tomatoes was greater, at 26.5 mg. Moreover, the data show that, when the tangerine tomato sauce was fed first (Group 1), the plasma concentration of *tetra-cis* lycopene remained high after the 1-week washout period. When this was then followed by a week of feeding red tomato sauce, a more rapid increase in *trans* lycopene occurred, compared with levels attained when red tomato sauce was fed first (Group 2). Plasma levels of *trans* lycopene after the red sauce feeding attained the highest level measured in our study.

These data suggest that the *tetra-cis* lycopene remaining in the plasma may have facilitated absorption of *trans* lycopene subsequently ingested. Therefore, our data suggest that *tetra-cis* lycopene is both more bioavailable than and facilitates the absorption of the *trans* isomer in similar foods. The data on 'other' *cis* isomers of lycopene indicate conversion of *trans* to *cis* lycopene after absorption. Thus, elevated concentrations of *tetra-cis* lycopene resulting from the first red tomato sauce feeding (Figure 2), in turn resulting in large increases in the concentration of other *cis*-lycopene isomers in plasma after feeding tangerine tomato sauce in Group 2 (Figure 3). The increase in other *cis*-lycopene isomers parallels the increase in *trans* lycopene and occurs to an even greater extent. This suggests that conversion of *trans*-lycopene to *cis*-lycopene isomers after absorption is very rapid—more evidence that *cis*-lycopene isomers are the preferred form in human plasma. It also suggests that, if lycopene is absorbed primarily by an active transport mechanism, *tetra-cis*-lycopene and *trans*-lycopene isomers might have different affinities because of their differing geometries.

Superoxide dismutase concentrations did not change in this study, probably because this test is carried out in erythrocytes, which have a long lifespan compared with our short-term intervention. However, oxidative damage, as estimated by malondialdehyde concentrations estimated by TBARS, decreased after both tomato sauce treatments. When results from the red and tangerine tomato sauce treatments were combined, the tangerine tomato sauce treatment was more effective at decreasing oxidative damage (Figure 5). Thus, the decrease in oxidative damage paralleled increases in serum lycopene and not the amount of lycopene eaten in the diet.

Volunteers in this study were typical healthy adults. Cholesterols, triacylglycerols, protein, hematocrit levels, and body composition indices were all within the normal range for the US adult population (Table I). These tests suggest that volunteers did not have illnesses, fat mal-absorption, or intestinal disorders that might have influenced lycopene-isomer absorption during this study. Several of these health indices have been shown to influence carotenoid status or metabolism, with especially

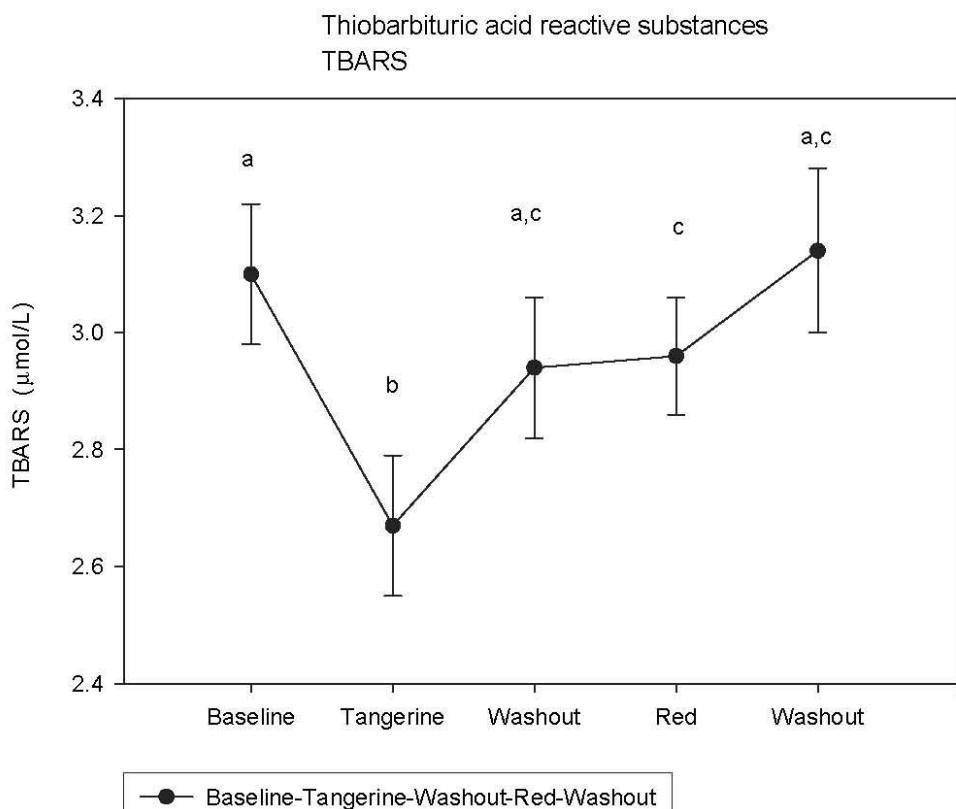


Figure 5. Effect of tomato sauce interventions on malondialdehyde concentrations as estimated by TBARS. Interventions that were significantly different at $P < 0.05$ have different superscripts.

strong evidence for CRP (Ford et al. 2003) and cholesterol (Gross et al. 2003). However, these had no effect on our results, presumably because they did not vary greatly within our study population. The age, sex, and body composition of these subjects did not appear to influence lycopene metabolism, which is consistent with results of other studies in healthy adults (Edwards et al. 2003; Cohn et al. 2004).

Previous studies show that lycopene from tomato juice or fresh tomato is absorbed less well than lycopene from tomato sauce (Boileau et al. 2002; Tyssandier et al. 2002; Allen et al. 2003; Cohn et al. 2004; Frohlich et al. 2006). Including fat with carotenoids increases absorption (Boileau et al. 2002; Brown et al. 2004; Tang et al. 2005). Our chili meals were provided with bread, butter, and salad with full fat dressing to provide approximately 38% fat in the diet, with a P:S ratio of 0.6. Thus, our meals have the relatively high fat content of a typical western diet. Our results therefore can be extended to other western-style diets, but probably not to diets that are very low in fat content.

The meals had relatively high fiber contents, and concentrations of phytate of 0.22 and 0.25%, respectively, in the tangerine and red tomato-based chili meals. Fiber decreased β -carotene utilization by female human subjects (Rock and Swendseid 1992; Riedl et al. 1999). The presence of relatively high fiber and phytate, resulting from simultaneous ingestion of the beans included in our meals, may account for the

14 B. J. Burri et al.

somewhat small changes of lycopene in these subjects, but it is probable that any impact of fiber and phytate would be similar for all isomers of lycopene. One might expect greater lycopene absorption in a meal in which, for example, pasta is substituted for beans.

Feeding *tetra-cis*-rich lycopene tomato sauce to our volunteers did not change any test of general health or cholesterol status. Thus, no signs of potentially deleterious effects occurred in these subjects when they ate tangerine tomato-based chili on our study. The major effect of substituting tangerine tomato sauce for red tomato sauce in the diet of these subjects is that the subjects absorbed proportionately more lycopene when fed tangerine tomato sauce-based chili. Our data show that tangerine tomatoes are a better source of bioavailable lycopene, as expected. They suggest that lycopene absorption can be increased by substituting tangerine tomatoes for common red tomatoes in the diet. The apparent added benefit of consuming tangerine tomatoes is that the *cis* isomers present might facilitate the absorption of accompanying *trans* lycopene. This study demonstrates an advantage of using fruits that have specific compositional differences in human nutrition research, instead of pure supplements and placebos.

Acknowledgements

The authors thank Robert Marquardt for his expert assistance in preparing tangerine tomato sauce. They thank Ellen Bonnel, Mary Rivera, Sara Stoffel and the WHNRC Ragle staff for their assistance with recruiting and feeding subject on this study. The authors also thank Dr Bruce Mackey, Pacific West Area, USDA for statistical analyses. Autumn olive (*Elaeagnus umbellata* Thunberg) plants were a gift from Beverly A. Clevidence, Beltsville Human Nutrition Research Center, USDA.

References

- al-Delaimy WK, van Kappel AL, Ferrari P, Sliman V, Steghens JP, Bingham S, Johansson I, Wallstrom P, Overvad K, Tjonneland A, et al. 2004. Plasma levels of six carotenoids in nine European countries: Report from the European Prospective Investigation into Cancer and Nutrition (EPIC). *Public Health Nutr* 7:713–722.
- Allen CM, Schwartz SJ, Craft NE, Giovannucci EC, De Groff VL, Clinton SK. 2003. Changes in plasma and oral mucosal lycopene isomer concentrations in healthy adults consuming standard servings of processed tomato products. *Nutr Cancer* 47:48–56.
- Bertram JS, Pung A, Churley M. 1991. Diverse carotenoids protect against chemically induced neoplastic transformation. *Carcinogenesis* 12:671–678.
- Boileau AC, Merchen NR, Wasson K, Atkinson CA, Erdman SW Jr. 1999. *Cis*-lycopene is more bioavailable than *trans*-lycopene in vitro and in vivo in lymph-cannulated ferrets. *J Nutr* 129:1176–1181.
- Boileau TWM, Boileau A, Erdman JW Jr. 2002. Bioavailability of all-*trans* and *cis*-isomers of lycopene. *Exp Biol Med* 227:914–919.
- Brown MJ, Ferruzzi MG, Nguyen ML, Cooper DA, Eldridge AL, Schwartz SJ, White WS. 2004. Carotenoid bioavailability is higher from salads ingested with full-fat than with fat-reduced salad dressings as measured with electrochemical detection. *Am J Clin Nutr* 80:396–403.
- Burri BJ, Nelson MD, Neidlinger TR. 2003. Measurement of the major isoforms of vitamins A and E and carotenoids in the blood of people with spinal cord injuries. *J Chromatogr A* 987:359–366.
- Clifford AJ, Burri BJ. 2004. Carotenoid and retinoid metabolism: insights from isotope studies. *Arch Biochem Biophys* 430:110–119.
- Cohn W, Thurman P, Tenter U, Aebischer C, Schierle J, Schalch W. 2004. Comparative multiple dose plasma kinetics of lycopene administered in tomato juice, tomato soup or lycopene tablets. *Eur J Nutr* 43:304–312.

- Di Mascio P, Kaiser S, Sies H. 1989. Lycopene as the most efficient biological carotenoid singlet oxygen quencher. *Arch Biochem Biophys* 274:532–538.
- Diwadkar-Navsariwala V, Novotny JA, Gustin DM, Sosman JA, Rodvold KA, Crowell JA, Stacenicz-Sapuntzakis M, Bowen PE. 2003. A physiological pharmacokinetic model describing the disposition of lycopene in healthy men. *J Lipid Res* 44:1927–1939.
- During A, Dawson HD, Harrison EH. 2005. Carotenoid transport is decreased and expression of the lipid transporters SR-BI, NPC1L1, and ABCA1 is downregulated in Caco-2 cells treated with ezetimibe. *J Nutr* 135:2305–2312.
- Edwards AJ, Vinyard BT, Wiley ER, Brown ED, Collins JK, Perkins-Veazie P, Baker RA, Clevidence BA. 2003. Consumption of watermelon juice increases plasma concentrations of lycopene and β-carotene in humans. *J Nutr* 133:1043–1050.
- Etminan M, Takkouche B, Caamano-Isorna F. 2004. The role of tomato products and lycopene in the prevention of prostate cancer: A meta-analysis of observational studies. *Cancer Epidemiol Biomark Prevent* 13:340–345.
- Ford ES. 2000. Variation in serum carotenoid concentrations among United States adults by ethnicity and sex. *Ethn Dis* 10:208–217.
- Ford ES, Gillespie C, Ballew C, Sowell A, Mannino DM. 2002. Serum carotenoid concentrations in US children and adolescents. *Am J Clin Nutr* 76:818–827.
- Ford ES, Liu S, Mannino DM, Smith SJ. 2003. C-reactive protein concentration and concentrations of blood vitamins, carotenoids, and selenium among United States adults. *Eur J Clin Nutr* 57:1157–1163.
- Frohlich K, Kaufmann K, Bitsch R, Bohm V. 2006. Effects of ingestion of tomatoes, tomato juice and tomato puree on contents of lycopene isomers, tocopherols and ascorbic acid in human plasma as well as on lycopene isomer pattern. *Br J Nutr* 95:734–741.
- Ganji V, Kafai MR. 2005. Third National Health and Nutrition Examination Survey, 1998–1994. Population determinants of serum lycopene concentrations in the United States: Data from the Third National Health and Nutrition Examination Survey, 1988–1994. *J Nutr* 135:567–572.
- Gartner C, Stahl W, Sies H. 1997. Lycopene is more available from tomato paste than from fresh tomatoes. *Am J Clin Nutr* 66:116–122.
- Giovannucci E, Ascherio A, Rimm EB, Stampfer MJ, Colditz GA, Willett WC. 1995. Intake of carotenoids and retinol in relation to risk of prostate cancer. *J Natl Cancer Inst* 87:1767–1776.
- Gross M, Yu X, Hannan P, Prouty C, Jacobs DR Jr. 2003. Lipid standardization of serum fat-soluble antioxidant concentrations: The YALTA study. *Am J Clin Nutr* 77:458–466.
- Hadley CW, Clinton SK, Schwartz SJ. 2003. The consumption of processed tomato products enhances plasma lycopene concentrations in association with reduced lipoprotein sensitivity to oxidative damage. *J Nutr* 133:727–732.
- Heber D, Lu QY. 2002. Overview of mechanisms of action of lycopene. *Exp Biol Med (Maywood)* 227:920–923.
- Hollander D, Ruble PE Jr. 1978. Beta-carotene intestinal absorption: Bile, fatty acid, pH, and flow rate effects on transport. *Am J Physiol* 235:E686–E691.
- Isaacson T, Ronen G, Zamir D, Hirschberg J. 2002. Cloning of *tangerine* from tomato reveals a carotenoid isomerase essential for the production of β-carotene and xanthophylls in plants. *Plant Cell* 14:333–342.
- Ishida BK, Chapman MH. 2004. A comparison of carotenoid content and total antioxidant activity in catsup from several commercial sources in the United States. *J Agric Food Chem* 52:8017–8020.
- Ishida BK, Ma J, Chan B. 2001a. A simple, rapid method for HPLC analysis of lycopene isomers. *Phytochem Anal* 12:194–198.
- Ishida BK, Ma JC, Chan BG, Bartley GE, Grossman JN. 2001b. A modified method for simple, rapid HPLC analysis of lycopene isomers. *Acta Hort* 542:235–242.
- Ishida BK, Roberts JS, Chapman MH, Burri BJ. 2007. Processing Tangerine tomatoes. Effects on lycopene-isomer concentrations and profile. *J. Food Sci* 72:C307–C312.
- Johjima T. 1993. Determination of *cis* and *trans* carotenes of tangerine and yellowish tangerine tomatoes by micro-thin-layer chromatography. *J Jpn Soc Hort Sci* 62:567–574.
- Lee BM, Park KK. 2003. Beneficial and adverse effects of chemopreventive agents. *Mutat Res* 523:265–278.
- Levy J, Bosin E, Feldman B, Giat Y, Miinster A, Danilenko M, Sharoni Y. 1995. Lycopene is a more potent inhibitor of human cancer cell proliferation than either alpha-carotene or beta-carotene. *Nutr Cancer* 24:257–266.
- Lin YM, Burri BJ, Neidlinger TR, Muller HG, Dueker SR, Clifford AJ. 1998. Estimating the concentration of beta-carotene required for maximal protection of LDL in women. *Am J Clin Nutr* 67:837–845.

- Moraru C, Lee TC. 2005. Kinetic studies of lycopene isomerization in a tributyrin model system at gastric pH. *J Agric Food Chem* 53:8997–9004.
- Novotny JA. 2005. What can pharmacokinetic models tell us about the disposition of lycopene and the potential role of lycopene in cancer prevention? *J Nutr* 135:2048S–2049S.
- Patrick L. 2000. Beta-carotene: The controversy continues. *Altern Med Rev* 5:530–545.
- Pomerleau J, McKee M, Knai C. 2003. The burden of disease attributable to nutrition in Europe. *Pub Health Nutr* 6:423–424.
- Rao AV. 2002. Lycopene, tomatoes, and the prevention of coronary heart disease. *Exp Biol Med (Maywood)* 227:908–913.
- Re R, Fraser PD, Long M, Bramley PM, Rice-Evas C. 2001. Isomerization of lycopene in the gastric milieu. *Biochem Biophys Res Commun* 281:576–581.
- Riedl R, Linseisen J, Hoffmann J, Wolfram G. 1999. Some dietary fibers reduce the absorption of carotenoids in women. *J Nutr* 129:2170–2176.
- Rock CR, Swendseid MA. 1992. Plasma β -carotene response in humans after meals supplemented with dietary pectin. *Am J Clin Nutr* 55:96–99.
- Tang G, Ferreira AL, Grusak MA, Qin J, Dolnikowski GG, Russell RM, Krinsky NI. 2005. Bioavailability of synthetic and biosynthetic deuterated lycopene in humans. *J Nutr Biochem* 16:229–235.
- Tyssandier V, Cardinault N, Caris-Veyrat C, Amiot MJ, Grolier P, Bouteloup C, Azais-Braesco V, Borel P. 2002. Vegetable-borne lutein, lycopene, and beta-carotene compete for incorporation into chylomicrons, with no adverse effect on the medium-term (3-wk) plasma status of carotenoids in humans. *Am J Clin Nutr* 75:526–534.
- Unlu NZ, Bohn T, Francis D, Clinton SK, Schwartz SJ. 2007. Carotenoid absorption in humans consuming tomato sauces obtained from Tangerine or high- β -carotene varieties of tomatoes. *J Agric Food Chem* 55:1597–1603.
- Yagi K. 1976. A simple fluorometric assay for lipoperoxide in blood plasma. *Biochem Med* 15:212–216.
- Zechmeister L, LeRosen AL, Went FW, Pauling L. 1941. Prolycopene, a naturally occurring stereoisomer of lycopene. *Proc Natl Acad Sci USA* 2:468–471.
- Ziegler RG. 1991. Vegetables, fruits, and carotenoids and the risk of cancer. *Am J Clin Nutr* 53:S251–S259.

This paper was first published online on iFirst on 9 April 2008.