Cis-Lycopene Is More Bioavailable Than *Trans*-Lycopene In Vitro and In Vivo in Lymph-Cannulated Ferrets[1]

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ABSTRACT Lycopene is the predominant carotenoid in tomatoes and tomato-based foods and is also a predominant carotenoid in human serum and tissues. Intake of lycopene-rich foods was recently associated with decreased risk for several chronic diseases. The observation that serum and tissue lycopene is more than 50% *cis*-lycopene, whereas tomatoes and tomato-based foods contain mainly all-*trans*-lycopene, has led to the hypothesis that *cis*-isomers of lycopene are more bioavailable. We tested this hypothesis both in vitro (study 1) and in vivo (study 2). In study 1, bile acid micelles containing crystalline lycopene were prepared. The crystalline lycopene used for these analyses was 54.4% *cis*-lycopene. The optically clear micelle preparation contained 75.9% *cis*-lycopene in repeated analyses . In study 2, mesenteric lymph duct cannulated ferrets were used to study the in vivo absorption of lycopene from Lycored™ (an ethyl acetate extract of tomatoes containing 5% lycopene by weight; of which 91% was all-*trans* lycopene). Before being anesthetized, male ferrets ($n = 7$) were dosed orally with 40 mg lycopene per kg body weight in soybean oil. Lymph secretions were collected, on ice, for 2 h. The residual stomach and small intestinal contents, mucosa lining, lymph secretion and serum were analyzed by HPLC. Whereas the dose, stomach and intestinal contents contained 6.2–17.5% *cis*-lycopene, the mesenteric lymph secretions contained significantly more, 77.4%, *cis*-lycopene ($P < 0.01$). These studies demonstrate that in ferrets, *cis*-isomers of lycopene are more bioavailable than *trans*-lycopene probably because *cis*-isomers are more soluble in bile acid micelles and may be preferentially incorporated into chylomicrons. J. Nutr. 129: 1176–1181, 1999.

KEY WORDS: • *absorption* • *ferrets* • *lycopene* • *micelles*

Lycopene, the predominant carotenoid in tomatoes, is among the major carotenoids in human serum (Khachik et al. 1995 and 1997, Parker 1989, Stahl et al. 1992), liver (Kaplan et al. 1990, Schmitz et al. 1991), testes (Kaplan et al. 1990, Stahl et al. 1992), and the prostate (Clinton et al. 1994 and 1996). Recent interest in lycopene and its potential cancer preventing properties began when Giovannucci et al. (1995) demonstrated an inverse relationship between dietary intake of lycopene and prostate cancer incidence. More recently, lycopene concentrations in adipose were inversely associated with risk for myocardial infarction (Kohlmeier et al. 1997). *Cis*-isomers of lycopene make up >50% of the total lycopene in human serum and tissues (Clinton et al. 1996, Stahl et al. 1992). This is in contrast to the food sources where they originate; in tomatoes and tomato-based food products, all-*trans* lycopene comprises 79–91% of total lycopene (Clinton et al. 1996).

Serum and tissue profiles for β-carotene and its isomers also demonstrate a contrasting pattern. While 9-*cis* β-carotene is present in human tissues and in heat-processed foods (Chandler and Schwartz 1987), it is not present in human serum to any appreciable extent (Stahl et al. 1992 and 1995, Stahl and Sies 1994). The recent demonstration that 9-*cis* β-carotene isomerizes to all-*trans* β-carotene during absorption in humans has provided some insight into this observation (You et al. 1996).

It was suggested that *cis*-isomers of lycopene may be better absorbed than their all-*trans* parent structure (Britton 1995, Stahl and Sies 1992). This may be the result of greater solubility of *cis*-isomers in mixed micelles and a lower tendency of *cis*-isomers to aggregate. The objective of the current work was to explore the mechanisms by which *cis*-isomers may be better absorbed. Lymph-cannulated ferrets were used previously to study the intestinal absorption of β-carotene (Wang et al. 1992 and 1993). We present both in vitro data (*cis*-isomer composition of micelles, study 1) and in vivo data (lymph-cannulated ferrets, study 2) concerning the micellar solubility and intestinal absorption of *cis*-isomers of lycopene.

The objective of the first study was to evaluate the solubility of crystalline lycopene isomers in an in vitro bile acid micelle preparation. The isomeric composition (*cis*-lycopene vs. *trans*-lycopene) of the micelle preparation and the crystalline lycopene standard was evaluated. The possibility that the preparation procedures (including sonication) could be causing isomerization of *trans*-lycopene to *cis*-lycopene was also addressed.

The objective of the second study was to monitor the

[1] Supported by NRI-USDA program agreement #95–37206–1685.
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Manuscript received 17 December 1998. Initial review completed 21 January 1999. Revision accepted 5 March 1999.

isomeric composition of lycopene during digestion and intestinal absorption in lymph-cannulated ferrets.

MATERIALS AND METHODS

Study 1. Bile acid micelles containing lycopene were prepared using crystalline lycopene that had been purified by HPLC from Lycored™ (Makhteshim Chemical Works, Beer-Sheva, Israel). Lycored™, an ethyl acetate extract from tomatoes that contains ~5 g lycopene/100 g; 3 g β-carotene and carotenoid oxidation products/100 g; 72 g fatty acids/100 g as glycerides; and 20 g phospholipids, sterols, and unknown compounds/100 g (Vaida et al. 1996), was saponified with 500 g KOH/L in distilled H_2O at 70°C for 30 min. Following hexane extraction, lycopene was purified by using a 250 mm x 10 mm YMC C30 column (YMC., Wilmington, NC) with a mobile phase of 38% methyl-tert butyl ether (MTBE[3], Fisher Scientific, Pittsburgh, PA) in methanol (Fisher Scientific), a flow rate of 3.5 mL/min, and detection at 470 nm. A Rainin Dynamax™ Gradient HPLC system (Varian, Walnut Creek, CA) was used for both lycopene purification and all other quantitative HPLC analyses described in this paper. Lycopene was collected as it eluted from the column, the solvent removed under argon, and suspended in hexane (Fisher Scientific) containing 0.1 g BHT/L (Sigma Chemical, St. Louis, MO). The resulting lycopene standard was composed of 54.4% *cis*-lycopene, representing the equilibrium distribution of lycopene isomers reported by Nguyen and Schwartz (1998).

The following samples were prepared with each having a total final volume of 300 μL: lycopene standard alone (suspended in hexane/BHT) in a disposable glass test tube; lycopene standard + an oleic acid standard, suspended in CH_2Cl_2 (Sigma Chemical); lycopene standard + mono-oleate standard, suspended in CH_2Cl_2 (Sigma Chemical); lycopene standard + both oleic acid and mono-oleate standards; lycopene standard (hexane evaporated to dryness) + 12 mmol Na-taurocholate/L (Sigma Chemical), in the buffer containing 100 mmol mannitol/L, 5 mmol $CaCl_2$/L, 0.1 mmol $MgSO_4$/L, 100 mmol NaCl/L, 25 mmol KCl/L, 10 mmol N-tris[hydroxymethyl]-methyl-2-aminoethanesulfonic acid/L, (pH 7.2) used for making micelles. The samples were flushed with argon, and the test tubes capped with Parafilm (American National Can, Neenah, WI). The samples were sonicated for 60 min in a sonicating water bath (Fisher Scientific). At the same time, micelles containing lycopene were prepared using 12 mmol Na-taurocholate/L, 1 mmol mono-oleate/L, and 1 mmol oleic acid/L as previously described (Moore et al. 1996) with one modification. The procedures for incorporating lycopene into a micelle included 60 min of sonication. These preparation procedures produce micelles that were optically clear for up to 4 h.

The lycopene isomer profile of each test incubation and the micelle preparation was evaluated immediately following the 60-min sonication procedure by using the HPLC method of Yeum et. al. (1996). All incubations were dried (to remove solvents present during sonication) and reconstituted in hexane containing 0.1 g BHT/L before HPLC analysis. The micelle preparation was extracted with 4 mL hexane (0.1 g BHT/L) immediately before HPLC analysis. A Waters (Waters, Milford, MA) 991 photodiode array detector was used to verify *trans*- and *cis*-lycopene. This system included a Tracor (Finnegan Trametrics, Austin, TX) 995 isocratic HPLC pump, a 150 mm x 4.6 mm YMC C30, 3-μm HPLC column, and a mobile phase of 38% MTBE in methanol. All incubations were performed in triplicate on different days.

Study 2. *Animals.* Seven male ferrets (neutered and descented, Marshall Farms, New York, NY) with a mean weight of 916 g at the time of surgery were housed individually at the Edward R. Madigan Laboratory Animal Care Facility at the University of Illinois. Ferrets were allowed free access to both water and Purina Cat Chow® (Ralston Purina Company, St. Louis, MO) and socialized for at least 30 min/d. All procedures involving the use of animals were approved by the Laboratory Animal Care and Use Committee at the University of Illinois.

[3] Abbreviations used: MTBE, methyl-tert butyl ether; PLSD, protected least squares difference.

Surgical procedures. Food was withheld for 4 h before administration of a 2–3—mL dose of soybean oil containing Lycored™ (to provide 40 mg lycopene/kg body weight). Within 45 min, ketamine/acepromazine (30 mg/kg · 0.3 mg/kg^{-1}) was administered subcutaneously to allow preparation of the surgical site and induction of halothane anesthesia. Anesthesia was begun within 45 min administration of oral dose because the entire gastrointestinal transit time in ferrets is quite rapid (3–4 h) (Bernard et al. 1984). The surgical procedures for mesenteric lymph duct catheterization were previously described (Wang et al. 1992). These procedures were followed with modifications. Halothane (0.8–1.5% in oxygen) was used to maintain surgical anesthesia. The mesenteric lymph duct was catheterized using a 1.27 mm outside diameter x 1.02 mm inside diameter Micro-Renathane® tube (Braintree Scientific, Braintree, MA) and secured with a small amount of surgical glue (Nexaband, Veterinary Products Laboratories, Phoenix, AZ). Just before cannulation, the tubing was rinsed with heparin/saline and threaded with 0.53 mm diameter fishing line (Berkley Outdoor, Spirit Lake, IA). The fishing line was removed from the cannula lumen before securing the cannula with surgical glue. Lymph flow was collected by gravity for 2 h into a glass test tube on ice containing 150 μL of 150 mmol EDTA/L to prevent clotting. The tubing and surgical site was covered with a large sheet of foil to prevent light-induced isomerization of lycopene during lymph collection. Estimated fluid losses were replaced intravenously with sterile, warmed 25 g dextrose/L/4.5 g NaCl/L (Baxter Healthcare, Deerfield, IL). Deltaphase™ isothermal pads (Braintree Scientific) were used to maintain body temperature during the procedure. At the conclusion of the collection period, ferrets were killed by severing the caudal vena cava after removing a 20 mL sample of blood while under deep halothane anesthesia. A sample of residual stomach and small intestine contents was removed for analysis. A 10-cm section of proximal intestine was removed, sliced longitudinally, and flushed with 1 L of ice-cold saline before removing the mucosa for analysis. Mucosal cells were collected by scraping the intestine with a glass slide.

Sample analyses. The lycopene dose, stomach contents, small intestinal contents, lymph and serum were extracted in 0.1 g ethanol/L BHT with 9 mL hexane without saponification. Echinenone (generous gift from Hoffmann LaRoche, Basel, Switzerland) was used as an internal standard. Mucosal scrapings, liver, lung, and Purina Cat Chow® were saponified before extraction with 500 g KOH/L in distilled H_2O for 30 min at 60°C. Duplicate samples of lymph were saponified for comparison to nonsaponified samples. Samples were analyzed by HPLC within 24 h of collection by the method described above. As many as 7 *cis*-isomers of lycopene were separated by this HPLC method. All data are reported as total *cis*-lycopene and total *trans*-lycopene.

To evaluate the possibility that substantial isomerization of lycopene occurred during digestion, a total dose recovery was performed in one animal. All surgical procedures, lymph collection, and analysis were performed as described above. For this evaluation, all possible traces of residual digestive materials were carefully removed, weighed, and analyzed for total lycopene content.

Statistical analysis. In study 1, statistical analysis of the percent *cis*-lycopene (log base 10 transformed) obtained from the sample incubations was performed using one-way ANOVA and Fisher's protected least squares difference (PLSD) (Carmer and Swanson 1973) (Statview 4.5, Abacus Concepts, Berkeley, CA). In study 2, the same statistical tests were performed on the percent *cis*-lycopene obtained in all fractions analyzed. All values presented in the text are means ± SEM.

RESULTS

Study 1. In this study, we evaluated the solubility of crystalline lycopene isomers in an in vitro bile acid micelle preparation. When crystalline lycopene (54.4% *cis*-isomers, dissolved in hexane) was sonicated for 60 min, no isomerization occurred (**Table 1**). Limited isomerization occurred when the standard was sonicated in the presence of both oleic acid and mono-oleate, each dissolved in hexane and CH_2Cl_2. When the lycopene standard was sonicated with bile salts in a buffer,

TABLE 1

Cis-lycopene content of a lycopene standard and components of bile acid micelles following sonication for 60 min[1,2]

Fraction	Sonication conditions	*cis*-lycopene
		%
Standard	no sonication/hexane + BHT	54.4 ± 2.7[c]
Standards sonicated alone	hexane + BHT	53.9 ± 0.4[c]
Standard + monooleate	hexane + BHT/CH_2Cl_2	55.7 ± 0.1[c]
Standard + oleic acid	hexane + BHT/CH_2Cl_2	60.6 ± 3.3[b,c]
Standard + mono-oleate and oleic acid	hexane + BHT/CH_2Cl_2	65.5 ± 5.3[b]
Standard + 12 mmol/L taurocholic acid	buffer	55.7 ± 0.5[c]
Micelle preparation	buffer	75.9 ± 0.4[a]

[1] Values are means ± SEM for incubations from 3 separate experiments (n = 3) conducted on different days. Results with different superscripts are significantly different at $P < 0.05$ by one-way ANOVA and Fisher's PLSD.

[2] A crystalline lycopene standard comprised of 54.4% *cis*-lycopene was sonicated for 60 min under a variety of conditions. All individual components of the micelle preparation were combined with the crystalline lycopene standard (in hexane) and sonicated for 60 min. The resulting isomeric composition (expressed here as percentage *cis*-lycopene) was determined by HPLC analysis immediately following sonication. Bile acid micelles were simultaneously prepared using the same crystalline lycopene standard.

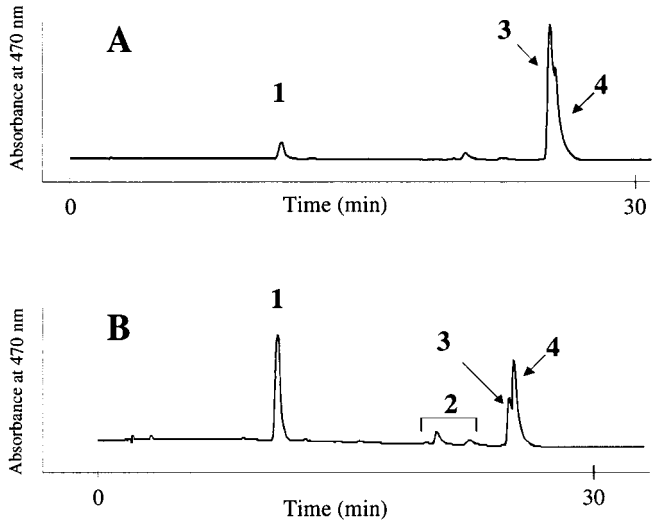

FIGURE 1 HPLC chromatogram of crystalline lycopene following 60 min of sonication and incorporation into bile acid micelles. (*A*) Crystalline lycopene (suspended in hexane/0.1 g BHT/L) immediately following 60 min sonication. (*B*) Bile acid micelle preparation made using the same crystalline lycopene standard shown in (*A*). In both chromatograms, peak numbers correspond to the following: (*1*) echinenone (used to verify peak relative retention times), (*2*) *cis*-lycopene isomers, (*3*) *trans*-lycopene, (*4*) 5-*cis* lycopene. The peaks were assigned *cis*-lycopene or *trans*-lycopene based on UV spectrum analysis and the HPLC method of Yeum et al. (1996).

no isomerization occurred. The resulting in vitro micelle preparation, however, had the largest enrichment of *cis*-lycopene at 75.9 ± 0.4%. Chromatograms of the standard sonicated alone (in hexane/BHT) for 60 min and the final micelle preparation made from this standard are shown in **Figure 1**.

Study 2. In this study, 40 mg lycopene/kg as Lycored™ mixed with soybean oil was fed orally to the ferrets. The *cis*-isomer composition (% *cis*-lycopene) of all fractions collected from the lymph cannulated ferrets is shown in **Figure 2**. As many as 7 *cis*-isomers of lycopene were separated in the individual fractions. Photodiode array analysis allowed us to verify which peaks corresponded to *trans*-lycopene, *cis*-lycopene, and 5-*cis* lycopene. The PDA spectra for the putative 5-*cis* isomer was identical to the spectra obtained for *trans*-lycopene and agrees with the report by Yeum et. al. (1996).

The dose fed to the ferrets contained 9.0 ± 2.8% *cis*-lycopene. The residual stomach and small intestine contents contained 6.2 ± 1.1% and 17.5 ± 5.7%, respectively. Mucosal scrapings contained a significantly greater proportion of *cis*-lycopene, 58.8 ± 8.6%. The lymph collections contained the highest proportion of *cis*-lycopene, at 77.4 ± 2.2%, of any fraction analyzed.

The serum, liver and lung tissues were also analyzed, and contained a small amount of lycopene. The ferrets apparently accumulated tissue lycopene from the small amount of lycopene that was present in the nonpurified diet. The serum, livers and lungs contained 52.0 ± 2.5%, 57.9 ± 4.3% and 47.3 ± 1.0% *cis*-lycopene, respectively.

Immediately following the 2-h lymph collection period, samples of residual stomach contents and small intestinal contents were removed. HPLC analysis revealed a lack of significant isomerization in these fractions. This observation was examined more closely in one ferret, (**Table 2**). In this dose-recovery study, 91.3% of the lycopene fed was recovered. The majority (91.1% of the total amount recovered) was contained in the residual stomach contents. HPLC analysis of this fraction revealed that it had the same isomeric composition as the original dose. The residual intestinal contents did show some increase in *cis*-isomers (31.0% *cis*-lycopene).

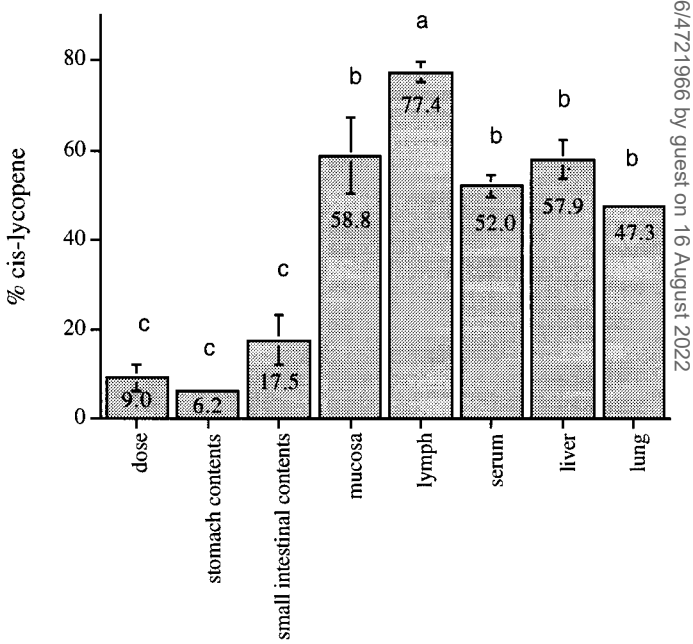

FIGURE 2 *Cis*-lycopene content of Lycored™ dose and tissues of ferrets (n = 7) fed an oral dose of lycopene (as Lycored™) providing ~40 mg/kg body weight. They were killed following a 2-h lymph collection period, and percentage *cis*-lycopene was determined in all digestive fractions by HPLC. Values are means ± SEM, n = 7. Bars with different letters are significantly different, $P < 0.01$ (one-way ANOVA and Fisher's PLSD).

TABLE 2

Quantitative recovery of cis- *and* trans-*lycopene from a Lycored™ dose following a 2-h lymph collection study in a ferret*[1,2]

Fraction	*trans*-lycopene	*cis*-lycopene	total lycopene	*cis*-lycopene
	nmol			%
Dose	79087.27	8333.80	87421.07	9.5
Residual stomach contents	72994.32	6616.37	79610.69	8.3
Residual small intestinal contents	115.49	52.16	167.65	31.1
Mucosa	0.11	0.41	0.52	78.8
Lymph, h 1	0.07	0.20	0.27	74.1
Lymph, h 2	0.43	1.65	2.09	78.9

[1] The data above demonstrate the recovery of a 40 mg lycopene/kg dose fed orally to a single ferret. A lymph cannula was placed in the mesenteric lymph, duct and lymph was collected on ice by gravity for 2 h before it was killed. All remaining stomach and intestinal contents were removed, weighed, and analyzed for total lycopene content.

[2] Data are expressed as total, *trans*-, and *cis*-lycopene. Percent *cis*-lycopene was calculated for each fraction and is shown above.

The β-carotene content of the fractions was also analyzed. In particular, the 9-*cis* β-carotene content of the individual fractions was calculated and is shown in **Table 3**. No 9-*cis* β-carotene was detected in the serum of the ferrets. Only a trace was detected in the lymph of one ferret out of the seven studied. The 9-*cis* content of the dose, stomach contents, intestinal contents, and mucosa ranged from 13–21% of total β-carotene. The lungs of the ferrets did not contain quantifiable 9-*cis*-β-carotene. Total β-carotene of this tissue was 0.03 nmol β-carotene/g tissue. Total lycopene contained in the lungs was also quite low at 0.03 nmol lycopene/g tissue.

Lycored™ was mixed with soybean oil that contained naturally occurring lutein and zeaxanthin. Preferential absorption of these xanthophylls was noted in both the lymph and mucosa fractions. Although the xanthophylls contributed <0.1% of the total carotenoids in the dose, they contributed 44.7% of the total carotenoids detected in lymph collections and 16.6% in mucosa scrapings (data not shown).

DISCUSSION

The majority of lycopene found in tomatoes and tomato products is present as all-*trans* lycopene. This is in contrast to the lycopene isomer profile reported for biological tissues. Human and animal tissues, including serum, contain significantly more *cis*-lycopene than the foods where the lycopene originated (Clinton et al. 1996, Stahl and Sies 1992). Human serum contains >50% *cis*-isomers of lycopene (Schierle et al. 1997, Stahl et al. 1992). Here we report that ferret serum contains a similar composition, 52% *cis*-lycopene.

The food matrix (i.e., the lipid and protein constituents of chromoplasts as well as the fiber contained within the tomato fruit) may contribute greatly to the stability of the all-*trans* form of lycopene in the fruit. This is supported by the observation that when tomatoes are heat processed, only minor isomerization is noted. For example, tomato sauce and tomato paste contain >90% *trans*-lycopene (Clinton et al. 1996, Nguyen and Schwartz 1998). It was also reported that heat treatment improves the bioavailability of lycopene without significantly changing the *cis*-isomer composition of the heat-treated foods (Gärtner et al. 1997, Stahl and Sies 1992).

During digestion and absorption, the food matrix is disrupted and lycopene must be incorporated into a micelle before absorption. It is plausible that once this disruption occurs, isomerization of *trans*-lycopene may occur. The data reported in these studies suggest that *cis*-lycopene is more bioavailable than *trans*-lycopene, most likely because of increased solubility in mixed micelles. We have conducted both in vitro and in vivo studies to examine this phenomenon.

TABLE 3

*All-*trans *and 9-*cis*-β-carotene concentration of ferret tissues, diet, and Lycored™ dose*[1]

Fraction	*trans*-β-carotene	9-*cis*-β-carotene	9-*cis*-β-carotene
			%
Diet,[2] *nmol/g*	0.31 ± 0.2	0.16 ± 0.01	34.4
Lycored™,[3] dose, *μmol/L*	474.63 ± 23.02	83.24 ± 17.51	14.9
Serum, *nmol/L*	18.06 ± 2.00	ND[4]	0.0
Liver, *nmol/g*	0.14 ± 0.04	0.03 ± 0.01	17.7
Lung, *nmol/g*	0.03 ± 0.20	NQ[5]	0
Residual stomach contents, *μmol/L*	71.79 ± 4.50	10.69 ± 3.05	13.0
Intestinal contents, *μmol/L*	172.70 ± 42.20	45.50 ± 15.60	20.9
Mucosa, *nmol/g*	0.40 ± 0.21	0.10 ± 0.08	20.0
Lymph, *nmol/L*	143.70 ± 36.10	0.20 ± 0.20	0.0

[1] Values are means ± SEM, $n = 7$.

[2] Purina Cat Chow®, Raltston Purina Company, St. Louis, MO.

[3] Makhteshim Chemical Works, Beer-Sheva, Israel.

[4] Not detected.

[5] Not quantitated.

Study 1 was designed following observations of a prior study examining competition between carotenoids for uptake into human brush border membrane vesicles. When working with crystalline lycopene in an organic solvent, we noticed that an ~50:50 equilibrium mixture of *trans-* and *cis*-lycopene existed, even when an all-*trans* lycopene fraction was purified by preparative HPLC. This observation was previously reported by another lab (Nguyen and Schwartz 1998). These investigators also purified 100% *trans*-lycopene and noted that the formation of an equilibrium mixture of *cis-* and *trans*-lycopene could be delayed by the presence of BHT or incubation at 4°C, but it could not be avoided by these conditions. In previous studies, we found that invariably the resulting micelle preparation contained >75% *cis*-lycopene, although the standard used to make the micelles contained only 50% *cis*-lycopene.

It was important to examine the possibility that the procedures used to make the micelles could have caused isomerization. The micelle preparation procedures included 60 min of sonication, which provides mechanical energy and produces heat. When 50:50 (*cis*:*trans*) lycopene (in hexane/BHT) was sonicated for 60 min, we found that the equilibrium *cis*-isomer composition had not changed (Table 1). This observation further strengthens the idea that a stable equilibrium is being formed among lycopene isomers in an organic solvent. Isomerization was observed following the incubation of crystalline lycopene with fatty acids suspended in a chlorinated solvent (Table 1). When lycopene was sonicated in taurocholate/buffer without the fatty acids, no isomerization was observed. This sample condition best simulates the actual conditions during the micelle preparation procedures because all traces of organic solvent were removed before the addition of the bile salts and buffer solutions.

We discovered that incorporating lycopene into a micelle in vitro is challenging relative to the ease with which β-carotene is incorporated. While the two carotenoids have identical molecular weights and empirical formulas, their structures are different. This difference in structure not only confers a difference in color (i.e. red lycopene vs. orange β-carotene), but also likely confers a difference in micellar solubility. Both compounds are hydrocarbons having very little or no solubility in water. β-carotene possesses a closed end ring structure, making it a shorter molecule than lycopene. *Trans*-lycopene is a longer molecule because of this extended chain. It is possible that the formation of a *cis*-conformation, for example 5-*cis* lycopene, shortens the effective chain length, rendering it more soluble. C*is*-isomers of carotenoids are less likely to crystallize than the extended *trans* conformations (Britton 1995). With this decreased tendency of *cis*-lycopene to form aggregates, the *cis*-isomers of lycopene would be more likely to be incorporated into a bile acid micelle. This hypothesis is consistent with our in vitro findings.

To directly study the absorption of *cis*-lycopene, a model that allowed collection of lymph after ingestion of lycopene was used. In study 2, Lycored™, a tomato oleoresin, containing ~5% (w/w) lycopene (Vaida et al. 1996), was chosen as the source of lycopene for the oral dose. Ferrets were chosen for this work because of their demonstrated utility in studying carotenoid absorption and metabolism (Gugger et al. 1992, Hébuterne et al. 1995, Ribaya-Mercado et al. 1989, Tang et al. 1993, Wang et al. 1992 and 1993, White et al. 1993, Zhou et al. 1996) and because they are sufficiently large to provide ample lymph collection for HPLC analysis.

The lycopene fed to the ferrets was ~90% all-*trans*, consistent with the isomer profile of tomato products. As predicted, the isomer profile of the lymph (representing newly absorbed lycopene) was similar to the profile of the in vitro micelle preparation, with >75% *cis*-lycopene. The isomer profile of the residual stomach contents did not differ from the original dose. The majority of the dose fed was recovered in the stomach (Table 2). The dose was fed in excess of what was expected to be absorbed and the use of halothane anesthesia may have dramatically decreased gastrointestinal transit time. The total gastrointestinal transit time for ferrets is reported to be between 3 and 4 h (Bernard et al. 1984). Approximately 1 h elapsed between oral dose administration and beginning the collection of lymph secretions. The second hour of lymph collection contained more lycopene than the first hour; however, the *cis*:*trans* ratio did not differ.

The residual contents of the small intestine are the best indicator of whether lycopene isomerizes during digestion. More *cis*-lycopene was detected (17.5 ± 5.7% *cis*-lycopene) in the residual small intestinal contents relative to the dose and residual stomach contents; however, this difference was not statistically significant ($P = .17$). It is possible that *cis*-lycopene, which comprised just under 10% of the total lycopene in the dose, is more easily liberated from the lipid milieu of the gastric contents into mixed micelles within the small intestine.

We observed a substantial shift in the *cis*-isomer profile between the small intestinal contents and the mucosa lining. A greater proportion of *cis*-lycopene in the mucosa could be the consequence of selective incorporation of *cis*-lycopene into bile acid micelles. The lycopene isomer profile of the lymph secretions (77.4% *cis*) was significantly greater than any of the tissues analyzed, including the intestinal mucosa (58.8% *cis*). It is suspected that at high doses, the absorption of carotenoids is dependent upon two concentration gradients: movement of carotenoid from the micelle to the brush border membrane and the removal of the carotenoid from the membrane into an intracellular location (Parker 1996). If *cis*-lycopene is more readily removed from the membrane for incorporation into chylomicrons, this might explain why there was a significantly greater proportion of *cis*-lycopene in the lymph secretions than in the mucosa scrapings.

The lycopene isomer profiles of the serum, liver, and lung were similar to what was reported in humans (Clinton et al. 1996, Stahl et al. 1992) and for rats and mice (Tom Boileau, University of Illinois, personal communication). It is of interest that the lymph contained >75% *cis*-lycopene while the storage tissues contained ~50% *cis*-lycopene. Lycopene is likely delivered to the liver by way of chylomicron remnants. It appears that at some point between absorption via lymph and storage in the liver, lycopene returns to a state of equilibrium between *trans-* and *cis*-isomers. This isomer profile is similar to what was observed when lycopene is stored in organic solvents (Nguyen and Schwartz 1998). The lycopene isomer profile of the liver would likely predict what the profile of other storage tissues, including serum, would be.

The β-carotene concentration of the ferret diet, lycopene dose, and tissues were also analyzed so that a comparison between *trans* β-carotene and 9-*cis* β-carotene could be made (Table 3). 9-*cis* β-carotene is not detected in human serum, even after 9-*cis* β-carotene is supplemented orally (Ben-Amotz and Levy 1996, Gärtner et al. 1996, Gaziano et al. 1995, Stahl et al. 1993 and 1995, Tamai et al. 1995). As expected, 9-*cis* β-carotene was not detected in ferret serum, but was detected in liver where it contributed 17.7% of total β-carotene. Of particular interest was the comparison between the 9-*cis* β-carotene concentration of the lycopene dose and the subsequent lymph secretions. In the dose, 9-*cis* β-carotene contributed nearly 15% of the β-carotene present. No 9-*cis* β-carotene

could be detected in the lymph secretions from six out of seven ferrets and only a trace in the seventh ferret.

HPLC analysis of the lymph collections revealed that preferential absorption of xanthophylls was occurring. The dose fed to the ferrets contained only a trace amount of lutein and zeaxanthin (<0.1% of carotenoids) contributed by the soybean oil. These oxy-carotenoids are more polar than the hydrocarbon lycopene and would be expected to behave differently during digestion and absorption. Preferential absorption of xanthophylls over β-carotene in humans was previously reported (Gärtner et al. 1996).

The data we have presented support the hypothesis that a high percentage of *cis-* lycopene is present in tissues because it is better absorbed than *trans*-lycopene. This is most likely because of enhanced solubility of *cis*-lycopene in bile acid micelles and possibly preferential incorporation into chylomicrons. It is possible that lycopene exists in both human and animal tissues as ~50% *cis*-lycopene because this mixture is the most stable and represents an equilibrium between *trans*-lycopene and its isomers. The food matrix that surrounds lycopene when it is present within the tomato seems to prevent this isomeric equilibrium from occurring.

ACKNOWLEDGMENT

The authors thank Laura Bauer for her technical assistance with the ferret surgery.

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