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Differential Bioavailability, Clearance, and Tissue Distribution of the Acyclic Tomato Carotenoids Lycopene and Phytoene in Mongolian Gerbils[1–3]

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Abstract

Lycopene (LYC) is the major tomato carotenoid and is the focus of substantial research. Phytoene (PE), a minor tomato carotenoid, is found in human blood and tissues in similar concentrations to LYC. To determine which metabolic differences underlie this phenomenon, Mongolian gerbils (*Meriones unguiculatus*, n = 56) were fed control or tomato powder (TP)-containing diets (to establish steady-state serum and tissue carotenoid concentrations similar to tomato-fed humans) for 26 d. The TP-fed gerbils were then provided either a single, oral, cottonseed oil (CO) vehicle dose and tissues were collected at 6 h or they were provided unlabeled PE or LYC in CO and tissues were evaluated at 6, 12, or 24 h. In vehicle-dosed, TP-fed gerbils, LYC was the major carotenoid (≥55% carotenoids) in liver, spleen, testes, and the prostate-seminal vesicle complex, whereas PE was the major serum and adipose carotenoid (≥37% total carotenoid) and phytofluene was the major carotenoid (≥38%) in adrenals and lungs. PE dosing increased hepatic, splenic, and serum PE concentrations compared with vehicle dosing ($P < 0.05$) from 6 to 24 h, whereas LYC dosing increased only serum LYC at 6 and 12 h ($P < 0.05$) compared with vehicle dosing. This suggested PE was more bioavailable and cleared more slowly than LYC. To precisely track absorptive and distributive differences, ^{14}C-PE or ^{14}C-LYC (n = 2/group) was provided to TP-fed gerbils. Bioavailability assessed by carcass ^{14}C-content was 23% for PE and 8% for LYC. Nearly every extra-hepatic tissue accumulated greater dose radioactivity after ^{14}C-PE than ^{14}C-LYC dosing. Thus, LYC and PE, which structurally differ only by saturation, pharmacokinetically differ in bioavailability, tissue deposition, and clearance. J. Nutr. 143: 1920–1926, 2013.

Introduction

The observed, disease-preventive properties of tomato consumption (1–5) are attributable in part to lycopene (LYC)[7] and other non-provitamin A carotenoids (6–9), including neurosporene, γ-carotene, ζ-carotene (ZC), phytoene (PE), and phytofluene (PF). These carotenoids are deposited in many human tissues and have also demonstrated bioactivity in a range of laboratory studies (10).

Though PE, PF, and ZC are generally present in tomatoes at much lower concentrations than LYC, they are found in similar $ng \cdot g^{-1}$concentrations as LYC in human tissues and blood (11), which may be due to pharmacokinetic differences between these carotenoids. For instance, the relative plasma response to these tomato carotenoids was previously shown to differ. After a 4-wk tomato juice intervention providing high LYC (75 mg/d) and low PE, PF, and ZC (6, 5, and 0.5 mg/d, respectively), human PF and PE plasma concentrations increased more (167% and 174%, respectively) than those of ZC (98%) and LYC (29%) (12). The resultant plasma concentrations of PF and LYC were very similar (0.72 and 0.75 $\mu mol \cdot L^{-1}$, respectively), as were PE and ZC (0.30 and 0.26 $\mu mol \cdot L^{-1}$, respectively) (12). However, PE and PF were largely found in VLDL and LDL, whereas LYC was primarily in LDL and HDL, suggesting that carotenoid distribution is linked to the dynamics of lipoprotein metabolism (12). Additional animal feeding studies showed that PE, PF, and ZC are relatively enriched in the adrenals, liver, prostate-seminal

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[3] Supplemental Figures 1–4 are available from the ''Online Supporting Material'' link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.
[7] Abbreviations used: CO, cottonseed oil; HEAT, hexane/ethanol/acetone/toluene; LSC, liquid scintillation counting; LYC, lycopene; PDA, photodiode array detector; PE, phytoene; PF, phytofluene; SRB1, scavenger receptor class B member 1; TP, tomato powder; ZC, ζ-carotene.
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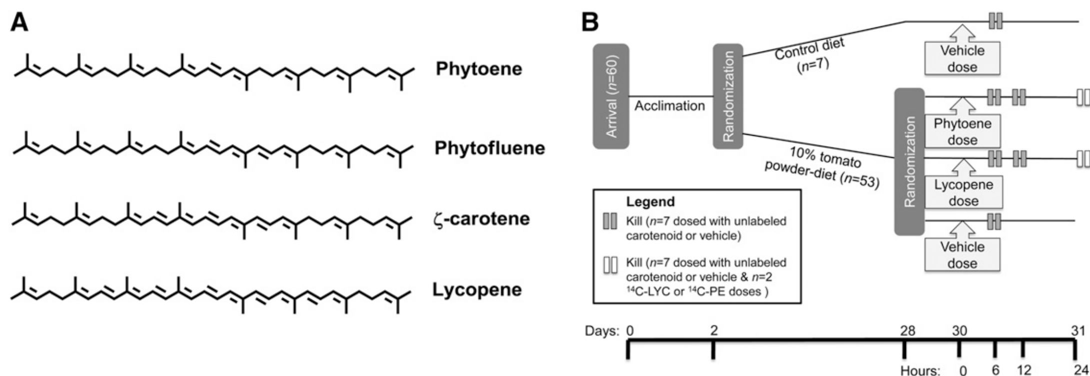

FIGURE 1 Tomato carotenoid structures (*A*) and Expt. 1 and 2 designs (*B*). During acclimation, gerbils received a semipurified powdered diet for 2 d. On d 28, the experimental diets were replaced with a control diet for 2 d and on d 30, gerbils received a single oral dose of either PE or LYC (PE dosed or LYC dosed), a CO carrier dose (vehicle-dosed groups) (Expt. 1), or ^{14}C-PE or ^{14}C-LYC (Expt. 2). TP-fed and control-fed, vehicle-dosed gerbils were killed 6 h after dosing, PE-dosed and LYC-dosed gerbils at 6, 12, and 24 h postdosing (Expt. 1), and ^{14}C-PE and ^{14}C-LYC gerbils at 24 h after dosing (Expt. 2). CO, cottonseed oil; LYC, lycopene; PE, phytoene; TP, tomato powder.

vesicle complex, and spleen compared with LYC (13,14). Thus, tomato product consumption leads to substantial circulating and tissue concentrations of PE, PF, and ZC, presenting these carotenoids, along with LYC, as potential mediators of biological actions associated with tomato feeding.

Carotenoid saturation and isomerization likely contributes to differences in bioavailability and/or metabolism (12–17). Although the tomato carotenoids bear the same tetraterpene, 40-carbon structures, PE, PF, and ZC are more saturated precursors of LYC (**Fig. 1***A*) and such differences in carotenoid physicochemical properties have been shown to affect carotenoid bioaccessibility (the ability for a carotenoid be extracted from a matrix and transferred to mixed micelles) and plasma response (18). Although a number of studies have all shown unique tissue distribution patterns of the tomato carotenoids, the mechanisms that underlie these patterns remain to be clearly elucidated.

Experimental model systems using labeled tracer technology may provide insight into pharmacokinetics relevant to humans and the design of future human studies. Among the rodent systems available for the evaluation of carotenoid pharmacokinetics, the Mongolian gerbil (*Meriones unguiculatus*) demonstrates tomato carotenoid absorption, metabolism, and distribution that more closely mimic human conditions (14,19,20). Thus, we compared the bioavailability, metabolism, and tissue distribution of LYC and its precursor, PE, in the gerbil system. This study utilized unlabeled and ^{14}C-labeled carotenoids, produced from tomato cell culture biolabeling and commercial sources, provided to gerbils fed a chronic, fixed dietary concentration of tomato powder (TP) to better mimic the human steady state.

Materials and Methods

Gerbils and experimental design

The animal protocol was approved by the University of Illinois Institutional Animal Care and Use Committee and all necessary procedures were followed to ensure ethical treatment of gerbils. To examine tomato carotenoid absorption and metabolism in gerbils, in Expt. 1, male, 40-d-old gerbils (38.1 ± 0.4 g body weight, (Charles River Laboratories, n = 56) were acclimated with a nonpurified diet (Teklad 8640, Harlan Laboratories) for 2 d, followed by a previously described control, semipurified, powdered diet for 2 d (21), and then randomly assigned to either control (n = 7) or 10% TP diet (n = 49) (Fig. 1). The TP diet (TP donated by Futureceuticals) provided *all-E*-LYC (214 $mg \cdot kg^{-1}$), *Z*-LYC isomers (83 $mg \cdot kg^{-1}$), PE (12 $mg \cdot kg^{-1}$), PF (4 $mg \cdot kg^{-1}$), ζC (6 $mg \cdot kg^{-1}$), and βC (3 $mg \cdot kg^{-1}$) and diets were balanced for energy, macronutrient composition, and fiber (drum-dried TP contained ~15.4 $kJ \cdot g^{-1}$, 0.01 g protein $\cdot g^{-1}$, 0.03 g fat $\cdot g^{-1}$, 0.16 g fiber $\cdot g^{-1}$, and 0.52 g carbohydrate $\cdot g^{-1}$). Randomly assigned, individually housed gerbils began the study diets and were subsequently provided fresh diet (15 g) every other day with free access to water. After 26 d, gerbils were fed a control diet for 2 d to minimize potential competition for absorption (22) between dosed and dietary carotenoids. Gerbils fed the TP diet were randomly assigned to receive doses while under isoflurane anesthesia of either PE in cottonseed oil (CO) [PE dosed; n = 21, 1.00 ± 0.01 mg (1.84 ± 0.02 mmol) PE in 202 ± 3 μL of CO], LYC in CO [LYC dosed; n = 21, 1.31 ± 0.05 mg (2.44 ± 0.09 mmol) LYC in 206 ± 3 μL of CO], or CO alone (vehicle-dosed group; n = 7, 206 ± 4 μL CO) or control diet-fed gerbils received CO alone. After dosing, gerbils were housed in clean cages with fresh control diet to minimize coprophagy. PE-dosed and LYC-dosed gerbils were killed by carbon dioxide asphyxiation followed by cardiac exsanguination at 6, 12, or 24 h (n = 7 per time point) and both TP and control-fed, vehicle-dosed gerbils were killed by carbon dioxide asphyxiation followed by cardiac exsanguination 6 h postdosing as previously described (23). The liver, adrenal glands, gonadal adipose, spleen, lungs, testes, and the prostate-seminal vesicle complex were collected, weighed, and snap frozen in liquid nitrogen and then stored at −80°C for carotenoid analysis.

In Expt. 2, gerbils (n = 4) followed the same dietary acclimation and TP-feeding regimen as in Expt. 1. Gerbils were dosed as above with either *^{14}C-PE* (n = 2) or *^{14}C-LYC* (n = 2) and killed 24 h later. In addition to the tissues listed above, the stomach, small intestine, and large intestine were flushed with cold PBS and these tissues and digesta were collected, as were the eyes, brain, thymus, skin, hind leg muscles, heart, the remaining carcass, and feces from cages.

Carotenoid analysis of diet

The TP and control diets (0.025 g) were analyzed by HPLC as previously published (14,24,25). Extinction coefficients and characteristic absorption spectra of carotenoids and respective geometrical isomers were previously published (26–31).

Preparation of purified carotenoid doses

Expt. 1. Unlabeled Doses. General precautions were taken to minimize carotenoid degradation and artifact formation during the extraction and analytical processes (32). PE was isolated from PE-rich oil (derived from *Blakeslea trispora* and suspended in oil, donated by Vitan) and LYC was isolated from Redivivo 10% LYC beadlets (DSM) extracts using a previously described, semipreparatory HPLC system (33) to remove other noncarotenoid components. The geometric isomer composition of purified LYC was 52 ± 2% all-*E* with the remainder as various *Z* isomers, while 100% of the PE was *15-Z* [the major isomer found in tomato (34)]. Carotenoid doses were transferred to CO as previously described (13). The targeted carotenoid dose was 1 mg in 200 μL of CO. Delivered doses were 1.00 ± 0.01 mg (1.84 ± 0.02 mmol)

PE in 202 ± 3 μL CO, 1.31 ± 0.05 mg (2.44 ± 0.09 mmol) LYC in 206 ± 3 μL of CO, and 206 ± 4 μL CO.

Expt. 2. ^{14}C-Labeled doses. A previously described system for biolabeling and extracting tomato carotenoids was utilized (24,35) to generate ^{14}C-PE or ^{14}C-LYC, which were not commercially available. PE was isolated in 2 steps, first as above (33), then by 10 × 250 mm, 5-μm particle size C30 (YMC) column using a published HPLC gradient method (flow rate, 3.8 mL · min^{-1}) (31). Total radioactivity was determined by liquid scintillation counting (LSC) (LS6500, Beckman-Coulter) and chromatographic purity was determined by peak area at 286 nm for PE and 472 nm for LYC. Radiopurity was determined by LSC quantitation from 1-min fractions of the HPLC eluate obtained by a Waters Fraction Collector I mixed with 5 mL of Biosafe II scintillation cocktail (RPI). The targeted dose mass was lowered to 0.3 mg in 200 μL of CO to maximize absorption and the intended radioactivity was ~0.35 μCi as limited by the tomato cell culture yields of ^{14}C-PE and LSC detection. ^{14}C-LYC yield from cell cultures was less than that of ^{14}C-PE, so the cell culture-derived ^{14}C-LYC was combined with a small mass of purified ^{14}C-LYC previously donated by BASF. Delivered ^{14}C-LYC doses were 0.76 μCi in 0.24 mg (0.45 nmol) in 202 μL oil and ^{14}C-PE doses were 0.36 μCi in 0.32 mg (0.58 nmol) in 209 μL oil. Radioactivity results were normalized to the percent of dose radioactivity administered to correct for delivered radioactivity differences.

Serum and tissue carotenoid HPLC analyses

Serum and tissue carotenoid extraction methods were previously described (13). Carotenoids were identified and quantitated by HPLC-photodiode array detector (PDA) as described for dietary carotenoid analysis.

^{14}C-tissue analysis and metabolite identification

To extract potential PE or LYC breakdown products in addition to intact carotenoids for ^{14}C-analysis, a plasma LYC metabolite extraction protocol [HEAT (hexane/ethanol/acetone/toluene; 10:6:7:7 v:v:v:v) method] previously described by Kopec et al. (36) was modified for tissues. Briefly, liver (0.5 g) and lung (0.2 g) were homogenized (Powergen, Fisher Scientific) in 5 mL of saturated aqueous NaCl. Serum (300 μL) was extracted without homogenization. The homogenate was mixed with 5 mL of ethanol containing 0.1% butylated hydroxytoluene and extracted 3 times with 10 mL of the HEAT extraction solvent, with centrifugation between each step for 10 min at 4°C at 600 × *g* (CR3i, Jouan, Thermo-Scientific), and the upper layers were reserved, pooled, and dried under reduced pressure. The extracts were stored at −20°C, analyzed within 6 h by dissolution in 100 μL of mobile phase B, 80–95 μL was injected for HPLC analysis, and resultant 1-min fractions were analyzed by LSC.

Tissue and digesta ^{14}C-analysis

Tissue radioactivity analysis by LSC was previously described with the exception of several modifications (37). Samples (0.05–0.1 g of minced tissue or 0.07–0.1 g ground feces) were prepared for solubilization by 1 mL of TS-2 Tissue and Gel Solubilizer (Research Products International) per the manufacturer's instructions. Samples of gastric, small intestinal, and large intestinal digesta and the remaining carcass were homogenized and similarly solubilized. Total radioactivity for each tissue or compartment was calculated based on tissue weight or calculated plasma total volume (38). Carcass samples were analyzed in 5 replicates per gerbil, digestive tract tissues and digesta in triplicate, liver and testes in duplicate, and all other tissues by one sample per animal. Only one spleen was collected from the ^{14}C-PE–dosed gerbils.

Statistical analyses

Tissue and serum carotenoid concentrations of PE-dosed and LYC-dosed gerbils at 6, 12, and 24 h postdosing were compared with those of the vehicle-dosed group (Expt. 1). Group comparisons were made by 1-factor ANOVA and significant differences were determined by Tukey's studentized range test (α = 0.05) (Expt. 1). When the assumptions of normality and homogeneity of variance of ANOVA were not met due to a violation of homogeneity of variance for serum PE, spleen PE, and lung PE concentration data, the Kruskal-Wallis nonparametric *t* test was used (α = 0.05) (Expt. 1). The statistical analysis software SAS v. 7.1 (SAS Institute) was used. Results are presented as a mean of the analyses ± SEM when possible. In Expt. 2, limited ^{14}C-PE from tomato cell cultures had to be considered in tandem with expected limitations of LSC sensitivity [estimated from (33,39)], limiting group sizes to n = 2. Therefore, statistical mean separation tests could not be performed (Expt. 2), but averages were compared.

Results

Gerbil weight gain

Gerbil final weights in Expt. 1 did not differ by diet, dosing group, or kill time point (mean final weight, 67.8 ± 0.5 g). The final mean gerbil weight for Expt. 2 was 65.6 ± 1.2 g.

Expt. 1: tomato carotenoid biodistribution in vehicle-dosed gerbils

All of the tomato carotenoids were detectable in the vehicle-dosed serum but were more similar in concentration than those found in the diet, with PE being the major serum carotenoid followed by total LYC, PF, ζC, and trace amounts of βC (**Table 1**).

LYC was the major carotenoid in 5 of the 7 tissues assayed, whereas PE was most prominent in the adipose and PF in the adrenals (Table 1). The total carotenoid concentrations in the liver and adrenals were ~100- and 10-fold greater, respectively, than in the spleen. Carotenoids were most concentrated in the liver, where LYC was predominant, followed by the adrenals (Table 1). The proportions of tomato carotenoids accumulated in tissues (Table 1) substantially differed from those found in the TP (67% *all-E* LYC, 26% *Z*- LYC isomers, 3% PE, 2% ZC, 1% PF, and 1% β-carotene). Splenic tissue primarily accumulated LYC followed by PF. PF and LYC were the major carotenoids in the lung tissue and PE was primary in gonadal adipose. In both the testes and the prostate-seminal vesicle complex, LYC was most prominent, whereas PE was not detectable in either tissue (Table 1). Carotenoids were not detected in control-fed, vehicle-dosed gerbil serum or tissues or control diets and are not discussed further.

Expt. 1: Effect of PE or LYC dosing on serum and tissue carotenoid concentrations

Serum PE concentrations in the PE-dosed gerbils (283 ± 40, 199 ± 49, and 315 ± 68 nmol · L^{-1}, at 6, 12, and 24 h) were greater (P < 0.05) than those of vehicle-dosed gerbils (73 ± 16 nmol · L^{-1}) (**Fig. 2**). Serum total LYC was greater (P < 0.05) in LYC-dosed gerbils 6 and 12 h after dosing (122 ± 17 and 113 ± 5 nmol · L^{-1}, respectively) compared with vehicle-dosed gerbils (62 ± 11 nmol · L^{-1}) but by 24 h had returned to the concentration found in the vehicle-dosed gerbils (Fig. 2). These changes in serum concentrations relate to elevation of the PE concentration in the serum by 570%, 340%, and 650% and LYC elevations of 97%, 80%, and 39% at 6, 12, and 24 h, respectively.

Hepatic and splenic PE increased in PE-dosed gerbils over 24 h (Fig. 2); however, LYC was unchanged in either tissue in LYC-dosed gerbils. Similarly, at 24 h, pulmonary PE was greater in PE-dosed gerbils than in those vehicle dosed (Fig. 2), whereas pulmonary LYC did not increase in LYC-dosed gerbils. PE was not detected in the testes or prostate-seminal vesicle complex in PE-dosed gerbils during the 24 h time-course.

Expt. 2: 24-h absorption of ^{14}C-PE and ^{14}C-LYC

Dose radioactivity distribution in tissues. After 24 h, 23% of PE dose radioactivity was detected in the total carcass in contrast

TABLE 1 Serum and tissue carotenoid concentrations and proportions in vehicle-dosed, tomato-fed gerbils (Expt. 1)[1]

	Serum	Liver	Adrenals	Spleen	Lung	Gonadal adipose	Testes	Prostate-seminal vesicle complex
	nmol · L^{-1} (%)	*nmol · g^{-1} (%)*	*nmol · g^{-1} (%)*	*nmol · g^{-1} (%)*	*nmol · g^{-1} (%)*	*nmol · g^{-1} (%)*	*nmol · g^{-1} (%)*	*nmol · g^{-1} (%)*
PE	73 ± 16 (38)	24 ± 6 (10)	15.7 ± 2.1 (34)	0.17 ± 0.06 (3)	0.02 ± 0.01 (6)	0.08 ± 0.04 (37)	n/d	n/d
Total LYC	62 ± 11 (32)	134 ± 30 (55)	7.7 ± 1.3 (17)	2.64 ± 0.30 (56)	0.16 ± 0.03 (39)	0.07 ± 0.02 (31)	0.40 ± 0.08 (65)	0.12 ± 0.02 (65)
PF	39 ± 9 (20)	67 ± 13 (28)	19.1 ± 2.0 (42)	1.71 ± 0.24 (36)	0.15 ± 0.02 (38)	0.03 ± 0.01 (14)	0.17 ± 0.03 (28)	0.02 ± 0.01 (13)
ZC	20 ± 2 (10)	12 ± 2 (5)	2.9 ± 0.4 (6)	0.22 ± 0.04 (5)	0.07 ± 0.01 (17)	0.04 ± 0.01 (18)	0.05 ± 0.01 (7)	0.04 ± 0.003 (23)
β-Carotene	Trace	5 ± 1 (2)	0.6 ± 0.04 (1)	n/d	n/d	n/d	n/d	n/d

[1] Values are means ± SEMs, n = 6–7. Lower limit of detection = 0.02 nmol PE · g^{-1} tissue or 190 nmol PE · g^{-1} and 0.5 nmol B-carotene · g^{-1} tissue or 370 nmol B-carotene · L^{-1} of injection carrier. LYC, lycopene; n/d, not detectable; PE, phytoene; PF, phytofluene; ZC, ζ-carotene.

with 8% of the ^{14}C-LYC dose radioactivity (**Table 2**). Forty-seven percent of the ^{14}C-PE and 58% of the ^{14}C-LYC dose radioactivity were recovered from the digesta and feces and presumed to have been unabsorbed. Twenty-nine percent of the ^{14}C-PE dose and 34% of the ^{14}C-LYC were not recovered.

The 24-h distribution of ^{14}C-PE and ^{14}C-LYC radioactivity differed by tissue. The small and large intestinal tissues contained the greatest percent of ^{14}C-PE dose radioactivity, whereas the liver and stomach tissues contained the greatest percent of ^{14}C-LYC radioactivity (**Table 3**). Across all tissue dose radioactivity uptake analyses, the mean within-group difference was 83% and 101% for ^{14}C-PE– and ^{14}C-LYC–dosed gerbils, respectively, whereas the mean absolute percent difference between ^{14}C-PE– and ^{14}C-LYC–dosed gerbil groups was 368%, indicating that between-group differences were larger than within-group variability. The percent of ^{14}C-PE dose radioactivity was greater than that from ^{14}C-LYC in nearly all tissues except the liver, stomach, and bladder (Table 3). Tissue deposition of ^{14}C-PE radioactivity was greater than ^{14}C-LYC by 9-fold in the peritoneal adipose and the adrenals, 6-fold in the lungs, and 4-fold in the hind leg muscle. The ^{14}C-PE percent dose radioactivity per gram was greatest in adrenals (1.8 % · g^{-1} tissue) followed by the liver and the spleen (0.76 and 0.49% · g^{-1}, respectively). The ^{14}C-LYC percent dose radioactivity per gram was greatest in the liver (1% · g^{-1} tissue) followed by the spleen and the eyes (0.34 and 0.19% · g^{-1}, respectively) (Table 3). Notably, the percent of ^{14}C-PE dose radioactivity per gram in the skin was 12 times greater than that from ^{14}C-LYC.

Tissue ^{14}C-carotenoid HPLC-PDA-LSC analysis. The hexane-extracted, radioactive, nonpolar compounds from the ^{14}C-PE and ^{14}C-LYC–dosed gerbil livers were determined to be largely associated with intact PE and LYC, respectively, based on carotenoid dose chromatograms and radioactivity plots (**Supplemental Fig. 1**). HEAT-extractable, polar, and nonpolar liver ^{14}C-compounds were detected in ^{14}C-PE–dosed gerbil liver (**Supplemental Fig. 2**) at elution times corresponding to intact PE (24 min) and a more polar compound eluting between 17 and 22 min, which was not present in the dose (Supplemental Fig. 1). Radioactivity of HEAT extract of ^{14}C-LYC–dosed gerbil liver (**Supplemental Fig. 3**) was associated with intact LYC isomers (32–45 min) as well as more polar fractions (5 and 15–30 min), which were not present in the dose (Supplemental Fig. 1). The specific identities of these peaks could not be resolved by HPLC-PDA.

Radioactivity was detected in the HEAT extracts of from ^{14}C-PE– but not ^{14}C-LYC–dosed gerbil lung tissue (**Supplemental Fig. 4**). Radioactivity in the lungs of ^{14}C-PE–dosed gerbils eluted near PE standard elution time (~22 min); however, the characteristic PE absorption spectra could not be definitively identified, likely due to coeluting compounds. Serum was also HEAT extracted and HPLC fractionated; however, the radioactivity signal was below LSC detection.

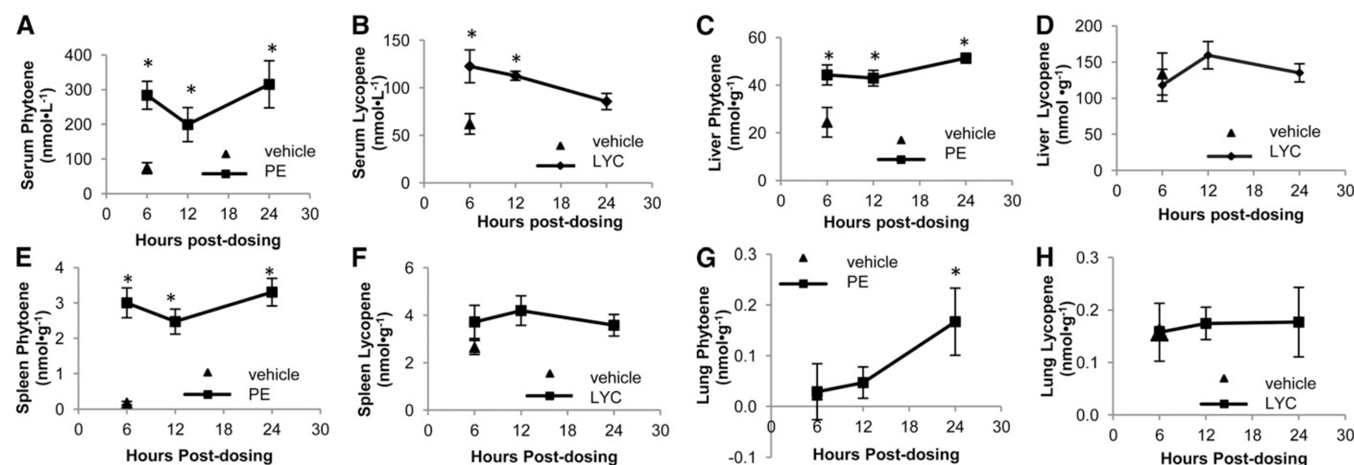

FIGURE 2 Changes in carotenoid concentrations in serum (*A,B*), liver (*C,D*), spleen (*E,F*), and lung (*G,H*) in TP-fed gerbils that were administered vehicle, PE (*A,C,E,G*), or LYC (*B,D,F,H*) (Expt. 1). Values are means ± SEMs, n = 6 or 7. *Different from the vehicle-dosed group at 6 h, $P < 0.05$ by ANOVA between either PE-dosed or LYC-dosed gerbils at 6-, 12-, or 24-h time point compared with the vehicle-dosed, 6-h time point. Note that *y*-axes differ. LYC, lycopene; PE, phytoene; TP, tomato powder; vehicle, vehicle-dosed gerbils.

TABLE 2 Percent distribution of dosed ^{14}C-PE and ^{14}C-LYC radioactivity in digesta and tissues of TP-fed gerbils (Expt. 2)[1,2]

Compartment	^{14}C-PE	^{14}C-LYC
	% total dose radioactivity delivered	*% total dose radioactivity delivered*
Feces	29.4	42.3
Large intestinal contents	16.0	12.9
Tissues and serum	17.7	5.9
Carcass	4.9	1.6
Stomach contents	2.2	3.3
Small intestinal contents	0.4	0.2
Unrecovered	29.8	34.0

[1] Values are means, n = 2. LYC, lycopene; PE, phytoene; TP, tomato powder.
[2] 24 h after dosing TP-fed gerbils.

Discussion

Here we demonstrate that PE and LYC, which structurally differ by only 4 double bonds, exhibit profound differences in their short-term distributive and metabolic fates. By examining the comparative pharmacokinetics of unlabeled and ^{14}C-labeled PE and LYC doses, we found that a greater proportion of PE was absorbed than LYC; LYC appeared to be more rapidly cleared from the liver, the 24-h serum clearance of PE was slower than LYC, and radioactivity from ^{14}C-PE was more highly deposited and retained than from ^{14}C-LYC in nearly all extra-hepatic tissues.

Differential tissue biodistribution of tomato carotenoids in tomato-fed, vehicle-dose gerbils. TP feeding led to distinct patterns of PE and LYC tissue and serum distribution. As in humans, the adrenals and liver of gerbils accumulated very high concentrations of LYC and other carotenoids (11,40–42), potentially because both tissues express scavenger receptor class B member 1 (SRB1), a transporter also involved in intestinal LYC uptake (43,44). In contrast to the adrenals, intact PE was not detected in the testes. In gerbils, PE was the major carotenoid in adipose tissue (Table 1), which is a major depot for lipophilic carotenoids (45). In gerbils, LYC was the prominent carotenoid in both the testes and prostate-seminal vesicle complex (Table 1), agreeing with previous reports in humans and animals (11, 13,40,41,46).

Relative bioavailability and metabolism of PE vs. LYC. Although the diets provided 3% PE and 93% LYC (percent total carotenoids), we observed that PE was a major carotenoid (>30%) in TP-fed, vehicle-dosed gerbils' serum, adrenals, and adipose, yet it was not detected in the testes or prostate-seminal vesicle complex in Expt. 1. Accordingly, the relative pharmacokinetics of single doses of PE or LYC in tomato-fed gerbils substantially differed. The marked pharmacokinetic differences observed in Expt. 1 raised the following questions: *1*) do hepatic and testicular tissue LYC and PE responses differ due to clearance or uptake; *2*) is tissue uptake of PE greater than that of LYC; and *3*) how does the total body uptake of PE and LYC differ? To help investigate these questions, gerbils were dosed with ^{14}C-PE and ^{14}C-LYC in Expt. 2.

First, we examined ^{14}C-PE compared with ^{14}C-LYC uptake into the liver and androgen-sensitive tissues. ^{14}C was detectable in livers of ^{14}C-LYC–dosed gerbils, suggesting uptake of either intact or metabolized ^{14}C-LYC. In the liver, a large portion of this radioactivity was associated with intact LYC (Supplemental Fig. 3). Thus, although LYC dosing in Expt. 1 did not increase hepatic LYC concentrations (Fig. 2*D*), intact LYC is deposited in the liver within 24 h based on Expt. 2. This suggests that LYC is

TABLE 3 Dose radioactivity deposited in tissues and serum of TP-fed gerbils (Expt. 2)[1,2]

Compartment	^{14}C-PE		^{14}C-LYC	
	% dose radioactivity in compartment	% dose radioactivity · g tissue^{-1}	% dose radioactivity in compartment	% dose radioactivity · g tissue^{-1}
Adrenals	0.07	1.80	0.01	0.19
Bladder	0.00	0.02	0.00	0.08
Brain	0.02	0.02	0.01	0.01
Eyes	0.08	0.36	0.04	0.19
Gonadal adipose	0.03	0.04	0.01	0.02
Heart	0.02	0.06	0.01	0.04
Kidneys	0.10	0.16	0.02	0.04
Large intestine	6.87	0.85	0.90	0.11
Liver	2.36	0.76	3.20	1.04
Lungs	0.11	0.32	0.01	0.05
Muscle	0.07	0.02	0.02	0.00
Peritoneal adipose	0.02	0.13	0.00	0.01
Prostate-seminal vesicle complex	0.03	0.10	0.01	0.07
Serum	0.36	0.10	0.11	0.03
Skin	0.01	0.07	0.00	0.01
Small intestinal mucosa	0.33	1.34	0.09	0.24
Small intestine	6.95	0.99	1.12	0.15
Spleen	0.03	0.49	0.02	0.34
Stomach	0.21	0.40	0.26	0.48
Testes	0.03	0.03	0.01	0.01
Thymus	0.01	0.08	0.00	0.02

[1] Values are means, n = 2. LYC, lycopene; PE, phytoene; TP, tomato powder.
[2] In gerbils 24 h after ^{14}C-PE or ^{14}C-LYC dosing.

cleared from the liver at a rate such that dosing in Expt. 1 replaced metabolic losses but did not increase LYC concentrations. Evidence of extensive LYC metabolism in our model is supported by a recent report in which the 18.6-d plasma half-life of total radioactivity (from ^{14}C-LYC and ^{14}C-LYC metabolic products) in 2 men after consuming a microdose of ^{14}C-LYC was substantially longer than the 5-d plasma half-life of ^{14}C-LYC (47). Furthermore, the maximal serum concentration of total radioactivity in these men was 2.6-fold greater than the maximal serum concentration of radioactivity from ^{14}C-LYC (47). LYC metabolism remains poorly understood; however, LYC may be an in vivo substrate for the β,β-carotene 9′,10'-oxygenase or β,β-carotene 15,15'-monooxygenase enzymes (23,48,49). In the current study, we could not definitively identify ^{14}C-LYC metabolites in the liver tissue by HPLC-PDA, but LYC metabolites were previously detected in rat liver as well as human plasma (11,36,50).

Though intact PE was undetected in the testes and prostate-seminal vesicle complex in Expt. 1, in Expt. 2, these tissues actually accumulated a greater percent of ^{14}C-PE dose radioactivity than of ^{14}C-LYC (Table 3). This suggests PE is rapidly metabolized in these tissues or metabolites are retained or are taken up by these tissues. Previously, we found that in vitro prostate cancer cells accumulated polar ^{14}C-PE products, suggesting PE is metabolized in cells or non-enzymatic oxidative PE products are taken up by these cells (34). To the authors' knowledge, this is the first in vivo report suggesting that PE metabolites may be present in the prostate and testes.

Next, we found that all tissues except for the liver, bladder, and stomach, assayed in the Expt. 2, accumulated a greater percent of dose radioactivity from ^{14}C-PE than from ^{14}C-LYC (Table 3). To the authors' knowledge, this is the first report indicating that PE or its products were accumulated, and to a greater extent than LYC, in the eyes, muscle, brain, heart, thymus, intestinal tissues, and adipose (Table 3). Extensive extra-hepatic distribution of PE and/or its metabolites warrants future investigation to identify metabolites and characterize PE and metabolite bioactivities in these tissues.

^{14}C-PE was much more bioavailable than ^{14}C-LYC based on greater total body deposition (23% vs. 8%, respectively) (Table 3). As in previous studies of ^{14}C-LYC dosing in rats (33,39), a large percent of both the ^{14}C-PE and ^{14}C-LYC radioactivity was unrecovered. Detectable ^{14}C was found in the breath and urine of men who received ^{14}C-LYC microdoses within hours of consumption (47), and nearly 1% of dose radioactivity from LYC was detected in rat urine collected during 24 h after ^{14}C-LYC dosing (39). Although neither urine nor exhaled air was assayed in the current study, future tomato carotenoid mass-balance studies should measure radioactivity in these compartments.

Elevated plasma PE compared with LYC after dosing was likely due to several factors. First, Expt. 1 indicated a faster clearance rate of serum LYC than PE by steady serum LYC depletion during 24 h. Then, Expt. 2 indicated greater bioavailability of PE based on greater relative serum radioactivity response to ^{14}C-PE than ^{14}C-LYC. The relatively slower rate of PE clearance from the liver compared with LYC, suggested by a sustained elevation in liver PE concentrations after dosing (Fig. 2*D*) (Expt. 1), may have also contributed to a larger pool of intact PE for efflux into the circulation and subsequent uptake by extra-hepatic tissues. A much greater amount of ^{14}C from the ^{14}C-PE than from the ^{14}C-LYC dose was also recovered in intestinal tissues, suggesting greater enterocyte uptake and a slow release of PE over time may have also contributed to the sustained plasma PE concentrations observed in Expt. 1. Extensive metabolism of LYC may have caused the higher percent of ^{14}C-LYC dose radioactivity in the bladder, most likely in the form of water-soluble LYC metabolites in the urine (Table 3), as was previously observed in humans and rats (39,47).

Limitations of the current study extend from the amount of ^{14}C-labeled carotenoid available due to the expense and complexity of producing high yields of this unique and valuable tool. Thus, we are limited to small but strategic studies of limited statistical power to test bioavailability and tissue distribution. Methodological improvements to improve yield and the use of stable isotopes are already being made and will enhance the ability of investigators to elucidate the metabolism of bioactive carotenoids and their biological importance (25,31,51). Stable isotope carotenoids are better suited for future metabolite identification based on signal strength, potential for analysis in shared MS facilities, and safety in humans.

In summary, these results demonstrated that compared with LYC, PE was more bioavailable and more slowly cleared from serum and liver than LYC. Additionally, extensive extra-hepatic distribution of PE was shown. PE appears to be more bioavailable and more slowly cleared than LYC, which may suggest LYC is preferred to PE for enzymatic cleavage. These pharmacokinetic differences likely underlie the phenomenon of relatively lower amounts of dietary PE than LYC resulting in similar PE and LYC blood and tissue concentrations. Further studies to characterize the absorption of LYC and PE and identify the metabolites of LYC and PE should be pursued. Careful pharmacokinetic investigation of different dietary phytochemicals reveals that even structurally similar compounds are handled quite differently and likely result in different biological effects.

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