The Biodistribution of a Single Oral Dose of [14C]-Lycopene in Rats Prefed Either a Control or Lycopene-Enriched Diet

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ABSTRACT Lycopene (lyc) has emerged as a primary candidate for dietary interventions of prostate cancer; however, research regarding its absorption, tissue distribution, and metabolism is limited. Previously, we evaluated the biodistribution (3–168 h) of a single oral dose of 14C-lyc in rats prefed lyc for 30 d. The liver was the primary depot for lyc, and the 14C and 14C-polar products appeared in tissues as early as 3 h after dosing. In the current study, F344 rats (n = 48) were randomly assigned to 1 of 4 groups prefed either a control or lyc-enriched diet (0.25 g lyc/kg diet) for 30 d and killed at 5 or 24 h after receiving a single oral dose of 14C-lyc. The percentage of the 14C dose absorbed at 24 h was lower (5.5 ± 0.5%) in lyc-prefed (LP) rats than in control-prefed (CP) rats (6.9 ± 0.4%, P < 0.04). Hepatic total 14C and 14C-lyc in CP rats was greater than in LP rats at 24 h (P < 0.005). A portion of 14C was delivered to extrahepatic tissues as early as 5 h, irrespective of diet. Of the tissues analyzed, an increase in the percentage in 14C-polar products occurred between 5 and 24 h only in the prostate and seminal vesicles, suggesting increased accumulation of 14C-polar products in these tissues, irrespective of prior dietary treatment. These data suggest that lyc absorption, tissue uptake, and catabolism were affected by prefeeding and that lyc can be partially taken up by extrahepatic tissues from the postprandial triglyceride-rich fraction. J. Nutr. 135: 2212–2218, 2005.

KEY WORDS: lycopene • lycopene metabolites • tissue biodistribution • prostate • rats

The carotenoid lycopene (lyc),4 the red pigment in tomatoes, has emerged as a candidate for dietary interventions of prostate cancer (1–5) and cardiovascular disease (6–9). Among the North American population, lyc is often the primary carotenoid in human tissues and serum (10–12). Epidemiologic studies showed that the consumption of lyc-rich foods is inversely related to prostate cancer incidence (4,5,13). It was reported that the consumption of 7–10 servings/wk of vegetables is inversely related to prostate cancer incidence (4,5,13). In vitro studies provided additional evidence supporting antioxidant (14) and nonantioxidant (15) mechanisms by which lyc may protect the prostate. Lyc is a strong singlet-oxygen quencher; it is 10 times more potent than α-tocopherol and 2 times more potent than β-carotene (14). Its ability to efficiently quench singlet oxygen may be associated with its 11 conjugated double bonds. Lyc was also shown to inhibit cell cycle progression from the G0/G1 to the S phase (15), inhibit insulin-like growth factor-1 (IGF-1) (15,16), enhance gap junctional communication via connexin 43 (17), and activate the phase II detoxification enzyme, glutathione transerase (18); all of these may be associated with a decrease in the risk of cancer.

Despite broad interactions between lyc and prostate cancer risk, there has been limited research evaluating the absorption, biodistribution, and metabolism of lyc. Our laboratory previously investigated the time-course biodistribution of a single dose of 14C-lyc over 168 h in rats fed a lyc-containing diet for 30 d before dosing (19). Approximately 7% of the dose was absorbed, the liver was the primary depot for lyc, and 14C was detectable in all measured tissues 3 h after the dose. The results also suggested that a substantial percentage of the radiolabeled lyc in the prostate was metabolized. As a result of these findings, the current study was designed to compare the effects of prefeeding control or lyc-containing diets to rats on the absorption and appearance of lyc metabolites, cis and trans isomers of newly consumed 14C-lyc in tissues. We hypothesized that prefeeding lyc would reduce the absorption of a single 14C-lyc dose and would result in enhanced metabolism and increased production of lyc metabolites compared with control-prefed (CP) rats.

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4 Abbreviations used: ARE, antioxidant response element; 14C, 14C-lycopene; CP rats, control-prefed rats; GI, gastrointestinal tract; IGF-1, insulin-like growth factor-1; lyc, lycopene; LP rats, lycopene-prefed rats; PDA, photodiode array; SR-B1, scavenger receptor class B type 1; T-rad, total radioactivity; TRL, triglyceride-rich lipoprotein.
MATERIALS AND METHODS

Chemicals. Lyc (5% water dispersible lyc beadlets), radiolabeled lyc, and echinone (crystalline form) standards were gifts from DSM. The standards were solubilized in hexane and the absorbance was determined spectrophotometrically. Lyc was measured at 472 nm with an extinction coefficient \(E_{1cm}^{1%=3400}\) of 2450 (20). Echinone was measured at 452 nm with an extinction coefficient \(E_{1cm}^{1%=2158}\). HPLC-photodiode array (PDA) analyses determined the purity of lyc and echinone to be 97 and 98%, respectively. The specific activity of 6,7,6,7'-[¹⁴C]-lyc was equal to 6761 kBq/mg and was purified using a YMC C₁₀ preparatory column (details below). Scintillation cocktail fluid, Biosafe II (Research Products International) was utilized to quantify radioactive levels within tissues using either an on-line radioactive detector (β-gram detector, INUS Systems) or an off-line scintillation counter. DMSO was purchased from Mallinckrodt.

Diet and ¹⁴C-Lyc doses. Rats consumed an AIN-93G semipurified pelleted control or lyc-enriched diet (0.25 g lyc/kg diet; DYETS) ad libitum throughout the study. The composition of the vitamin and mineral mixes was described previously (21). The lyc administered was purified to eliminate impurities, mainly major products. The purified dose was >98% pure and was composed of 90%, all-trans + 5-cis lyc and 8.4% other cis isomers of lyc. The radioactive lyc dose was prepared by solubilizing 6,7,6,7'-[¹⁴C]-lyc and an equivalent amount of nonradioactive lyc (250 g of control diet) in 25 mL of control oil for a final specific activity of 2775 kBq/μg lyc, detailed earlier (19). Each rat was administered 421.8 kBq of ¹⁴C-lyc, which provided 0.152 mg total lyc (including ¹⁴C-lyc and nonradioactive lyc). Rats consumed ~8 mg lyc/d from the diet, which provided a daily lyc intake that was ~50 times greater than that provided in the dose. A medium-sized raw tomato (~100 g) contains ~3 mg of lyc; thus, the amount of lyc consumed daily from the diet was ~2.7 tomatoes, and the amount of lyc dose was equivalent to about a slice of a tomato.

Preparatory HPLC method. The HPLC system used to purify and quantify the ¹⁴C-lyc was comprised of a Waters 991 PDA detector (Millipore), a Rainin Dynamics gradient pump system model SD-200, a Varian Prostar model 210, and a YMC C₁₀ Preparatory 10×250-mm column. The separation of all-trans-lyc, other cis-lyc, and polar impurities was accomplished using a modified method described by Yeum et al. (22). The modified gradient procedure had a flow rate of 4.73 mL/min. It was monitored from 250 to 550 nm and had the following gradient: 10% B held for 8.33 min, 20 min linear gradient to 65% B, 20 min linear gradient to 95% B, 1.7 min hold at 95% B, 1.7 min linear gradient to 10% B, and 5.3 min hold at 10% B for a final time of 57 min. The cis and trans isomers of lyc present in the ¹⁴C-dose were collected into a glass vial for purification and then dried in a Speedvac concentrator (model AS160; Savant).

Animals and study design. The University of Illinois Laboratory Animal Care and Use Committee approved the animal protocol and all principles of laboratory care were followed. Male F344 rats (n = 48) were purchased from Harlan. Rats were housed individually in wire-bottomed cages in a controlled facility and were acclimated to their new environment. At 40 d of age, rats were assigned to 4 groups (n = 12) based on body weight (113 ± 2 g). They were weighed and provided with fresh AIN-93G control or AIN-93G-lyc-enriched diet (0.25 g lyc/kg) (22) every other day for 30 d. Treatment groups were assigned as follows: 1) control diet for 30 d and killed 5 h after the ¹⁴C dose; 2) control diet for 30 d and killed 24 h after the ¹⁴C dose; 3) lyc diet for 30 d and killed 5 h after the ¹⁴C dose; 4) lyc diet for 30 d and killed 24 h after the ¹⁴C dose. At 70 d of age, rats weighed 254 ± 7 g; they were deprived of food for 6 h and then orally intubated with a single dose of 421.8 kBq of ¹⁴C-labeled control in 0.5 mL cornseed oil. Immediately after dosing, rats were placed in metabolic cages to facilitate urine and feces collection at 3, 6, and 24 h and were provided with their respective diets. Termination procedures (CO₂ asphyxiation), tissue collection, and storage methods were followed as previously described (19).

Analysis of total ¹⁴C in tissues, serum, and feces. Total ¹⁴C in tissues, serum, and feces was measured as described previously (19).

Analytical method for quantifying nonradioactive and radioactive chemical forms of lyc and lyc metabolites. The HPLC-PDA system was used to measure the nonradioabeled lyc concentrations in tissue and serum samples as previously described (19,22,24). A β-gram online radioactive detector was placed in series with an HPLC-PDA, which allowed for measurement of ¹⁴C-lyc and ¹⁴C-labeled products. The analytical HPLC analysis was identical to the preparatory HPLC method, except that an analytical YMC C₁₀ column (4.6 × 50 mm) was used. The gradient method was followed as described by Yeum et al. (22). The β-gram method included mixing 3 mL/min of Biosafe II with the eluent from the HPLC system (1 mL/min) and collecting radioactivity data every 6 s for a total of 38 min/sample. To maximize accuracy and reliability for recovery of radioactivity in the testes and serum, 2 samples within groups were pooled, resulting in a sample size of 6. For analysis of nonradioactive lyc in the prostate, adrenals, and seminal vesicles, 6 rats/group were pooled, resulting in a sample size of 2. The 2 samples for each tissue were analyzed individually for lyc using HPLC, and the eluent for the 2 samples was collected into the same vial in 30-s intervals, resulting in a sample size of 1 for radioactive analysis of the prostate, adrenals, and seminal vesicles using an external scintillation counter. Serum total radioactivity was calculated by assuming that serum accounts for 5.46 g/100 g total body weight (25).

Assignment of all-trans, cis-lyc and ¹⁴C-labeled products. Standards, spectra, and literature were used to identify the HPLC peaks (20,22,23,26,27). Peaks were combined into the following 3 groups: hexane soluble polar products (1–5.5 min); other cis isomers, such as 9-, 13-, and 15-cis lyc (20.5–26 min); and all-trans + 5-cis lyc (27–30 min).

Statistical analysis. The experiment was conducted as a post-hoc 2 × 2 factorial arrangement of treatments with 2 diets (control and lyc) and 2 times (5 or 24 h). The method of statistical analysis used was multiple linear regression in which the full model consisted of the main effects and interaction effects. The following regression equation was used: \( Y = B_0 + B_1 (\text{time}) + B_2 (\text{diet}) + B_3 (\text{time} \times \text{diet}) \). The data were log₁₀ transformed when the homogeneity of variance assumption was violated. If the interaction term was not significant at \( P < 0.05 \), it was removed and the model equation used was \( Y = B_0 + B_1 (\text{time}) + B_2 (\text{diet}) \). The model, main effects, and interactions were considered significant when \( P \leq 0.05 \). In addition, to assess the differences between dietary groups at a specific time point, t tests were performed where indicated in the text, and differences were considered significant when \( P < 0.05 \). Due to the small weight of tissues, prostate, adrenals, and seminal vesicles were pooled within groups for quantification of nonradioactive lyc (n = 2) or radioactive lyc (n = 1) for each tissue; thus statistical analysis could not be performed for these tissues. Values are means ± SEM.

RESULTS

Food intake and weight gain. Food intake and weight gain did not differ between treatment groups during the 30 d prefeding period (data not shown). No adverse effects of diet or dose administration were apparent other than the red color observed in the liver and feces of lyc-prefed (LP) rats.

Accumulation of nonradioabeled lyc in liver, prostate and serum. As early as 5 h, all tissues in both LP and CP rats contained measurable lyc. Serum total lyc increased over time in both dietary groups (Table 1); however, the concentration was greater in LP rats than in CP rats, irrespective of time (\( P < 0.05 \)). The hepatic lyc concentration in CP rats was significantly less than in the LP rats, irrespective of time (\( P \leq 0.05 \)). Hepatic lyc increased from 5 to 24 h in CP rats (\( P \leq 0.05 \)) but not in LP rats (Table 1).

\* Composition of modified AIN-93G purified placebo- and lycopene-containing diets are described (g/kg diet): 200 g casein, 579.83 g corn starch, 115.75 g dextrose, 100 g sucrose, 50g cellulose, 100 g soybean oil, 0.02 g t-butylihydroquinone, 35 g salt mix #2100251, 10 g vitamin mix #3100251, 3 g c-lysine and 1.4 g choline chloride. The placebo diet also included 5 g placebo beadlets which are identical in composition to the lycopene beadlets but do not contain lycopene. The lycopene-containing diet included 5 g of lycopene beadlets which are composed of 5 g lycopene/100 g beadlets. The vitamin and mineral mix compositions are described in (21).
Absorption, excretion, and recovery of the $^{14}$C-dose. Each rat was administered 421.8 kBq of $^{14}$C-lyc in 0.5 mL of cottonseed oil. The percentage of the $^{14}$C dose absorbed at 24 h in the LP rats was 5.5 ± 0.5%, which was less than the 6.9 ± 0.4% absorbed in CP rats ($P < 0.04$). The total recovery of the dose from all tissues, intestinal contents, urine, and feces was 75 ± 6 and 57 ± 6.5% from LP and CP rats, respectively ($P < 0.03$).

Elimination of radioactivity from rats was primarily through the feces, with a minor portion eliminated through urine. The percentage of the $^{14}$C dose excreted through feces in the LP and CP rats, respectively. The percentage of the $^{14}$C dose excreted through urine was 0.088 ± 0.009 and 0.091 ± 0.0087% in LP and CP rats, respectively. We assumed that all of the $^{14}$C in urine was absorbed and metabolized $^{14}$C-labeled metabolic products.

Total radioactivity in the gastrointestinal tract, serum, and tissues. The gastrointestinal (GI) tract was divided into the stomach wall, small intestinal wall, cecum, large intestinal wall, stomach contents, small intestinal contents, and large intestinal contents. Total radioactivity (T-rad) in each section of the GI-tract wall (Table 2) changed over time; i.e., it decreased in the stomach and increased in the cecum and large intestine. The concentration of T-rad in the stomach contents of LP rats was greater ($P < 0.01$) than that of CP rats, irrespective of time. When both time points were considered,

### TABLE 1

<table>
<thead>
<tr>
<th>Diet</th>
<th>Time</th>
<th>Serum</th>
<th>Hepatic tissue</th>
<th>Seminal vesicles</th>
<th>Dorsolateral plus anterior prostate</th>
<th>Ventral prostate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>h</td>
<td>nmol/L</td>
<td>nmoL/g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LP</td>
<td>5</td>
<td>314 ± 58</td>
<td>116 ± 5.6</td>
<td>0.063</td>
<td>0.18</td>
<td>0.18</td>
</tr>
<tr>
<td>CP</td>
<td>5</td>
<td>2.3 ± 0.87</td>
<td>0.47 ± 0.03</td>
<td>0.012</td>
<td>0.05</td>
<td>0.06</td>
</tr>
<tr>
<td>LP</td>
<td>24</td>
<td>600 ± 97</td>
<td>107 ± 6.8</td>
<td>0.101</td>
<td>0.24</td>
<td>0.19</td>
</tr>
<tr>
<td>CP</td>
<td>24</td>
<td>6.9 ± 0.63</td>
<td>0.64 ± 0.03</td>
<td>0.026</td>
<td>0.05</td>
<td>0.04</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM.  
2 Significant increase over time in both dietary groups, $P < 0.05$. Significantly greater in LP rats than CP rats, regardless of time, $P < 0.05$, $n = 6$.  
3 Significantly greater in LP rats than CP rats, irrespective of time, $P < 0.05$. The increase in hepatic lyc over time was significant in CP rats, $P < 0.05$, $n = 12$.  
4 $n = 2$.

### TABLE 2

<table>
<thead>
<tr>
<th>Tissue</th>
<th>CP-5 h</th>
<th>LP-5 h</th>
<th>CP-24 h</th>
<th>LP-24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>kBq/L</td>
<td>kBq/L</td>
<td>kBq/g</td>
<td>kBq/g</td>
</tr>
<tr>
<td>Stomach contents</td>
<td>2988 ± 541</td>
<td>4755 ± 677</td>
<td>747 ± 307</td>
<td>805 ± 228</td>
</tr>
<tr>
<td>Small intestinal contents</td>
<td>412 ± 118</td>
<td>201 ± 176</td>
<td>343 ± 148</td>
<td>234 ± 72</td>
</tr>
<tr>
<td>Large intestinal contents</td>
<td>915 ± 155</td>
<td>370 ± 64</td>
<td>3634 ± 401</td>
<td>2558 ± 670</td>
</tr>
<tr>
<td>Cecum</td>
<td>564 ± 224</td>
<td>318 ± 45</td>
<td>1365 ± 248</td>
<td>1333 ± 320</td>
</tr>
<tr>
<td>Serum</td>
<td>32 ± 4</td>
<td>37 ± 6</td>
<td>104 ± 22</td>
<td>108 ± 24</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, $n = 12$.  
2 Significant effect of time, $P < 0.05$.  
3 Significant effect of diet, $P < 0.05$.  
4 Significant effect of diet $\times$ time, $P < 0.05$.
CP rats contained more T-rad/L in the large intestinal contents (P < 0.002) than LP rats.

Hepatic T-rad concentration was greater (P < 0.01, t test) in CP rats at 24 h compared with LP rats. The serum T-rad in both groups increased between 5 and 24 h after administration of the dose (P < 0.003).

All extrahepatic tissues examined contained measurable amounts of T-rad at both time points, but the concentrations varied depending on the tissue and time (Table 2). Diet affected the distribution of T-rad in the spleen, heart, and hindbrain in that CP rats had more T-rad than LP rats, irrespective of time (P < 0.05). Although the T-rad in the spleen increased from 5 to 24 h in both dietary groups (P < 0.05), CP rats had more T-rad at 24 h than LP rats (P < 0.02, t test).

At 5 h after dosing, levels of T-rad in the prostate, adrenals, and seminal vesicles were low but detectable in both groups (Fig. 1). Although the seminal vesicles were at least 3 times larger in size than the prostate and adrenals, the T-rad/g in the prostate and adrenal was at least 2 times greater than the T-rad in the seminal vesicles.

\[ 14^C \text{-lyc and } 14^C \text{-polar products in the GI tract and tissues.} \]

The radioactivity associated with \( 14^C \)-all-trans and cis isomers of lyc (collection of 20–30 min from HPLC) or hexane-soluble \( 14^C \)-polar products of \( 14^C \)-lyc (collection from 1 to 5.5 min) and the individual percentages of these \( 14^C \)-products were measured in the GI tract, serum, tissues, and feces (Table 3). It is noteworthy that the total concentration of \( 14^C \)-lyc + \( 14^C \)-polar products increased substantially from 5 to 24 h in the liver (P < 0.0001). Levels tended to increase in the mucosal cells (P < 0.08) and serum (P < 0.1) between 5 and 24 h. However, the percentage of \( 14^C \)-polar products was largely unchanged over time in these tissues irrespective of prior dietary treatment. Although it was not possible to analyze these data statistically, the total concentration of \( 14^C \)-lyc + \( 14^C \)-polar products decreased from 5 to 24 h in the prostate and seminal vesicles, but the percentage of \( 14^C \)-polar products increased, irrespective of diet. The amount of \( 14^C \)-lyc + \( 14^C \)-polar products also decreased with time in the adrenal in both dietary groups. The predominant forms of \( 14^C \) in the adrenal glands and feces were \( 14^C \)-polar products regardless of the diet consumed (Table 3).

The small intestinal contents of LP rats had greater \( 14^C \)-lyc + \( 14^C \)-polar product concentrations than the CP rats, irrespective of time (P < 0.0001, Table 3). Interestingly, the small intestinal wall contained an equal amount of \( 14^C \)-lyc and \( 14^C \)-polar products in both dietary groups (Table 3). The concentration of polar products in serum was approximately the same as that of the other \( 14^C \)-cis isomers in both treatment groups, irrespective of time.

**DISCUSSION**

The protective effects of tomato consumption on prostate cancer (4,5) and cardiovascular disease risk (6–9,28) have often been attributed to its primary carotenoid, lyc. Despite this enthusiasm, fundamental research in the area of uptake, tissue distribution, and metabolism of lyc is limited. The current study was designed to determine whether absorption and biodistribution of total \( 14^C \)-lyc and \( 14^C \)-polar products were altered by prior lyc consumption. Here, we examined the effect of feeding either a control or lyc-enriched diet for 30 d before administration of a single oral dose of \( 14^C \)-lyc.

The following can be concluded from the data presented: 1) the surfet of lyc in the GI tract and in tissues of LP rats resulted in decreased absorption of a single oral dose of \( 14^C \)-lyc; 2) prefeeding lyc resulted in differential concentrations of \( 14^C \), and to a lesser extent of \( 14^C \)-lyc isomers or \( 14^C \)-polar products in tissues; and 3) compared with other tissues, there was a percentage increase in the \( 14^C \)-polar products in the prostate and seminal vesicles, suggesting increased accumulation of \( 14^C \)-polar products in these tissues, irrespective of prior dietary treatment. In addition, these data along with findings from the previous study (19) suggest that extrahepatic tissues take up \( 14^C \)-lyc from the postprandial triglyceride-rich lipoprotein (TRL) fraction.

We evaluated total \( 14^C \) in tissues by solubilizing tissue samples with tissue solubilizer to measure T-rad (Table 2). In addition, we evaluated the hexane-soluble extract from tissues, serum, and feces to enable us to focus on the lyc and hexane-soluble lyc metabolites. For example, ~67% of the T-rad in the liver of LP rats was accounted for in the hexane extract (data not shown), and hexane-soluble compounds in the feces of LP and CP rats accounted for only 10% and 27% of the total, respectively. Separation and analysis of more \( 14^C \)-polar products from the ethanol:water extract was not carried out.

**Differential concentrations of T-rad among tissues.**

Prefeeding lyc resulted in differential concentrations of radioactivity in the small intestinal mucosa, spleen, heart, and hindbrain, and LP rats contained less radioactivity than CP rats. Of the postabsorptive tissues analyzed, the liver and spleen contained the greatest concentration of T-rad/g, irrespective of diet. The liver weight was considerably higher than that of the spleen (~16 times greater in this study); thus, T-rad was much greater in whole-liver tissue. The differential uptake of lyc into tissues is likely due to both LDL receptor–dependent (10,12) and LDL receptor–independent pathways, as seen with \( \alpha \)-tocopherol.

\( \alpha \)-Tocopherol tissue uptake is affected by the presence of scavenger receptor class B type 1 (SR-B1). SR-B1 is responsible for selective uptake of lipids into tissues (29); it is highly concentrated in human and rat liver and in steroidogenic tissues (29,30).

In SR-B1–deficient mice, \( \alpha \)-tocopherol uptake into the liver, spleen, kidney, and adipose tissue was reduced (31).
TABLE 3
Concentration of \(^{14}\)C-lyc plus \(^{14}\)C-polar products and the individual percentages of these \(^{14}\)C-products in the GI tract, tissues, and feces of rats prefed a lyc-enriched (LP) or control (CP) diet for 30 d before administration of a single oral dose of \(^{14}\)C.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Diet</th>
<th>Time</th>
<th>(^{14})C-polar metabolites</th>
<th>Total (^{14})C-polar metabolites</th>
<th>All-trans + 5-cis (^{2})</th>
<th>Other cis lyc (^{2})</th>
<th>Polar metabolites (^{2})</th>
<th>% of total (^{14})C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small intestinal contents</td>
<td>CP</td>
<td>5</td>
<td>19 ± 7</td>
<td>34 ± 2</td>
<td>31 ± 2</td>
<td>36 ± 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>LP</td>
<td>5</td>
<td>334 ± 47</td>
<td>41 ± 2</td>
<td>34 ± 2</td>
<td>25 ± 1</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>CP</td>
<td>24</td>
<td>22 ± 3</td>
<td>36 ± 3</td>
<td>36 ± 1</td>
<td>27 ± 3</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>LP</td>
<td>24</td>
<td>247 ± 20</td>
<td>40 ± 2</td>
<td>38 ± 1</td>
<td>23 ± 1</td>
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<tr>
<td>Small intestinal wall</td>
<td>CP</td>
<td>5</td>
<td>124 ± 33</td>
<td>52 ± 3</td>
<td>35 ± 1</td>
<td>14 ± 2</td>
<td></td>
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<tr>
<td></td>
<td>LP</td>
<td>5</td>
<td>131 ± 29</td>
<td>53 ± 3</td>
<td>32 ± 2</td>
<td>15 ± 2</td>
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</tr>
<tr>
<td></td>
<td>CP</td>
<td>24</td>
<td>138 ± 26</td>
<td>45 ± 3</td>
<td>35 ± 2</td>
<td>20 ± 3</td>
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<tr>
<td></td>
<td>LP</td>
<td>24</td>
<td>158 ± 24</td>
<td>53 ± 2</td>
<td>32 ± 2</td>
<td>15 ± 1</td>
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<tr>
<td>Serum</td>
<td>CP</td>
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<td>52 ± 6</td>
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<td>LP</td>
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<td>57 ± 17</td>
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<td>90 ± 36</td>
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<td>LP</td>
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<td>72 ± 30</td>
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<tr>
<td>Liver</td>
<td>CP</td>
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<td>362 ± 32</td>
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<td>26 ± 1</td>
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1 Values are means ± SEM; liver, feces, small intestinal wall and contents, n = 12; serum, n = 6.
2 n = 1.
3 Significantly greater \(^{14}\)C-lyc + \(^{14}\)C-polar metabolites in LP rats than CP rats, \(P < 0.001\). Prefeeding lyc increased \(^{14}\)C-polar product concentration and reduced the percentage of polar products compared with the CP rats at 5 and 24 h, \(P < 0.0001\).
4 Significant increase in \(^{14}\)C-lyc + \(^{14}\)C-polar metabolites over time, \(P < 0.001\).
5 Based on HPLC retention time of standards, peaks were combined into 3 groups: polar products (1–5.5 min); other cis lyc isomers (20.5–26 min); and all-trans + 5-cis lyc (27–30 min).
6 Accumulation of hexane soluble \(^{14}\)C-polar metabolites of lyc in the prostate and seminal vesicles in the 2 treatment groups. Interestingly, the percentage of \(^{14}\)C-polar products increased over time in the prostate and seminal vesicles, such as \(\beta\)-carotene, lutein, and zeaxanthin were thought to utilize a binding and/or transport protein for absorption and/or uptake into tissues (32–37). Although a binding protein specific to lyc has not yet been verified, it is plausible that one exists, which could explain the preferential uptake of \(^{14}\)C in some tissues of CP rats.

This suggests that the selective tissue uptake of \(\alpha\)-tocopherol may be the result of 2 separate pathways, and the predominant pathway for uptake is dependent on tissue type. These findings are interesting because in the current study, the liver, spleen, kidney, and androgen-dependent tissues were the tissues most highly concentrated with T-rad, which suggests selective uptake of T-rad into tissues, potentially mediated through 2 separate pathways (SR-B1 and LDL receptor).

The observed concentration differences in tissues may also suggest the presence of a lyc-binding protein. Carotenoids such as \(\beta\)-carotene, lutein, and zeaxanthin were thought to utilize a binding and/or transport protein for absorption and/or uptake into tissues (32–37). Although a binding protein specific to lyc has not yet been verified, it is plausible that one exists, which could explain the preferential uptake of \(^{14}\)C in some tissues of CP rats.
whereas the total concentration of \(^{14}C\)-lyc + \(^{14}C\)-polar products decreased, irrespective of diet (note that these were pooled samples due to small tissue weights). This suggests an increased accumulation of \(^{14}C\)-polar products in the prostate and seminal vesicles, but not in the liver or mucosal cells, where the percentage of \(^{14}C\)-polar products was unchanged over time. The \(^{14}C\)-lyc + \(^{14}C\)-polar products also decreased in the adrenals in both groups, but the percentage of \(^{14}C\)-polar products, which was already high, did not change.

We previously reported that polar products of \(^{14}C\)-lyc appeared in the prostate of rats prefed lyc for 30 d as early as 3 h after the single oral dose (19). We suggested that in addition to the singlet-oxygen quenching capacity of intact lyc, the \(^{14}C\)-polar products in the prostate may act at the gene level to modulate the expression of androgen-related genes (19). A recent publication indirectly supported this hypothesis by demonstrating that after \(\sim 60\) d of lyc feeding to Copenhagen rats, there was depressed expression of several androgen-metabolizing enzymes, IGF-I, and a number of basal inflammatory signals in the prostate compared with control-fed rats (38).

Although the authors of that study did not suggest that the observed transcriptional changes were a result of lyc metabolites, we suggest that transcriptional regulation is more likely to occur as a result of lyc metabolites than intact lyc. The lyc concentration in the total rat prostate tissue in their study (38) was \(0.46\) nmol/g, essentially identical to that in the current study (0.43 nmol/g at 24 h).

**Uptake of \(^{14}C\) from triglyceride-rich fractions into extrahepatic tissues.** Within the enterocyte of the small intestine, lyc is incorporated into the chylomicrons and VLDL (TRL fraction) and then secreted into the lymph system for transportation into the bloodstream (39). Upon entering the bloodstream, the activation of lipoprotein lipase results in the delipidation of the TRL fraction, before the uptake of chylomicron remnants by the liver. It was suggested that before hepatic uptake, carotenoids may be partially taken up by extrahepatic tissues during delipidation and passage through extrahepatic tissues (40).

The monitoring of carotenoids in the postprandial TRL has been a valuable tool with which to study the relative bioavailability and bioefficacy of carotenoids such as dietary \(\beta\)-carotene and newly formed retinol (41). Research showed that lyc is most concentrated in the postprandial TRL at 6 h in humans (42) and between 4 and 8 h in rats (43). Newly absorbed lyc is repackaged in the liver as VLDL and LDL and peaks in serum at \(\sim 24–27\) h in humans (42,44) and 24 h in rats (43). Because lyc is primarily in the postprandial TRL between 4 and 8 h in rats, the presence of \(^{14}C\) in tissues as early as 3 h [the previous study, (19)] and 5 h (the current study) strongly supports the hypothesis that TRL delivers a portion of the newly absorbed lyc to the extrahepatic tissues. Preliminary kinetic analysis using compartmental modeling of lyc absorption, distribution, and elimination in rats from our earlier study (19) suggests transport of lyc directly from TRL to extrahepatic tissues such as lung, adipose, adrenal, heart, kidney, testes, and prostate (45).

Lyc degradation products formed during freezer storage were shown to activate the antioxidant response element (ARE) in vitro (46), which suggests that metabolic products of lyc may also activate the ARE and potentially activate phase II detoxification enzymes (46). Furthermore, a potential lyc metabolite, acyclo-retinoic acid, was shown to activate the transcription of a retinoic acid receptor (47), a receptor responsible for modulating numerous target genes. The results from our biodistribution studies [(19) and current study] and molecular-based studies (38,46,47) support the theory that lyc and lyc metabolites may play a role in reducing the risk of prostate cancer by modulating the expression of relevant genes.

Our findings lead us to conclude that routine consumption of lyc decreases absorption and thus the accumulation of \(^{14}C\) from a single oral dose of \(^{14}C\)-lyc in most tissues. These data also support our earlier observation (19) that extrahepatic tissues can take up \(^{14}C\) from the postprandial TRL fraction. The relative accumulation of \(^{14}C\)-polar products and/or the rate of metabolism of \(^{14}C\)-lyc was greater in the prostate, seminal vesicles, and adrenals than in other tissues, regardless of dietary treatment. These results indirectly suggest that the absorption of \(^{14}C\), \(^{14}C\)-lyc, and \(^{14}C\)-polar products is a facilitated mechanism. Interestingly, we observed that androgen-dependent tissues and the spleen [related to immune function (10,12,48)] tended to have high concentrations of lyc. Ongoing research in our laboratory includes studies designed to identify lyc metabolites formed in vivo and to determine which enzymes are responsible for the observed catabolism of lyc.

**ACKNOWLEDGMENTS**

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**LITERATURE CITED**


