ABSTRACT: The effect of different oils on the absorption of carotenoids was investigated in mesenteric lymph duct cannulated rats. Sixteen treatment emulsions containing increasing concentrations of either lycopene (LYC) or astaxanthin (AST) (5, 10, 15, 20 µmol/L) were prepared with olive oil or corn oil and continuously infused into the duodenum of the rat. Absorption of carotenoids into the mesenteric lymph duct was determined. Absorption of LYC and AST from both oils increased with the amount infused into the duodenum. The average recovery of AST in the lymph from the olive oil emulsion was 20% but was decreased to 13% from emulsions containing corn oil. Lycopene was not as well absorbed as AST. The average recovery of LYC was 6% from olive oil emulsions but only 2.5% when infused with corn oil. The LYC used in this study was isolated from tomato paste and was primarily in the all-trans form. We did not observe any significant isomerization of all-trans LYC to 9-cis LYC during absorption. We conclude that the type of oil with which a carotenoid is consumed can influence its absorption.


A Comparison of Lycopene and Astaxanthin Absorption from Corn Oil and Olive Oil Emulsions

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Compared to other dietary lipids, carotenoids are not well absorbed, and factors influencing carotenoid absorption are poorly understood (see recent reviews 1–4). Because carotenoids are lipid soluble, the amount and type of lipid with which they are consumed may influence their absorption. Several studies have shown that concurrent consumption of dietary lipid significantly increases carotenoid absorption (5–8). Although the presence of dietary lipid appears to be a key factor in carotenoid absorption, information on the effect of different types of lipid on absorption of carotenoids is relatively sparse.

Hollander and Ruble (9) measured the disappearance of β-carotene from micellar perfusates in rat intestinal loops. The disappearance rate from perfusates containing polyunsaturated fatty acids (PUFA) (linoleic acid and linolenic acid) was lower than when oleic acid was added to the perfusate (9). In assuming that disappearance rates are an index of absorption, these results suggest that the unsaturated fatty acid composition of a diet can influence carotenoid absorption. The purpose of this study was to further investigate the influence of dietary lipid on carotenoid absorption by comparing the absorption of lycopene (LYC) and astaxanthin (AST) from a polyunsaturated triacylglycerol emulsion containing corn oil to their absorption from a monounsaturated triacylglycerol emulsion made with olive oil.

MATERIALS AND METHODS

Animals and surgical procedure. Male Holtzman albino rats obtained from Harlan Sprague Dawley (Indianapolis, IN) and weighing 300–350 g at the time of surgery were used. A feeding tube was placed into the duodenum and the major mesenteric lymph duct was cannulated. Surgery and animal care were conducted as previously described (10) and were approved by The University of Connecticut Institutional Animal Care and Use Committee.

Following surgery, the animals were placed in a warm, dark environment and allowed to recover for about 36 h. During recovery animals had access to water and received intraduodenally a glucose/electrolyte solution (Pedialyte; Ross Laboratories, Columbus, OH) at 2.0 mL/h. After recovery, treatment emulsions were infused into the duodenum for 12 h at a rate of 2.0 mL/h. The lymph was collected during the final 6 h of infusion for analysis. Lymph was stored at −70°C until analyzed.

Preparation of carotenoid stock solutions. Stock solutions of the two carotenoids were prepared and used within 48 h. Lycopene was purified from tomato paste on a 5% water-weakened alumina column eluted with hexane/ethyl acetate (95:5 vol/vol). Solvent was removed under reduced pressure and a stock solution of LYC prepared in dichloromethane. Astaxanthin was a gift from Dr. Harry Frank (University of Connecticut, Storrs, CT). A stock solution of AST also was prepared with dichloromethane. The concentration of carotenoid in each stock solution was determined by absorption spectrophotometry. Astaxanthin concentration was estimated at 466 nm (ε<sub>1% cm</sub> = 2135) and LYC concentration was estimated at 472 nm (ε<sub>1% cm</sub> = 3450) (11).

Treatment emulsions. The basic treatment emulsion consisted of a buffer solution (115 mmol/L NaCl, 5.0 mmol/L KCl, 6.8 mmol/L Na<sub>2</sub>HPO<sub>4</sub> · 2H<sub>2</sub>O, and 16.5 mmol/L NaH<sub>2</sub>PO<sub>4</sub>), 10 mmol/L sodium taurocholate (Sigma Chemical, St. Louis, MO) and 3.0% (wt/vol) olive oil or tocopherol-stripped corn oil (ICN, Costa Mesa, CA) with different concentrations of AST or LYC.

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Abbreviations: AST, astaxanthin; CRBP, cellular retinol-binding protein type II; HPLC, high-pressure liquid chromatography; LYC, lycopene; PUFA, polyunsaturated fatty acid.
Treatment emulsions were prepared by placing olive oil or corn oil in a round-bottomed flask and adding an appropriate amount of carotenoid stock solution to the oil. The carotenoid, either AST or LYC, and oil were mixed, and the solvent was removed with a stream of nitrogen. Sodium taurocholate and buffer were added to the lipid/carotenoid mixture. The contents of the flask then were emulsified using a probe sonicator producing approximately 40 watts output for 15 s, repeated four or five times until no lipid droplets were observed. An aliquot of the emulsion was extracted three times with hexane and the final concentration of carotenoid in the emulsion determined by high-pressure liquid chromatography (HPLC) as described below for lymph analysis.

**Carotenoid analysis of lymph.** Lymph samples were thawed to room temperature and an aliquot taken for analysis. To extract the carotenoids, dichloromethane/methanol (2:1, vol/vol) containing an internal standard, ethyl-β-apo-8′-carotenoate (Fluka, Ronkonkoma, NY), and lymph were placed in a separatory funnel at a solvent to lymph ratio of 9:1 (vol/vol) and stored in the dark at 4°C for 4–6 h. The bottom phase was removed and saved. Methanol at 1.5 times and dichloromethane at six times the original volume were added to the upper phase. The separatory funnel was again stored for several hours in the dark at 4°C. After phase separation the bottom phase was removed and combined with the original bottom phase. Preliminary studies showed that further extraction of the upper phase with a salt solution and solvents, as called for in the original extraction method of Folch et al. (12), did not improve the recovery of carotenoids from lymph.

Samples were prepared for injection into the HPLC by removing the solvent from the lipid extract under reduced pressure and redissolving the residue in dichloroethane/2-propanol (1:1 vol/vol). Carotenoids then were separated by HPLC using a Waters C18 Resolve column (15 cm × 3.9 mm; Millipore, Milford MA.) with an Upchurch C18 guard column (Upchurch Scientific, Oak Harbor, WA). An isocratic mobile phase consisting of acetonitrile/dichloromethane/methanol/n-butanol/ammonium acetate (90:15:10:0.1:0.1, by vol) was used for analysis of LYC and an isocratic mobile phase of methanol/water (98:2, vol/vol) was used for AST analysis. The carotenoids and ethyl-β-8′-carotenate were identified and quantified at a wavelength of 450 nm.

### Fatty acid analysis of treatment emulsions and lymph

A 200-µL aliquot of treatment emulsion or lymph was used for fatty acid analysis. Fatty acids in the aliquot were prepared for analysis by direct transmethylation in methanol/hexane (4:1, vol/vol) in the presence of acetyl chloride (13). The fatty acid methyl esters were separated by gas–liquid chromatography on a Supelcowax 10 fused-silica capillary column (30 m, 0.53 mm i.d.: Supelco, Bellefonte, PA), and identification of individual fatty acids was based on comparison of retention times with known standards.

**Statistical arrangement of treatments.** This study was a completely randomized design with treatments in a $2 \times 2 \times 4$ factorial arrangement. The 16 treatment emulsions contained either olive oil or corn oil with one of four different concentrations of LYC or AST (5, 10, 15, 20 µmol/L). There were three rats per treatment. Data were analyzed as a three-way analysis of variance using the General Linear Model procedure in SAS (14).

### RESULTS AND DISCUSSION

The carotenoids used in this study were chosen because they have very different physical properties. Astaxanthin, a $3′$ dihydroxy-4,4′-diketo derivative of β-carotene, was selected because it is a polar xanthophyll. Astaxanthin is a major carotenoid in marine animals with strong quenching activity against singlet oxygen and is an active scavenger of reactive oxygen species (15). Although AST is important in aquaculture, very little is known about its absorption by mammals. Mice fed a diet containing AST have significant levels of AST in the plasma which suggests that AST is readily absorbed (16). The second carotenoid used in this study was LYC, a nonpolar open-chained isomer of β-carotene associated with numerous health benefits such as prevention of cardiovascular disease and cancers of the prostate or gastrointestinal tract (17).

The fatty acid composition of a representative test emulsion containing each of the oils and the lymph collected from the rats intraduodenally infused with each of these emulsions is shown in Table 1. The fatty acids in the lymph reflect the fatty acids of the treatment emulsions. However, the lymph contained a greater proportion of palmitic acid and arachidonic

### TABLE 1

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Treatment emulsions</th>
<th>Mesenteric lymph</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Olive oil</td>
<td>Corn oil</td>
</tr>
<tr>
<td>16:0</td>
<td>12.0</td>
<td>11.2</td>
</tr>
<tr>
<td>16:1n-7</td>
<td>0.8</td>
<td>0.2</td>
</tr>
<tr>
<td>18:0</td>
<td>3.8</td>
<td>2.2</td>
</tr>
<tr>
<td>18:1n-9</td>
<td>65.3</td>
<td>26.3</td>
</tr>
<tr>
<td>18:2n-6</td>
<td>14.5</td>
<td>58.2</td>
</tr>
<tr>
<td>18:3n-3</td>
<td>0.8</td>
<td>0.9</td>
</tr>
<tr>
<td>20:4n-6</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

*Values are weight percentage (wt%) distributions of fatty acid methyl esters.*