Keto-Carotenoids Are the Major Metabolites of Dietary Lutein and Fucoxanthin in Mouse Tissues

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Abstract

Fucoxanthin, a xanthophyll present in brown algae consumed in Eastern Asia, can suppress carcinogenesis and obesity in rodents. We investigated the metabolism, tissue distribution, and depletion of fucoxanthin in ICR mice by comparison with those of lutein. The experiments comprised 14-d dietary supplementation with lutein esters or fucoxanthin, followed by 41- or 28-d, respectively, depletion periods with carotenoid-free diets. After lutein ester supplementation, 3′-hydroxy-e,e-caroten-3-one and lutein were the predominant carotenoids in plasma and tissues, accompanied by e,e-carotene-3,3′-dione. The presence of these keto-carotenoids in mouse tissues is reported here for the first time, to our knowledge. Lutein and its metabolites accumulated most in the liver (7.51 μmol/kg), followed by plasma (2.11 μmol/L), adipose tissues (1.01–1.44 μmol/kg), and kidney (0.87 μmol/kg). The half-life of the depletion (t_{1/2}) of lutein metabolites varied as follows: plasma (1.16 d) < liver (2.63 d) < kidney (4.44 d) < adipose tissues (>41 d). Fucoxanthinol and amarouciaxanthin A were the main metabolites in mice fed fucoxanthin and partitioned more into adipose tissues (3.13–3.64 μmol/kg) than into plasma, liver, and kidney (1.29–1.80 μmol/kg). Fucoxanthin metabolites had shorter t_{1/2} in plasma, liver, and kidneys (0.92–1.23 d) compared with those of adipose tissues (2.76–4.81 d). The tissue distribution of lutein and fucoxanthin metabolites was not associated with their lipophilicity, but depletion seemed to be slower for more lipophilic compounds. We concluded that mice actively convert lutein and fucoxanthin to keto-carotenoids by oxidizing the secondary hydroxy groups and accumulate them in tissues. J. Nutr. 140: 1824–1831, 2010.

Introduction

Epidemiologic studies have associated the consumption of carotenoid-rich foods with a reduced risk of cancer and cardiovascular diseases (1). Owing to their extended system of conjugated double bonds, the carotenoids can physically quench singlet oxygen and scavenge peroxyl radicals, alleviating the oxidative stress that is associated with the onset and progression of chronic diseases. Xanthophylls are carotenoids possessing at least 1 oxygenated functional group. Some of the major carotenoids found in the human diet belong to the xanthophyll class, such as lutein, zeaxanthin, and β-cryptoxanthin. Lutein (Fig. 1A) is the predominant carotenoid in egg yolks and yellow and green leafy vegetables. Lutein and zeaxanthin accumulate in the macula lutea of the retina and may play a protective role against age-related macular degeneration by filtering blue light, quenching singlet oxygen and/or acting as chain-breaking antioxidants (2). More polar xanthophylls such as capsanthin, astaxanthin, and fucoxanthin are present in paprika, salmon, and edible algae, respectively. Fucoxanthin is a marine carotenoid with an allenic bond and a 5,6-epoxide in the molecule (Fig. 1B). This xanthophyll is among the most abundant carotenoids in nature, found in brown algae largely consumed in Asian countries, such as wakame (Undaria pinnatifida) and hijiki (Hizikia fusiformis). Recent reports have shown that fucoxanthin inhibits proliferation of various cancer cell lines (3–5), suppresses angiogenesis ex vivo (6), and has antiinflammatory (7), antiobesity, and anti-diabetic (8,9) effects in mice and/or rats. We have found that fucoxanthin is hydrolyzed to fucoxanthinol in the intestinal tract and oxidized to amarouciaxanthin A by liver microsomal dehydrogenase in mice (10), suggesting that xanthophylls are actively metabolized in mammals.

To assess tissue distribution, metabolic transformations and rate of depletion of fucoxanthin in mice by comparison with those of a typical dietary xanthophyll, lutein, we conducted 2 experiments consisting of dietary supplementation with lutein or fucoxanthin, followed by depletion periods. We evaluated the rate of depletion of lutein, fucoxanthin, and their respective metabolites in plasma, liver, kidney, and adipose tissues, and...
identified 2 lutein metabolites whose presence in mice is reported here for the first time, to our knowledge.

Materials and Methods

Carotenoids. Lutein esters were isolated from a marigold extract suspended in soybean oil (Xangold 15%, a gift from Cognis, Tokyo, Japan; Supplemental Method 1) containing a minimum of 15% lutein esters and other minor carotenoids, which yielded, upon saponification, 7.57% lutein, 0.39% zeaxanthin, and 0.04% cryptoxanthin (data from the supplier). Fucoxanthin was isolated from dry wakame (Undaria pinnatifida) as previously published (11), with modifications (Supplemental Method 2).

HPLC standards of lutein (12), fucoxanthin (13), fucoxanthinol, and amarouciaxanthin A (10) were prepared as previously reported and lactucaxanthin ([3R,6R,3‘R,6‘R]-e,e-carotene-3,3‘-dione; alternative name ‘tunaxanthin F’) was isolated from lettuce (Lactuca sativa; Supplemental Method 3).

Carotenoid-supplemented diets. Solutions containing purified carotenoids were concentrated under low pressure, below 35°C, and added to soybean oil containing tert-butylhydroquinone at 200 mg/kg. The remaining solvents in the carotenoid/oil mixtures were removed by constantly stirring in vacuo for several hours until constant weight was achieved. The diets were prepared under dim yellow light and were based on the AIN-93G diet composition (14) with 2 modifications: the soybean oil was replaced by the carotenoid-supplemented soybean oil (prepared as described above) and fiber was replaced by corn starch to avoid possible interference with carotenoid absorption. The carotenoid-supplemented diets were stored at 4°C in vacuum-sealed packages and consumed within 3 wk. The feeds were analyzed for carotenoids after exhaustive extraction with dichloromethane:methanol 2:1 (v:v). Extracts containing lutein esters were saponified (as described below for biological samples) and analyzed with HPLC setup 1. Fucoxanthin-containing feeds were extracted and analyzed using HPLC setup 2 without saponification.

Animals and experimental design. Five-week-old male ICR mice were obtained from Charles River Laboratories Japan and housed in individual wire cages under a 12 h-light/12-h-dark cycle at 24°C. During the 5- to 7-d acclimatization period, the mice had free access to tap water and a commercial rodent diet (MF; Oriental Yeast). Preliminary experiments showed that a 14-d feeding period with lutein- or fucoxanthin-containing diets was sufficient to load measurable amounts of carotenoid metabolites into the liver of mice. Thus, the lutein supplementation/depletion experiment was comprised of a 14-d carotenoid supplementation in which the mice (n = 45) consumed ad libitum a powdered AIN-93G-based diet supplemented with lutein esters, followed by a 41-d depletion period when the mice were fed a carotenoid-free pelleted AIN-93G diet (Oriental Yeast). During the depletion period, groups of 5 mice were randomly chosen and killed at 0 h, 6 h, 12 h, 1 d, 3 d, 7 d, 14 d, 21 d, and 41 d. The fucoxanthin supplementation/depletion experiment followed the same protocol described above, but the last sampling was done at 28 d. Every 2 d, body weight and feed intake were measured and a fresh supply of feed was offered to each mouse. At each time point, without prior feed deprivation, mice were anesthetized with diethyl ether and blood was collected with heparinized syringes from the caudal vena cava. Liver, kidneys, and epididymal, inguinal and interscapular fat depots were excised, blotted dry, and weighed. All experiments were conducted in accordance with the basic guidelines of the Ministry of Agriculture, Forestry and Fisheries for laboratory animal studies.

Source of the human plasma sample. A plasma sample from a healthy volunteer was obtained in a previous study in our laboratory (15) and stored at −80°C until analyses. The blood was collected after overnight fast before the supplementation period (baseline). None of the participants had taken any carotenoid-containing supplement during the year before the study began (15).

Preparation of biological samples. To determine the concentration of lutein and their metabolites, samples of plasma (200 μL) and tissues (60–70 mg) were saponified with 1000 μL of 4.5% KOH containing 9.5% pyrogallol in 95% ethanol in screw-capped tubes with argon headspace. After the 30-min reaction at 60°C, 2 mL of water was added to each tube and carotenoids were extracted 3 times with 3 mL diethyl ether:hexane 70:20:20 (v:v:v). The supernatants were combined with 60 nmol of 1,2,3,6-butylhydroquinone at 200 mg/kg. The solutions containing purified carotene or carotenoids in feed and biological samples were performed on a 0.2-μm membrane, and subjected to analysis by reversed-phase HPLC (setup 1). The samples were vortexed and centrifuged, and the hexane layer was discarded and the process repeated 3–4 times, with fresh portions of hexane, to ensure removal of the lipophilic material. The methanol:water phase was dried under reduced pressure, redissolved in methanol, and analyzed by reversed-phase HPLC (setup 1). The recovery of lutein spiked to mouse tissues was higher than 97%. To evaluate the possible formation of artifacts and loss of lutein metabolites during saponification, selected samples of mouse tissues and human plasma were also analyzed without saponification (Supplemental Method 4).

The concentration of fucoxanthin and its metabolites was determined in extracts without saponification. Plasma (160 μL) or tissue samples (50–100 mg) were combined with the internal standard (neoxanthin) and methanol and hexane were added to the final ratio of hexane:methanol:water 1:1:0.1 (v:v:v). The samples were vortexed and centrifuged, and the hexane layer was discarded and the process repeated 3–4 times, with fresh portions of hexane, to ensure removal of the lipophilic material. The methanol:water phase was dried under reduced pressure, redissolved in methanol:dimethyl sulfoxide:water 70:20:20 (v:v:v), filtered through a 0.2-μm membrane, and subjected to analysis by reversed-phase HPLC (setup 2). The recovery of fucoxanthin metabolites and neoxanthin spiked to mouse tissue samples was higher than 90%.

HPLC setups for quantitative analyses. Quantitative analyses of carotenoids in feed and biological samples were performed on a
Shimadzu HPLC system (Shimadzu) consisting of a LC-10AD pump set at 0.2 mL/min, a SPD-M10A photodiode array detector at a 250- to 550-nm range, and a CTO-10AS column oven at 25°C. To achieve satisfactory peak resolution between lutein and its metabolites, the HPLC conditions were adapted from Hudon et al. (16) and consisted of 2 tandem TSK gel ODS-80Ts columns (2 × 250 mm, Tosoh) attached to an ODS1 guard column (2 × 10 mm, Tosoh), run with an acetonitrile: methanol 96:4 (v/v) mobile phase containing 0.1% (w/v) ammonium acetate (HPLC setup 1). Fucoxanthin and its metabolites were analyzed with a single 2 × 250 mm TSK gel ODS-80Ts column (Tosoh) attached to an ODS1 guard column (2 × 10 mm, Tosoh) and a mobile phase consisting of acetonitrile:methanol:water 71.25:14.25:14.5 (v/v/v) containing 0.1% ammonium acetate (HPLC setup 2). The peak areas of all compounds were taken at their absorbance maxima in the mobile phase. The concentrations were calculated from the calibration curves of known standards (lutein, fucoxanthin, and fucoxanthinol). Due to the unavailability of standards, lutein metabolites were estimated from the lutein calibration curve and amarouciaxanthin A was estimated from the fucoxanthinol calibration curve.

Identification of carotenoids and their metabolites. The structural elucidation of carotenoids in biological samples was based on their UV-vis absorption spectra in the mobile phase, coelution with known standards, chemical derivatization tests for functional groups, iodine-catalyzed photoisomerization, and liquid chromatography-MS according to the amount of samples and availability of standard compounds (Supplemental Method 5).

Calculations and data analyses. Carotenoid concentration data are presented as mean ± SD or as percentage of total carotenoids. To calculate the half-lives ($t_{1/2}$)$^5$ of depletion, the mean carotenoid concentration in plasma and tissues throughout the depletion period was plotted against time ($n$ = 5 at each time point). As the plots showed that carotenoid depletion followed apparent first-order kinetics, the $t_{1/2}$ for each carotenoid was calculated by dividing ln 2 by the slope of the In-transformed concentration vs. time curve. The $t_{1/2}$ range was calculated from the highest and lowest slopes within a 95% CI. Half-lives from slopes that did not overlap the 95% CI were considered different.

Results

Body weight, feed, and carotenoid intake. The initial body weight of the ICR mice was 33.2 ± 1.4 g in the lutein experiment and 32.5 ± 1.4 g in the fucoxanthin experiment. During the 14-d lutein supplementation, daily consumption of feed was 5.06 ± 0.38 g, which corresponded to 2.73 ± 0.20 μmol lutein esters/d. For the fucoxanthin supplementation, feed intake was 5.18 ± 0.67 g/d, corresponding to 0.128 ± 0.016 μmol fucoxanthin/d, including 9.5% of a cis isomer. The lutein ester and fucoxanthin levels in feed were calculated based on preliminary (L. Yonekura and A. Nagao, unpublished data) experiments to yield comparable levels of their metabolites in tissues. We chose to feed the mice lutein esters instead of free lutein, because lutein esters are more easily dissolved in oil and more bioavailable than free lutein (17).

Identification of lutein metabolites. At the end of the 14-d dietary supplementation with lutein esters, the carotenoid profile of the mouse livers showed measurable amounts of all-trans-lutein, 13-cis and/or 13'-cis-lutein, and 3 metabolites: e,e-carotene-3,3’-dione, 3'-hydroxy-e,e-caroten-3-one, and cis-3'-hydroxy-e,e-caroten-3-one (Fig. 2). Common HPLC methods for tissue carotenoid analyses on reversed phase columns make use of mid-polarity mobile phases to elute lutein in relatively short retention times (Rt). Under such conditions, lutein would coelute with its oxidative metabolites and those compounds could remain unnoticed. In the present study, we used 2 tandem 25-cm long ODS columns to attain suitable chromatographic resolution between lutein and its metabolites.

The high-fat content of mouse liver and adipose tissue extracts did not allow direct analysis by reversed-phase chromatography (HPLC setup 1), because the fat disturbed reconstitution of carotenoids in the sample solvent for HPLC setup 1 and a stronger solvent would cause peak distortion. Therefore, the plasma and tissue samples were saponified before the analysis of lutein and its metabolites. To confirm if the presumed lutein metabolites were not artifacts formed during saponification, an extract from the same sample was also analyzed after defatting by means of a previous run through reversed-phase HPLC. The chromatograms of liver extracts with or without saponification are shown in Fig. 2A,B. The identity of the major carotenoids was the same in both saponified and unsaponified liver samples, as indicated by the same Rt (Fig. 2, peaks 1–5) and compatible UV-vis and atmospheric pressure chemical ionization (APCI)-MS spectra (Supplemental Fig. 1). However, the concentration of e,e-carotene-3,3’-dione (peak 1) was much lower in the saponified sample (as described in the following section). The identification of lutein and its metabolites was carried out on pooled extracts of mouse liver. Rt, UV-vis, and APCI-MS spectral data were obtained from the liquid chromatography-MS setup 1 (Supplemental Method 5), as follows:

5 Abbreviations used: APCI, atmospheric pressure chemical ionization; m/z, mass:charge ratio; Rt, retention time; RI, relative intensity; $t_{1/2}$, half-life of depletion.

FIGURE 2 Representative reversed-phase HPLC profiles of carotenoids in saponified (A) and unsaponified (B) liver extracts from mice fed diets containing lutein esters for 14 d and in unsaponified extracts of human plasma sample (C). Peaks were identified as e,e-carotene-3,3’-dione (1), 3'-hydroxy-e,e-carotene-3-one (2), cis-3'-hydroxy-e,e-carotene-3-one (3), 3'-hydroxy-B,e-carotene-3-one (4), all-trans-lutein (4), 13-cis and/or 13'-cis lutein (5). Peaks with the same number in different chromatograms had similar UV-vis and MS spectra, shown in Supplemental Figure 1.
3′-Hydroxy-e,e-carotene-3-one. The peak 2 at Rt 23.2 min corresponded to the most abundant carotenoid in mouse liver. (Fig. 2, peak 2) Its UV-vis spectrum with main absorbance at 442 nm and well-defined vibrational bands (Supplemental Fig. 1A2) was consistent with that of authentic luteoxanthin [(3R,6R,3′R,6′R)-e,e-carotene-3,3′-diol], indicating a polyene structure with 9 conjugated double bonds. The APCI-MS (Supplemental Fig. 1B2) showed peaks at masscharge ratio (m/z) 567 [M+H]+ (relative intensity in percentage, RI, 5.5) and m/z 549 [M+H-18]+ (base peak), and no further loss of water in the MS-MS spectrum of the m/z 549 fragment, indicating the presence of 1 hydroxyl group and presumably 1 keto group in a C40 carotene backbone.

NaBH₄-assisted reduction of this compound produced 2 new peaks separated by normal-phase HPLC (Supplemental Fig. 2A, B). One of the reduced compounds coeluted with authentic luteoxanthin (Supplemental Fig. 2C,D), which is 1 of the 10 stereoisomers of tunaxanthin (e,e-carotene-3,3′-diol) that includes 6 diastereomers that can be separated by normal-phase HPLC (18). Mass spectra of the reduced compounds showed 2 fragment ions corresponding to the loss of water at m/z 551 [M+H-18]+ and 533 [M+H-36]+, as did those of luteoxanthin. In addition, the UV-vis spectra of the reduced compounds were consistent with those of luteoxanthin. The 2 peaks produced by NaBH₄ reduction were then assigned as tunaxanthin diastereomers, indicating that the parent compound had a structure similar to tunaxanthin except for 1 keto group instead of hydroxyl attached to the ε-end ring. Moreover, the UV-vis spectra of the parent compound featured well-defined vibrational bands, indicating that the keto group was not conjugated to the main polyene chain. The parent compound could also be methylated by methanolic HCl, indicating the presence of a secondary allylic hydroxyl group. Therefore, peak 2 was identified as 3′-hydroxy-e,e-carotene-3-one (Fig. 1A).

cis-3′-Hydroxy-e,e-carotene-3-one. The peak 3 at Rt 24.1 min had the same response in chemical derivatization tests and APCI-MS (Supplemental Fig. 1B3) as those of peak 2. However, the UV-vis spectrum presented a strong cis peak with ε₂ε₅ = 5.385 at 330 nm (Supplemental Fig. 1A3), so the peak 3 was identified as cis-3′-hydroxy-e,e-carotene-3-one. After sample handling (solvent evaporation and storage at ~20°C), part of the isolated peak 3 spontaneously converted to a compound devoid of the cis peak in the UV-vis spectrum and unchanged APCI-MS, indicating isomerization from cis to all-trans configuration. Because peak 2 had the same Rt as the peak formed spontaneously from peak 3, peak 2 can be regarded as all-trans-3′-hydroxy-e,e-carotene-3-one (Fig. 1A).

e,e-Carotene-3,3′-dione. Peak 1 at Rt 21.0 min had UV-vis absorbance maxima and vibrational bands (Supplemental Fig. 1A1) very similar to those of peak 2. The APCI-MS (Supplemental Fig. 1B1) showed a base peak at m/z 565 [M+H]+ and no peaks due to water loss, in agreement with a MS profile of a diketocarotenoid. The NaBH₄-assisted reduction of peak 1 generated 2 peaks separated by normal-phase HPLC (setup 3; Supplemental Method 5). Their Rt, UV-vis, and APCI-MS spectra were comparable to those of NaBH₄-treated peak 2, indicating formation of tunaxanthin diastereomers from the parent compound. Thus, peak 1 was identified as e,e-carotene-3,3′-dione (Fig. 1A).

All-trans-lutein [(3R,3′R,6′R)-β,β-carotene-3,3′-diol, Fig. 2, peak 4] at Rt 26.7 min was identified by APCI-MS (Supplemental Fig. 1B4), UV-vis spectral data (Supplemental Fig. 1A4), and coelution with authentic lutein. The absence of carbonyl groups was confirmed by the negative response to NaBH₄-assisted reduction.

13-cis-Lutein and 13′-cis-lutein (Fig. 2, peak 5), at Rt 29.9 min, was identified by APCI-MS (Supplemental Fig. 1B5), UV-vis spectral data (Supplemental Fig. 1A5), and coelution with lutein isomers produced by iodine-catalyzed photoisomerization. The blue-shift of 6 nm in absorption maximum from that of all-trans-lutein (Supplemental Fig. 1A, spectra 4 and 5) and the intensity of the cis peak, ε₂ε₅ = 0.427, suggested that C13 or C13′ was the location of isomerization (19). However, 13-cis-lutein and 13′-cis-lutein could not be resolved with the HPLC conditions used in this study.

The peak 3′ of the human plasma chromatogram (Fig. 2C), with Rt very close to that of peak 3 (cis-3′-hydroxy-e,e-carotene-3-one) of the mouse liver chromatograms (Fig. 2A,B), was identified as 3-hydroxy-β,β-carotene-3′-one from the following features: the APCI-MS spectrum showed peaks at m/z 567 [M+H]+ (base peak) and m/z 549 [M+H-18]+ (RI 24.5), which were very similar to previously published MS for that compound (19,20), whereas m/z 549 [M+H-18]+ was predominant in the fragmentation pattern of cis-3′-hydroxy-e,e-carotene-3-one (Supplemental Fig. 1B3); UV-vis absorbance maximum was 5 nm red-shifted relative to that of 3′-hydroxy-e,e-carotene-3-one and the spectrum did not feature a cis peak. e,e-Carotene-3,3′-dione and 3′-hydroxy-e,e-carotene-3-one were also present in the human plasma extract (Fig. 2C, peaks 1 and 2) but at much smaller amounts relative to all-trans-lutein, the most abundant carotenoid in the human plasma chromatogram.

Identification of fucoxanthin metabolites. At the end of the fucoxanthin supplementation period, the mouse plasma had measurable amounts of fucoxanthinol and amarouciaxanthin A, consistent with our previous report (10). We also found 3 additional metabolites of fucoxanthin in the present study (Fig. 3; peaks 1, 2, and 5). Peak 5 at Rt 19.8 min had a UV-vis absorbance maximum at 458 nm, unresolved vibrational fine structure, and a cis peak at 340 nm (ε₂ε₅ = 0.590; Supplemental Fig. 3A5). APCI-MS fragmentation was compatible with that of amarouciaxanthin A (Supplemental Fig. 3B5), and the compound coeluted with one of the isomers formed by iodine-catalyzed photoisomerization of amarouciaxanthin A. Thus, peak 5 was assigned as cis-amarouciaxanthin A. Peak 1 at Rt 7.5 min had absorbance maximum at 470 nm with an unresolved vibrational structure and APCI-MS base peak at m/z 445. MS-
MS of the m/z 445 peak (R1 35.7) showed a fragment ion at m/z 427 (base peak). Peak 2 at Rt 10.5 min had an absorbance maximum at 460 nm with unresolved vibrational structure and APCI-MS ions at m/z 445 (base peak) and 427 (R1 85.1). The limited amounts of these 2 compounds did not allow a complete identification.

**Distribution of carotenoids in selected mouse tissues and their depletion.** At the end of the dietary supplementation with lutein esters, mouse tissues and plasma had surprisingly high concentrations of 3'-hydroxy-\(\text{e,e}\)-caroten-3-one and its cis isomer, which are assumed to be oxidative metabolites of lutein. The concentrations of those metabolites were even higher than those of lutein and its isomers in liver and epididymal, inguinal, and interscapular adipose tissues (Table 1).

The losses of lutein and its metabolites during saponification were evaluated by analyzing the extracts from saponified and untreated samples. Both extracts were defatted by passing through a short ODS column. The ratios of carotenoid concentration between saponified and untreated samples were: 94.0% for the sum of all-trans, 13-cis-\(\text{e,e}\)-lutein, and 13 ' -cis-lutein; 71.3% for the sum of 3'-hydroxy-\(\text{e,e}\)-caroten-3-one and cis-3'-hydroxy-\(\text{e,e}\)-caroten-3-one, and 53.9% for \(\text{e,e}\)-carotene-3,3'-dione. The negligible loss of lutein by saponification was very consistent with the high recovery of lutein spiked to tissues, whereas the ketocarotenoids were unstable. Due to the large loss of \(\text{e,e}\)-carotene-3,3'-dione by saponification, we are not reporting the concentrations of this carotenoid.

Considering the total amount of lutein and its metabolites, we observed the highest concentration in the liver, followed by plasma, all adipose tissues, and the kidney (Table 1). In particular, the liver accumulated large amounts of 3'-hydroxy-\(\text{e,e}\)-caroten-3-one and \(\text{e,e}\)-carotene-3,3'-dione. 3'-Hydroxy-\(\text{e,e}\)-caroten-3-one was also predominant in the adipose tissues (Table 1). On the other hand, in the kidney, all-trans-lutein and 3'-hydroxy-\(\text{e,e}\)-caroten-3-one are present in comparable amounts, while 13-cis- and 13'-cis-lutein are the most abundant carotenoids in plasma. The \(t_{1/2}\) for the total lutein metabolites (sum of the 4 major compounds found in tissues) varied greatly between tissues, in the following order: plasma < liver < kidney < adipose tissues (Table 2). The depletion of lutein and its metabolites in epididymal, inguinal, and interscapular fat depots was much slower than in other tissues, but the \(t_{1/2}\) could not be determined, because the concentrations of lutein metabolites were not significantly reduced even at 41 d of depletion. Within the same tissue, the compound-specific \(t_{1/2}\) values were similar for all 4 metabolites analyzed (Table 2).

After the dietary supplementation with fucoxanthin, we observed a higher concentration of total fucoxanthin metabolites in mouse adipose tissues than in plasma, liver, and kidney (Table 3). Amarouciaxanthin A and its cis isomer were preferentially accumulated in adipose tissues. Among the epididymal, inguinal, and interscapular fat tissues, the distribution of fucoxanthin metabolites was similar, with 52.8–56.6% amarouciaxanthin A, 22.6–26.5% cis-amarouciaxanthin A, and 18.8–24.1% fucoxanthinol (Table 3). Unlike the profile in adipose tissues, fucoxanthinol was the most abundant metabolite in liver and kidney, followed by amarouciaxanthin A and cis-amarouciaxanthin A. Amarouciaxanthin A was the most abundant carotenoid in the plasma samples, followed by fucoxanthinol and cis-amarouciaxanthin A. We did not detect fucoxanthin in the plasma and tissues analyzed in this study, consistent with our previous report (10).

Overall, \(t_{1/2}\) of fucoxanthin metabolites in plasma and tissues were shorter than those of lutein and its metabolites (Tables 3 and 4). Compared among tissues and plasma, the \(t_{1/2}\) of the total fucoxanthin metabolites were longer in adipose tissues compared with \(t_{1/2}\) in plasma, liver, and kidney (Table 4). In adipose tissues (Table 1).

### Table 1: Concentration of carotenoids in mouse plasma and tissues at the end of the 14-d dietary supplementation with lutein esters

<table>
<thead>
<tr>
<th>Tissue</th>
<th>3'-Hydroxy-(\text{e,e})-caroten-3-one</th>
<th>cis-3'-Hydroxy-(\text{e,e})-caroten-3-one</th>
<th>All-trans lutein</th>
<th>13-cis- and 13'-cis-Lutein</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>0.55 ± 0.22 (25.9)</td>
<td>0.22 ± 0.08 (10.5)</td>
<td>0.22 ± 0.07 (10.5)</td>
<td>1.12 ± 0.63 (53.0)</td>
<td>2.11 ± 0.80</td>
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<tr>
<td>Liver</td>
<td>3.81 ± 1.57 (50.7)</td>
<td>1.63 ± 0.52 (21.7)</td>
<td>0.61 ± 0.15 (8.1)</td>
<td>1.47 ± 0.33 (19.6)</td>
<td>7.51 ± 2.14</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.30 ± 0.09 (34.4)</td>
<td>0.10 ± 0.04 (11.3)</td>
<td>0.26 ± 0.08 (30.6)</td>
<td>0.20 ± 0.09 (23.7)</td>
<td>0.87 ± 0.24</td>
</tr>
<tr>
<td>Epididymal fat</td>
<td>0.51 ± 0.13 (50.1)</td>
<td>0.17 ± 0.07 (16.6)</td>
<td>0.20 ± 0.06 (19.7)</td>
<td>0.14 ± 0.08 (13.7)</td>
<td>1.01 ± 0.20</td>
</tr>
<tr>
<td>Inguinal fat</td>
<td>0.62 ± 0.19 (47.0)</td>
<td>0.20 ± 0.05 (15.1)</td>
<td>0.29 ± 0.08 (22.1)</td>
<td>0.21 ± 0.05 (15.8)</td>
<td>1.32 ± 0.30</td>
</tr>
<tr>
<td>Interscapular fat</td>
<td>0.73 ± 0.28 (50.8)</td>
<td>0.21 ± 0.08 (14.9)</td>
<td>0.29 ± 0.07 (20.3)</td>
<td>0.20 ± 0.03 (14.0)</td>
<td>1.44 ± 0.43</td>
</tr>
</tbody>
</table>

1 Values are mean ± SD, n = 5 (% of total carotenoids).

### Table 2: \(t_{1/2}\) of carotenoids in mouse plasma and tissues after 14-d dietary supplementation with lutein esters

<table>
<thead>
<tr>
<th>Tissue</th>
<th>3'-Hydroxy-(\text{e,e})-caroten-3-one</th>
<th>cis-3'-Hydroxy-(\text{e,e})-caroten-3-one</th>
<th>All-trans lutein</th>
<th>13-cis- and 13'-cis-Lutein</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>1.20 (0.88, 1.89)</td>
<td>1.45 (1.12, 2.07)</td>
<td>0.74 (0.53, 1.20)</td>
<td>1.15 (0.83, 1.86)</td>
<td>1.16 (0.88, 1.79)</td>
</tr>
<tr>
<td>Liver</td>
<td>2.68 (2.26, 3.28)**</td>
<td>2.84 (2.39, 2.94)**</td>
<td>2.25 (1.88, 2.78)**</td>
<td>2.63 (2.10, 3.50)**</td>
<td>2.63 (2.25, 3.16)**</td>
</tr>
<tr>
<td>Kidney</td>
<td>4.10 (3.50, 4.99)**</td>
<td>3.73 (2.98, 4.95)**</td>
<td>5.33 (3.85, 8.66)**</td>
<td>4.15 (3.43, 5.29)**</td>
<td>4.44 (3.63, 5.68)**</td>
</tr>
</tbody>
</table>

1 Values are \(t_{1/2}\) calculated from depletion curves drawn with ln-transformed mean concentrations against time (95% CI). *Depletion curves had 95% CI bands that did not overlap with those of plasma and liver. **Depletion curves had 95% CI bands that did not overlap with those of plasma and liver.

2 \(t_{1/2}\) of carotenoids in epididymal, inguinal, and interscapular fat tissues were assumed to be longer than 41 d.
tissues, \( t_{1/2} \) differed significantly according to the site, being the shortest in intercapsular fat, followed by inguinal fat and epididymal fat. Of the individual carotenoids within tissues, the \( t_{1/2} \) of fucoxanthin was generally shorter than that of amarouciaxanthin A, which in most cases was shorter than that of cis-amarouciaxanthin A (Table 4).

**Discussion**

**Metabolism.** The most surprising finding in this study was that keto-carotenoids were the most abundant metabolites in ICR mice fed lutein esters. 3'-Hydroxy-\( \varepsilon,\varepsilon \)-carotene-3-one (and its cis isomer) accounted for >50% of the carotenoids in the liver and adipose tissues. Another keto-carotenoid, \( \varepsilon,\varepsilon \)-carotene-3,3'-dione, was also present in appreciable amounts in all samples from lutein-supplemented ICR mice, but quantitative data are not reported here due to the loss of this compound during saponification. Previous reports have indicated that lutein was the predominant carotenoid found in BalbC mice after dietary supplementation with lutein esters (21,22), but the use of reversed-phase HPLC with single wavelength detection and conditions that did not allow chromatographic resolution of lutein and zeaxanthin may have hindered the detection of keto-carotenoids. Although there may be differences in carotenoid metabolism even among strains of the same species (2.3), we also detected the keto-carotenoids 3'-hydroxy-\( \varepsilon,\varepsilon \)-carotene-3-one and \( \varepsilon,\varepsilon \)-carotene-3,3'-dione as major carotenoids in the liver of BalbC mice fed diets containing lutein (L. Yonekura and A. Nagao, unpublished data). Khachik et al. (19,24–26) have also reported the presence of 3-hydroxy-\( \varepsilon,\varepsilon \)-carotene-3-one as well as 3'-hydroxy-\( \varepsilon,\varepsilon \)-carotene-3-one and \( \varepsilon,\varepsilon \)-carotene-3,3'-dione in human serum, breast milk, retina, and liver, and these keto-carotenoids were reported to amount to 34% of the lutein/zeaxanthin level in human serum (27). They also suggested that keto-carotenoids were metabolites of dietary lutein, because their concentrations were significantly raised from the baseline in individuals receiving lutein supplementation (27). In fact, we also detected a considerable amount of these keto-carotenoids in human plasma in the present study. Apart from their presence in human tissues, 3'-hydroxy-\( \varepsilon,\varepsilon \)-carotene-3-one and \( \varepsilon,\varepsilon \)-carotene-3,3'-dione have also been detected in the feathers of pin-tailed and golden-winged manakins (*llicura militaris*), where \( \varepsilon,\varepsilon \)-carotene-3,3'-dione was a major carotenoid (16), and in hen egg yolks as a minor carotenoid (28). Our results and the aforementioned reports suggest the existence of a common metabolic pathway for lutein oxidation (Fig. 1A) widely distributed among birds and mammals. Khachik et al. (27) and Matsuno et al. (28) suggested metabolic pathways for the transformation of dietary lutein, which encompassed oxidation of lutein to 3-hydroxy-\( \beta,\beta \)-carotene-3-one, double bond migration yielding 3'-hydroxy-\( \varepsilon,\varepsilon \)-carotene-3-one, and further oxidation to \( \varepsilon,\varepsilon \)-carotene-3,3'-dione. This may well be the pathway underlying the formation of metabolites we found in the present study. The reason why we could not detect 3'-hydroxy-\( \beta,\beta \)-carotene-3-one could be its faster isomerization to 3'-hydroxy-\( \varepsilon,\varepsilon \)-carotene-3-one in ICR mice compared with in humans.

We previously reported that dietary fucoxanthin is transformed to fucoxanthinol and amarouciaxanthin A (Fig. 1B) in mice (10,29). In the present study, we observed 2 additional peaks whose APCI-MS featured base peaks at m/z 445, which is also the most abundant fragment in the APCI-MS of amarouciaxanthin A. Further transformation of amarouciaxanthin A to more polar metabolites may be taking place in ICR mice.

Similarly to the oxidative metabolism we described here for lutein and fucoxanthin, other authors have reported the oxidation of 4,4'-dimethoxy-\( \beta \)-carotene at the 4 and 4' positions

**TABLE 3** Concentration of carotenoids in mouse plasma and tissues at the end of the 14-d dietary supplementation with fucoxanthin¹

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Fucoxanthinol</th>
<th>Amarouciaxanthin A</th>
<th>cis-Amarouciaxanthin A</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma, ( \mu \text{mol/l} ) (%)</td>
<td>0.34 ± 0.12 (26.7)</td>
<td>0.87 ± 0.26 (67.2)</td>
<td>0.08 ± 0.02 (6.1)</td>
<td>1.29 ± 0.40</td>
</tr>
<tr>
<td>Liver, ( \mu \text{mol/kg} ) (%)</td>
<td>0.85 ± 0.32 (46.9)</td>
<td>0.60 ± 0.18 (33.2)</td>
<td>0.36 ± 0.12 (19.9)</td>
<td>1.80 ± 0.62</td>
</tr>
<tr>
<td>Kidney, ( \mu \text{mol/kg} ) (%)</td>
<td>0.69 ± 0.10 (49.3)</td>
<td>0.44 ± 0.02 (31.5)</td>
<td>0.27 ± 0.02 (19.2)</td>
<td>1.40 ± 0.12</td>
</tr>
<tr>
<td>Epididymal fat, ( \mu \text{mol/kg} ) (%)</td>
<td>0.65 ± 0.10 (20.7)</td>
<td>1.65 ± 0.10 (52.8)</td>
<td>0.83 ± 0.04 (26.5)</td>
<td>3.13 ± 0.18</td>
</tr>
<tr>
<td>Inguinal fat, ( \mu \text{mol/kg} ) (%)</td>
<td>0.61 ± 0.14 (18.8)</td>
<td>1.82 ± 0.34 (56.6)</td>
<td>0.79 ± 0.17 (24.5)</td>
<td>3.22 ± 0.64</td>
</tr>
<tr>
<td>Interascapular fat, ( \mu \text{mol/kg} ) (%)</td>
<td>0.88 ± 0.17 (24.1)</td>
<td>1.94 ± 0.40 (53.3)</td>
<td>0.82 ± 0.18 (22.6)</td>
<td>3.64 ± 0.72</td>
</tr>
</tbody>
</table>

¹ Values are mean ± SD, \( n = 5 \) (% of total carotenoids).

**TABLE 4** \( t_{1/2} \) of carotenoids in mouse plasma and tissues after 14-d dietary supplementation with fucoxanthin¹

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Fucoxanthinol</th>
<th>Amarouciaxanthin A</th>
<th>cis-Amarouciaxanthin A</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma, ( d ) (95% CI)</td>
<td>0.76 [0.63, 0.96]</td>
<td>0.92 [0.72, 1.29]</td>
<td>1.46 (1.06, 2.29)</td>
<td>0.92 (0.72, 1.25)</td>
</tr>
<tr>
<td>Liver, ( d ) (95% CI)</td>
<td>0.64 [0.48, 0.96]</td>
<td>0.84 [0.75, 0.96]</td>
<td>2.02 (1.58, 2.81)</td>
<td>1.23 (0.96, 1.72)</td>
</tr>
<tr>
<td>Kidney, ( d ) (95% CI)</td>
<td>0.66 [0.51, 0.83]</td>
<td>0.99 [0.80, 1.30]</td>
<td>2.14 (1.77, 2.70)</td>
<td>1.04 (0.78, 1.56)</td>
</tr>
<tr>
<td>Epididymal fat, ( d ) (95% CI)</td>
<td>2.62 [2.44, 2.82]</td>
<td>4.88 [4.13, 5.68]</td>
<td>6.54 [4.53, 11.7]</td>
<td>4.81 [4.10, 5.87]</td>
</tr>
<tr>
<td>Inguinal fat, ( d ) (95% CI)</td>
<td>1.92 [1.89, 2.22]</td>
<td>3.67 [3.19, 4.30]</td>
<td>5.02 [4.39, 5.92]</td>
<td>3.76 [3.38, 4.25]</td>
</tr>
<tr>
<td>Interascapular fat, ( d ) (95% CI)</td>
<td>1.81 [1.38, 2.65]</td>
<td>2.55 [2.22, 3.00]</td>
<td>3.73 [3.28, 4.33]</td>
<td>2.76 [2.24, 3.35]</td>
</tr>
</tbody>
</table>

¹ Values are \( t_{1/2} \) calculated from depletion curves drawn with ln-transformed mean concentrations against time (95% CI). \( t_{1/2} \) values in a row without a common superscript letter had depletion curves without overlapping 95% CI bands. *Depletion curves had 95% CI bands that did not overlap with those of plasma, liver, and kidney.

**Oxidative transformation of xanthophylls in mice**

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to canthaxanthin (30) and at the 3’ position of capsanthin to capsanthone (31) after ingestion of those carotenoids by humans. Thus, mammals are able to oxidize secondary hydroxyl groups of various xanthophylls. In a previous study, we found that a liver microsomal NAD-dependent dehydrogenase played a role in the oxidation of fucoxanthinol to amarouciaxanthin A (10), but we still do not know whether lutein or capsanthin is oxidized by the same enzyme. Further studies are needed to determine the enzymes and mechanisms involved in the oxidative conversion of xanthophylls. The a,b-unsaturated carbonyl moiety of keto-carotenoids derived from lutein have a unique structure, which has high potential to react with nucleophilic molecules in biological tissues (32). Hence, the biological activities of lutein metabolites are worth investigating in terms of the beneficial effects of lutein on human health.

**Tissue distribution.** Surprisingly, lutein and its metabolites were more concentrated in the liver, whereas the less lipophilic fucoxanthin metabolites accumulated mainly in adipose tissues. In the present study, the total concentration of lutein and its metabolites in liver was 3.5-fold that in plasma, whereas in adipose tissues it ranged from 0.5- to 0.7-fold. In guinea pigs, the highest lutein concentration was found in the liver, followed by kidney and plasma, while there was no lutein in adipose tissues (33). In quail, the major site of lutein accumulation was found in the liver, followed by adipose tissue and serum (34). Previous studies in humans are conflicting, showing a highly variable partition of lutein into adipose tissues, ranging from 6-fold enrichment to concentrations much lower than those in plasma (35–37). Thus, adipose tissues cannot be assumed to be the major site for lutein accumulation.

Regarding the tissue distribution of fucoxanthin metabolites, we found that adipose tissues were the main site of accumulation, where concentrations were 2.2- to 2.6-fold relative to plasma. During the preparation of this manuscript, Hashimoto et al. (38) reported that fucoxanthin and its metabolites accumulated mainly in the adipose tissues, liver, and heart of mice. However, in their study, liver and adipose tissues had comparable concentrations of total fucoxanthin metabolites, which may be due to the shorter supplementation period (1 wk compared with 2 wk used in our study). To date, there is no data on the accumulation of fucoxanthin metabolites in human adipose tissue.

A recent report showed differences in carotenoid concentration according to the site of the adipose tissue (39). We determined the carotenoid concentration in 3 different sites: epididymal fat, which is the largest visceral fat depot in mice; inguinal fat, representative of the subcutaneous white adipose tissue; and interscapular fat, the major brown fat depot in mice. However, we did not observe differences in carotenoid concentrations among those sites.

The distribution profile of individual metabolites differed between tissues. 3’-Hydroxy-e,e-caroten-3-one and its cis isomer were more abundant than lutein (all-trans and its cis isomer) in liver and adipose tissues, whereas the opposite occurred in plasma and kidney. Lutein metabolites were highly accumulated in the liver, suggesting the oxidative conversion of lutein by liver enzymes. Regarding fucoxanthin metabolites, in plasma and adipose tissues, amarouciaxanthan A, including its cis isomer, was more abundant than fucoxanthinol, whereas the liver and kidneys had comparable amounts of the 2 metabolites. Amarouciaxanthan A accumulation in adipose tissue was remarkable. By contrast, the more lipophilic lutein and its metabolites accumulated mainly in the liver. The unique distribution of carotenoid metabolites in each tissue does not seem to be related to the lipophilicity of the metabolites and tissues and may be associated with other factors, including the existence of tissue-specific enzymatic transformation, different metabolic and transport rates for each compound, and the existence of compound- and tissue-specific transport mechanisms. The elucidation of such mechanisms, however, is beyond the scope of this study.

With regard to the proportion between carotenoid intake and their accumulation in tissues, we found a remarkable difference between lutein esters and fucoxanthin. In this study, mice ingested 20-fold more lutein esters than fucoxanthin, and the t1/2 of lutein and its metabolites was longer than those of fucoxanthin metabolites. Nonetheless, the levels of lutein and its metabolites in tissues did not exceed those of fucoxanthin. These results indicate that fucoxanthin is more readily absorbed than lutein esters in mouse intestine. However, we have previously reported that the bioavailability of dietary epoxyxanthophylls such as neoxanthin and fucoxanthin from spinach and algae is low in humans (15). Thus, the intestinal absorption of epoxyxanthophylls in humans and its difference among species are intriguing issues that deserve future study.

**Depletion rates.** The depletion of lutein and its metabolites occurred much more slowly than that of fucoxanthin metabolites. Because lutein and its oxidative metabolites are more lipophilic than fucoxanthin metabolites, our results suggest that the more lipophilic carotenoids have longer t1/2 in mice, especially in adipose tissues. In addition, we also observed longer t1/2 values for amarouciaxanthan A (all-trans and cis) compared with those for fucoxanthinol (less lipophilic than amarouciaxanthan A) in adipose tissues. Adipose tissues are generally regarded as potential sites to assess the long-term intake of lipophilic compounds (40). However, many authors have failed to find any evidence that the carotenoid concentration in adipose tissues reflects the long-term intake of these phytochemicals (41,42), which can be partially explained by the highly variable turnover rates of carotenoids found in the present study.

In summary, we identified the keto-carotenoids 3’-hydroxy-e,e-caroten-3-one and e,e-carotene-3,3’-dione as major metabolites of dietary lutein in ICR mice. Our study confirms several indications that the oxidation of xanthophylls’ secondary hydroxyl groups into carbonyl is an active metabolic pathway in mammals. Tissue accumulation and the distribution of lutein and fucoxanthin metabolites were not associated with their lipophilicity, but the depletion rates seemed to be slower for more lipophilic compounds.

**Acknowledgments**

L.Y. designed and conducted research, analyzed data, and wrote the paper; M.K. conducted carotenoid analyses; M.T. prepared carotenoid standards; and A.N. analyzed data and had primary responsibility for final content. All authors read and approved the final manuscript.

**Literature Cited**


