ABSTRACT:

Fucoxanthin, a major carotenoid in edible brown algae, potentially inhibits the proliferation of human prostate cancer cells via apoptosis induction. However, it has been postulated that dietary fucoxanthin is hydrolyzed into fucoxanthinol in the gastrointestinal tract before absorption in the intestine. In the present study, we investigated the further biotransformation of orally administered fucoxanthin and estimated the cytotoxicity of fucoxanthin metabolites on PC-3 human prostate cancer cells. After the oral administration of fucoxanthin in mice, two metabolites, fucoxanthinol and an unknown metabolite, were found in the plasma and liver. The unknown metabolite was isolated from the incubation mixture of fucoxanthinol and mouse liver preparation (10,000 g supernatant of homogenates), and a series of instrumental analyses identified it as amarouciaxanthin A [(3S,5R,6'S)-3,5,6'-trihydroxy-6,7-didehydro-5,6,7',8'-tetrahydro-β,β-carotene-3',8'-dione]. The conversion of fucoxanthinol into amarouciaxanthin A was predominantly shown in liver microsomes. This dehydrogenation/isomerization of the 5,6-epoxy-3-hydroxy-5,6-dihydro-β end group of fucoxanthinol into the 6'-hydroxy-3'-oxo-ε end group of amarouciaxanthin A required NAD(P)⁺ as a cofactor, and the optimal pH for the conversion was 9.5 to 10.0. Fucoxanthinol supplemented to culture medium via HepG2 cells was also converted into amarouciaxanthin A. The 50% inhibitory concentrations on the proliferation of PC-3 human prostate cancer cells were 3.0, 2.0, and 4.6 μM for fucoxanthin, fucoxanthinol, and amarouciaxanthin A, respectively. To our knowledge, this is the first report on the enzymatic dehydrogenation of a 3-hydroxyl end group of xanthophylls in mammals.

Fucoxanthin is a major nonprovitamin A carotenoid in brown algae (Haugan et al., 1992). Various types of brown algae, such as hijiki (Sargassum fusiforme), kombu (Laminaria japonica), and wakame (Undaria pinnatifida), are staples in the diet of East Asians. On the basis of animal experiments, the administration of brown alga powders or extracts was reported to suppress carcinogenesis (Yamamoto and Maruyama, 1985; Yamamoto et al., 1987; Funahashi et al., 1999, 2001). Fucoxanthin displayed beneficial effects on cancer chemoprevention in cell culture studies (Okuizumi et al., 1990; Hosokawa et al., 1999, 2001) and in animal experiments (Okuizumi et al., 1993; Kim et al., 1998). We recently demonstrated that epoxyxanthophylls such as fucoxanthin and neoxanthin reduce the viability of prostate cancer cells by inducing apoptosis to a greater extent than the other carotenoids present in foodstuffs (Kotake-Nara et al., 2001). Although the conversions of β-carotene and other provitamin A carotenoids into retinol are well known, little information is available on the metabolism of nonprovitamin A carotenoid. In particular, the mammalian metabolism of dietary epoxy-xanthophylls, including fucoxanthin, remains unknown despite their abundance in foodstuffs and the potential health benefits. Recently, we demonstrated in mice that orally administered fucoxanthin is incorporated into blood circulation as fucoxanthinol, a deacetylated metabolite (Sugawara et al., 2002). In the present study, we investigated further biotransformation of fucoxanthinol in mice and HepG2 cells and estimated the antiproliferative effect of fucoxanthin metabolites on PC-3 human prostate cancer cells.

Materials and Methods

Materials. Cholesterol esterase from porcine pancreas, lysophosphatidylcholine (1-palmitoyl-sn-glycero-3-phosphocholine), mono-olein, sodium taurocholate and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were purchased from Sigma-Aldrich (St. Louis, MO). Boiled and dried wakame was purchased from a local market in Tsukuba, Japan. Other chemicals and solvents were of reagent grade.
Apparatus. Visible absorption (VIS) spectra were measured on a U-3310 spectrophotometer (Hitachi, Ltd., Tokyo, Japan). High-resolution mass spectra were recorded on a Fourier transform ion cyclotron resonance mass spectrometer (APEX II 70e; Nikon Bruker Daltonics, Tsukuba, Japan) with an interface of electrospray ionization. $^1$H NMR spectra were recorded on a Bruker AVANCE instrument at 400 ppm in CDCl$_3$ (99.96%; Isotech, Miamisburg, OH). Chemical shifts (δ) were related to the signals of tetramethylsilane, and only relevant coupling constant values (J) were given. Circular dichroism (CD) spectrum was measured with a J-820 CD System (Jasco, Tokyo, Japan). Ellipticity (θ) was expressed as actual values in millidegrees observed with a heparinized syringe. Plasma was prepared from each blood sample by centrifugation of blood at 1000 g for 15 min at 4 °C. Immediately after each blood sample was taken, the liver was excised and rinsed with ice-cold saline.

For the intravenous injection study, an emulsion containing fucoxanthinol was prepared as follows. Appropriate amounts of fucoxanthol were dissolved with soybean oil (20 mg) and soybean phosphatidylcholine (5 mg) in a small amount of dichloromethane in a glass tube, and the solvent was dried under a stream of argon gas. After the residue was suspended in 0.5 ml of 0.15 M NaCl with a vortex mixer, the suspension was sonicated three times for 2 min each under ice-cold conditions, and then filtered with a 0.2-μm filter. The concentration of fucoxanthinol in the filtrated emulsion was confirmed by HPLC. The emulsion (1.5 nmol of fucoxanthol in 50 μl) was injected into the tail vein of each mouse. Two hours after the injection, plasma and liver samples were prepared as described above.

Metabolites of fucoxanthol were extracted as follows. Plasma (0.1 ml) diluted with 0.3 ml of phosphate-buffered saline (PBS) or 0.4 ml of liver homogenates (10% in PBS, w/v) was mixed with 1.5 ml of dichloromethane/methanol (1:2, v/v) and vortexed for 1 min. Then, dichloromethane (1.9 ml) and distilled water (1.2 ml) were added to the mixture and further vortexed for 1 min. After the centrifugation at 1000 g for 10 min, the dichloromethane phase was transferred and evaporated to dryness in vacuo. The residue was dissolved in a mixture of dimethyl sulfoxide (DMSO)/methanol (1:1, v/v), and an aliquot was subjected to HPLC analysis.

Isolation and Identification of Amaroouciaxanthin A. The unknown metabolite shown in mouse plasma and liver was synthesized by incubating fucoxanthol with liver homogenates. Livers of male ICR mice (8 weeks old) were excised immediately after cervical dislocation and rinsed with ice-cold saline. The livers were then homogenized in a Potter-Elvehjem homogenizer with 4 volumes of ice-cold 0.154 M KCl containing 50 mM Hepes-KOH, pH 7.4, 1.0 mM EDTA, and 0.1 mM diithiothreitol, and the homogenates were centrifuged at 10,000 g for 10 min at 4 °C. The 10,000 g supernatant (~7 ml) was incubated at 37°C for 15 min with 50 μM fucoxanthol, 2.4 mM NAD$^+$, and 0.2% Tween 20 (final concentrations) in 0.1 M glycine-KOH buffer, pH 10.0, in a total volume of 20 ml. After the incubation, the reaction mixture was mixed with 30 ml of dichloromethane/methanol (2:1, v/v), and the metabolite was extracted into the dichlormethane phase. The extract was then dried up under an argon gas stream, and the residue was applied to an alumina column. Neutral alumina (ICN Biomedicals Inc., Costa Mesa, CA) was deactivated to activity III before use. The metabolite was washed with increasing amounts of diethyl ether in hexane (2:8–5:5, v/v), and the final elution was obtained. It was necessary to wash out the nonpolar compounds on the column with methanol/ethanol acetate (70:30, v/v) for 15 min at a flow rate of 0.3 ml/min and then equilibrate the column with the mobile phase described above for 10 min before a subsequent injection.

Animal Studies. All procedures involving animals were conducted in accordance with the Guidelines for Experimental Animals of the National Food Research Institute, Japan. Male ICR mice (7 weeks old; Clea Japan, Inc., Tokyo, Japan) were housed at 25°C with a 12-h light/dark cycle and acclimated with free access to a standard rodent chow (MF, Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water. After 7 days of feeding, mice were deprived of food for 15 h before the administration trials described below.

For the oral administration study, mixed micelles containing fucoxanthol were prepared as described previously (Sugawara et al., 2002) with a slight modification. In brief, appropriate volumes of the stock solutions were transferred to a glass tube, and the solvent was dried up under a stream of argon gas. The residue was then dissolved in distilled water with a vortex mixer. The final concentrations were 12 mM sodium taurocholate, 2.5 mM mono-olein, 7.5 mM oleic acid, 1.25 mM lysophosphatidylcholine, and 0.2 mM fucoxanthol. The micellar solution (40 nmol of fucoxanthol in 0.2 ml) was administered to each mouse by direct stomach intubation. At 0 (untreated), 1, 2, 3, 6, or 9 h after the administration, each mouse was anesthetized with diethyl ether, and a blood sample was collected from the caudal vena cava by a heparinized syringe. Plasma was prepared from each blood sample by centrifugation of blood at 1000 g for 15 min at 4 °C. Immediately after each blood sample was taken, the liver was excised and rinsed with ice-cold saline.

For the intravenous injection study, an emulsion containing fucoxanthinol was prepared as follows. Appropriate amounts of fucoxanthol were dissolved with soybean oil (20 mg) and soybean phosphatidylcholine (5 mg) in a small amount of dichloromethane in a glass tube, and the solvent was dried under a stream of argon gas. After the residue was suspended in 0.5 ml of 0.15 M NaCl with a vortex mixer, the suspension was sonicated three times for 2 min each under ice-cold conditions, and then filtered with a 0.2-μm filter. The concentration of fucoxanthinol in the filtrated emulsion was confirmed by HPLC. The emulsion (1.5 nmol of fucoxanthol in 50 μl) was injected into the tail vein of each mouse. Two hours after the injection, plasma and liver samples were prepared as described above.

Metabolites of fucoxanthol were extracted as follows. Plasma (0.1 ml) diluted with 0.3 ml of phosphate-buffered saline (PBS) or 0.4 ml of liver homogenates (10% in PBS, w/v) was mixed with 1.5 ml of dichloromethane/methanol (1:2, v/v) and vortexed for 1 min. Then, dichloromethane (1.9 ml) and distilled water (1.2 ml) were added to the mixture and further vortexed for 1 min. After the centrifugation at 1000 g for 10 min, the dichloromethane phase was transferred and evaporated to dryness in vacuo. The residue was dissolved in a mixture of dimethyl sulfoxide (DMSO)/methanol (1:1, v/v), and an aliquot was subjected to HPLC analysis.

Analytical HPLC was carried out with a mobile phase of acetonitrile/methanol/water (75:15:10, v/v) containing 0.1% (w/v) ammonium acetate at a flow rate of 0.3 ml/min (system 1). The quantity of the unknown metabolite, identified as amaroouciaxanthin A in this report, was estimated from the peak area at 450 nm by use of the calibration curve of authentic fucoxanthol and amaroouciaxanthin A. The molar extinction coefficient of amaroouciaxanthin A could not be determined because an insufficient amount was obtained. It was necessary to wash out the nonpolar compounds on the column with methanol/ethanol acetate (70:30, v/v) for 15 min at a flow rate of 0.3 ml/min and then equilibrate the column with the mobile phase described above for 10 min before a subsequent injection.
terminated by adding 0.8 ml of dichloromethane/methanol (2:1, v/v). The mixture was incubated at 37°C for 60 min. The incubation was modified to use 0.2 ml. The subcellular fraction (H9262 0.5 mg of protein) in a total volume of 0.2 ml was centrifuged at 9000 g for 10 min at 4°C. The postnuclear supernatant was further centrifuged at 105,000g for 60 min at 4°C, and the precipitates of microsomes were washed by resuspension in the sucrose buffer and centrifuged at 9000 g for 10 min at 4°C. The postmitochondrial supernatant was centrifuged again at 105,000g for 90 min at 4°C, and the resulting supernatant was used as the cytosolic fraction. Prepared subcellular fractions were suspended in the sucrose buffer and were stored at −80°C. Protein concentrations were determined using a DC Protein Assay kit (Bio-Rad, Hercules, CA), and glucose 6-phosphatase activity was measured as a marker enzyme of microsomes (Swanson, 1955).

A standard incubation mixture unless otherwise stated contains 10 μM fucoxanthin, 2.4 mM NAD+, 0.1 M glycine-KOH buffer (pH 10.0), 0.2% Tween 20, and the subcellular fraction (~0.5 mg of protein) in a total volume of 0.2 ml. The mixture was incubated at 37°C for 60 min. The incubation was terminated by adding 0.8 ml of dichloromethane/methanol (2:1, v/v). The mixture was then vortexed for 1 min. After the centrifugation at 1000g for 10 min at 4°C, 0.4 ml of the dichloromethane phase was transferred and evaporated to dryness in vacuo. The residue was dissolved in a mixture of acetonitrile/methanol/water (50:25:25, v/v), an aliquot of which was subjected to HPLC analysis in a mobile phase of acetonitrile/methanol/water (50:25:25, v/v) containing 0.1% (w/v) ammonium acetate at a flow rate of 0.2 ml/min (system 2). The quantity of amarouciaxanthin A was estimated as a fucoxanthinol equivalence as described above.

Conversion of Fucoxanthin into Amarouciaxanthin A by HepG2 Cells. HepG2 human hepatoma cells (RIKEN BioResource Center, Tsukuba, Japan) were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% heat-inactivated fetal bovine serum, 4 mM t-glutamine, 40,000 U/l penicillin, and 40 mg/l streptomycin at 37°C in a humidified atmosphere of 5% CO2 in air. Cells that reached ~90% confluence in 60-mm dishes were cultured in fresh medium (4 ml/dish) supplemented with 1.0 μM fucoxanthinol and 0.4% DMSO (vehicle). After 24 h in culture, the media were transferred and filtered with a 0.2-μm filter to remove cell debris, and the cell layers were washed twice with ice-cold PBS (4 ml/dish). Amarouciaxanthin A was extracted from the medium by using 4 volumes of dichloromethane/methanol (2:1, v/v) and from the cell layers twice by using a mixture of hexane/2-propanol (3:2, v/v) (Brown et al., 1980). The quantity of amarouciaxanthin A was estimated by analytical HPLC (system 2).

Antiproliferative Activity on PC-3 Cells. PC-3 human prostate cancer cells (American Type Culture Collection, Manassas, VA) were cultured, and MTT assay was performed as previously reported (Kotake-Nara et al., 2001) with slight modifications. In brief, PC-3 cells were seeded at a density of 3 × 104 cells per well containing 100 μl of Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum in 96-well plates. After 24 h in culture, the medium was replaced with fresh medium supplemented with fucoxanthinol, fucoxanthinol, or amarouciaxanthin A. The carotenoids, dissolved in DMSO, were added to the culture medium at final concentrations of 0.625 to 20 μM. The final concentration of the vehicle (DMSO) was 0.4%, and the control culture received the vehicle alone. After 72 h in culture, cell viability was evaluated by MTT assay (Mosmann, 1983) with a modified extraction buffer (Hansen et al., 1989). The IC50 on PC-3 proliferation was calculated from the regression equation of a four-parameter sigmoid in SigmaPlot ver. 6.0 statistical software (SPSS Inc., Chicago, IL).

To determine the cellular uptake of carotenoids, the PC-3 cells that had reached up to ~90% confluence in 24-well plates were cultured in fresh medium (0.5 ml/dish) supplemented with 1.0 μM carotenoids and 0.4% DMSO. After 4 h in culture, the carotenoids were extracted from cells as described above and were then identified by the analytical HPLC (system 1).
Mice were administered fucoxanthin (40 nmol/mouse) solubilized in mixed micelles. Values are means ± S.D. of five mice. •, fucoxanthinol; ○, amarouciaxanthin A.

The incubation and HPLC (system 2) conditions are described under Materials and Methods. A, chromatogram obtained from the incubation mixture after 3-h incubation. B, spectra of fucoxanthin (solid line) and amarouciaxanthin A, which has been isolated from marine animals, a tunicate (Amaroucia pliciferum) (Matsumo et al., 1985a), and a bivalve (Paphia euglypta) (Matsumo et al., 1985b).

Amarouciaxanthin A [(3S,5R,6'S)-3,5,6'-trihydroxy-6,7-didehydro-5,6,7',8'-tetrahydro-β,ε-carotene-3',8'-dione]; VIS $\lambda_{\text{max}}$ (acetone) 465 nm, (ethanol) 467.5 nm; CD (nm) (θ) [ethanol, $A_{467.5}$ = 0.945 (l = 1 cm)] 215 (1.35), 225 (0), 246 (−6.99), 263 (0), 271 (2.89); high-resolution MS $m/z$ 637.3875 [M + Na]$^+$ (calc. C$_{40}$H$_{50}$NaO$_{5}$: 637.3864); LC/MS, shown in Fig. 4. $^1$H NMR (800 MHz, CDCl$_3$) $\delta$ = 1.05 s (3H, Me-17'), 1.07 s (3H, Me-17), 1.08 s (3H, Me-16'), 1.34 s (3H, Me-18), 1.34 s (3H, Me-16), 1.34 m (1H, H-2), 1.42 m (1H, H-4), 1.82 s (3H, Me-19), 1.91 s (3H, Me-18'), 1.95 m (1H, H-2), 1.96 s (3H, Me-19'), 1.99 s (3H, Me-20'), 2.01 s (3H, Me-20), 2.28 m (1H, H-4), 2.34 d ($J_{\text{gem}}$ = 18 Hz, 1H, H-2'), 2.49 d ($J_{\text{gem}}$ = 18 Hz, 1H, H-2'), 2.94 d ($J_{\text{gem}}$ = 15 Hz, 1H, H-7'), 3.05 d ($J_{\text{gem}}$ = 15 Hz, 1H, H-7'), 3.27 d ($J_{\text{gem}}$ = 15 Hz, 1H, H-7'), 4.04 d ($J_{\text{gem}}$ = 15 Hz, 1H, H-5), 4.27 d ($J_{\text{gem}}$ = 15 Hz, 1H, H-5), 4.33 br (1H, H-4), 4.35 br (1H, H-3), 5.95 s (1H, OH-6'), disappeared by additional D$_2$O), 6.04 s (1H, H-8), 6.13 d ($J_{10,11}$ = 11 Hz, 1H, H-10), 6.27 d ($J_{14,15}$ = 12 Hz, 1H, H-14), 6.36 d ($J_{12,11}$ = 15 Hz, 1H, H-12), 6.46 d ($J_{14,15}$ = 12 Hz, 1H, H-14'), 6.57 dd ($J_{11',10'}$ = 11 Hz, $J_{11',12'}$ = 15 Hz, 1H, H-11'), 6.62 dd (1H, H-11), 6.64 dd (1H, H-15'), 6.72 d ($J_{12',11'}$ = 15 Hz, 1H, H-12'), 6.76 dd (1H, H-15), and 7.11 d ($J_{10',11'}$ = 11 Hz, 1H, H-10'). The proton assignment in the $^1$H NMR spectrum was confirmed by double quantum-filtered correlation spectroscopy, heteronuclear single quantum correlation, heteronuclear multiple-bond correlation spectroscopy, and nuclear Overhauser effect spectroscopy spectra. The hydroxide/deuterium exchange performed in high-resolution MS with CH$_3$OD/D$_2$O (1:1, v/v) as solvent gave the shift of molecular ion (+ 3 u), $m/z$ 640.4089 [M$_2$+Na]$^+$ (calc. for C$_{40}$H$_{51}$D$_3$NaO$_5$: 640.4057); i.e., three exchangeable hydrogen atoms exist in the structure (three hydroxyl groups in amarouciaxanthin A). The reduction by sodium tetrahydroborate (Eugster, 1995) gave a hypsochromic shift with increased spectral fine structure in the VIS spectrum, heteronuclear single quantum correlation, heteronuclear multiple-bond correlation spectroscopy, and nuclear Overhauser effect spectroscopy spectra. The hydroxide/deuterium exchange performed in high-resolution MS with CH$_3$OD/D$_2$O (1:1, v/v) as solvent gave the shift of molecular ion (+ 3 u), $m/z$ 640.4089 [M$_2$+Na]$^+$ (calc. for C$_{40}$H$_{51}$D$_3$NaO$_5$: 640.4057); i.e., three exchangeable hydrogen atoms exist in the structure (three hydroxyl groups in amarouciaxanthin A). The reduction by sodium tetrahydroborate (Eugster, 1995) gave a hypsochromic shift with increased spectral fine structure in the VIS spectrum.

The proposed fragment pattern in the MS spectrum is indicated. Inset, total ion chromatogram (t$_b$ 9.3, amarouciaxanthin A).

Fig. 2. Time course changes of fucoxanthin metabolites in mouse plasma (A) and liver (B) after a single oral administration of fucoxanthin.

Fig. 3. HPLC chromatogram (A) and UV-visible spectrum (B) of amarouciaxanthin A produced by the incubation of fucoxanthinol with mouse liver homogenates.

Fig. 4. APCI-MS spectrum of purified amarouciaxanthin A in LC/MS analysis.

The proposed fragment pattern in the MS spectrum is indicated. Inset, total ion chromatogram (t$_b$ 9.3, amarouciaxanthin A).
incubation. No morphological changes were observed in HepG2 cells cultured with HepG2 cells of alloxan A was produced by incubating fucoxanthinol for 24 h with the cultured medium were 68 (4 nmol/dish), the amounts of amarouciaxanthin A in the cells and in the cultured medium. After 24 h in culture with fucoxanthinol, since fucoxanthin was hydrolyzed into fucoxanthinol, whereas NADP \(^{-}\) was less preferred (Fig. 5B). The optimal pH for the conversion was 9.5 to 10.0 (Fig. 5C).

**Conversion of Fucoxanthinol into Amarouciaxanthin A by HepG2 Cells.** When HepG2 cells were cultured in fucoxanthinol-supplemented medium, amarouciaxanthin A was produced in the cells and in the cultured medium. After 24 h in culture with fucoxanthinol (4 nmol/dish), the amounts of amarouciaxanthin A in the cells and in the cultured medium were 68 ± 1 and 31 ± 5 pmol/dish, respectively (means ± S.D. of three dishes). On the other hand, no amarouciaxanthin A was produced by incubating fucoxanthinol for 24 h with the medium only, regardless of whether the medium was fresh or conditioned with HepG2 cells of ~90% confluence for 24 h before the incubation. No morphological changes were observed in HepG2 cells cultured with fucoxanthinol (1.0 \(\mu M\)) for 24 h compared with the cells cultured with the vehicle alone.

**Antiproliferative Effect on PC-3 Cells.** Figure 6A shows the dose-dependent effects of fucoxanthin, fucoxanthinol, and amarouciaxanthin A on the viability of PC-3 human prostate cancer cells. The IC\(_{50}\) values on the proliferation of PC-3 cells were 3.0, 2.0, and 4.6 \(\mu M\) for fucoxanthin, fucoxanthinol, and amarouciaxanthin A, respectively.

The cellular uptakes of fucoxanthin, fucoxanthinol, and amarouciaxanthin A after 4 h in culture are shown in Fig. 6B. No morphological changes were seen in PC-3 cells cultured for 4 h with the carotenoids at the concentration of 1.0 \(\mu M\). The cellular uptake of fucoxanthin was estimated as a combined value of fucoxanthin and fucoxanthinol, since fucoxanthin was hydrolyzed into fucoxanthinol in PC-3 cells (Fig. 6B). No amarouciaxanthin A was generated in PC-3 cells cultured with fucoxanthin- or fucoxanthinol-supplemented medium.

**Discussion**

Several reports have noted that fucoxanthin, the major carotenoid in edible brown algae, has potential health benefits. However, little is known about the absorption and metabolism of dietary fucoxanthin in mammals. As reported previously (Sugawara et al., 2002), fucoxanthinol and an unknown metabolite, identified in the present study as amarouciaxanthin A, were confirmed in mouse plasma after the oral administration of fucoxanthin. Both metabolites were also found in the liver. Since these metabolites were detected in plasma for 9 h (Fig. 2), they and/or their further metabolites may act as functional molecules in vivo.

In the present study, no unchanged fucoxanthin was found in the plasma or liver of the fucoxanthin-administered mice. Two hours after the oral administration, unchanged fucoxanthin was not detected in the contents of the small intestine, whereas fucoxanthinol was present predominantly (data not shown). Orally administered fucoxanthin therefore seems to be rapidly hydrolyzed to fucoxanthinol in the gastrointestinal tract, although it could be in part hydrolyzed after absorption within 2 h after the administration. The enzymes that hydrolyze fucoxanthin in the gastrointestinal tract might be lipase, cholesterol esterase, carboxylesterase, or others. Fucoxanthin was actually deacetylated into fucoxanthinol in vitro by cholesterol esterase in the present study and by lipase in our previous study (Sugawara et al., 2002). It has been suggested that fatty acid esters of carotenoids are hydrolyzed in the small intestine in humans, because no esters have been detected in chylomicrons or serum (Khachik et al., 1992; Wingerath et al., 1995). Strand et al. (1998) reported that fucoxanthinol but not fucoxanthin was transferred to the egg yolks of laying hens fed a diet containing 15% brown alga meal. This hydrolysis of fucoxanthin is consistent with the general phenomenon that animals absorb carotenoids as a nonesterified form.

Amarouciaxanthin A was shown in plasma and liver of mice also after the i.v. injection of fucoxanthinol. This indicates that amarouciaxanthin A was generated from fucoxanthinol in vivo. In the present study, fucoxanthinol was actually converted into amarouciaxanthin A by the liver homogenate preparation. Although the conversion did not occur by a similar homogenate preparation of small intestinal mucosa (data not shown), the possibility of extrahepatic conversion of fucoxanthin into amarouciaxanthin A cannot be excluded. Also, in HepG2...
cell culture, amarouciaxanthin A was produced from fucoxanthinol. This suggests that fucoxanthinol is metabolized to amarouciaxanthin A also in human liver, since HepG2 is a highly differentiated cell line used in numerous studies on liver functions as a model of human hepatocytes. No amarouciaxanthin A was produced from fucoxanthinol by incubation with the medium only, even when the medium was conditioned with HepG2 cells. Therefore, amarouciaxanthin A in cultured medium should be secreted from HepG2 cells but should not be converted from fucoxanthinol in the medium by the secreted materials. From these findings, we propose the metabolic pathway of dietary fucoxanthin shown in Fig. 7. Until now, amarouciaxanthin A has been found only in two marine invertebrates, a tunicate (A. pliciferum) (Matsumo et al., 1985a) and a bivalve (P. euglypta) (Matsumo et al., 1985b). From the structural similarity, Matsumo et al. (1985a) hypothesized that amarouciaxanthin A would be one of the metabolites of fucoxanthin in A. pliciferum. However, there has been no experimental evidence for the conversion of fucoxanthin into amarouciaxanthin A. In particular, the metabolism of dietary fucoxanthin in mammals has been quite unknown until the present study.

From the structures of fucoxanthinol and amarouciaxanthin A, we presumed that dehydrogenase participates in the conversion (dehydrogenation/isomerization) of fucoxanthinol into amarouciaxanthin A. As shown in Fig. 5, the conversion was observed to occur predominantly in the microsomes when microsomal contamination into the mitochondrial fraction was taken into account. The cofactor and pH dependences indicate that NAD(P)⁺-dependent dehydrogenase participates in the conversion. In mammals, a number of NAD(P)⁺-dependent dehydrogenases are involved in steroid and retinoid metabolisms and belong to short-chain dehydrogenase/reductase (SDR) superfamily. To our knowledge, this is the first report on mammals on the enzymatic dehydrogenation of a 3-hydroxyl end group of xanthophylls. Khachik et al. (1997, 2002) reported the presence of (3R,6'R)-3-hydroxy-β-carotene-3'-one (3'-oxolutein) in human blood plasma and ocular tissues and presumed that 3'-oxolutein is converted from dietary lutein in vivo. Similar to the conversion medium found in the present study, the oxidation of lutein to 3'-oxolutein might be enzymatically mediated.

5,6-Epoxy-xanthophylls such as fucoxanthin, neoxanthin, andviolaxanthin are widely distributed in nature and represent a major portion of dietary carotenoids in a number of vegetables, fruits, and edible algae. Neoxanthin and violaxanthin are major carotenoids in leafy green vegetables. Recently, we demonstrated that fucoxanthin and neoxanthin remarkably reduce the viability of prostate cancer cells via apoptosis induction (Kotake-Nara et al., 2001). Apoptosis induction by fucoxanthin has also been observed in HL-60 human leukemia cells (Hosokawa et al., 1999). In the present study, two metabolites of fucoxanthin, fucoxanthinol and amarouciaxanthin A, also reduced the viability of PC-3 cells (Fig. 6A). Since ~50% of incorporated fucoxanthin was hydrolyzed to fucoxanthinol in PC-3 cells within the 4-h culture (Fig. 6B), the antiproliferative effect of fucoxanthin may be attributed to the action of fucoxanthinol or its metabolites. Since epoxides are generally supposed to be chemically reactive, the 5,6-epoxide in fucoxanthin was considered to play important roles in cytotoxicity. However, amarouciaxanthin A, a non-epoxy metabolite, also reduced the viability of PC-3 cells (Fig. 6A). This suggests that the 5,6-epoxide is not essential for the cytotoxicity of epoxy-xanthophylls, although the IC₅₀ value of amarouciaxanthin A is higher than those of fucoxanthin and fucoxanthinol. One or more other mechanisms not based on the cytotoxicity of epoxide should be involved in the antiproliferative effect of epoxy-xanthophylls on PC-3 cells.

Despite their abundance in foodstuffs and potential health benefits, little is known about the absorption and metabolism of dietary epoxy-xanthophylls. In the present study, we demonstrated that orally administered fucoxanthin is hydrolyzed into fucoxanthinol and subsequently converted into amarouciaxanthin A in mice. Therefore, fucoxanthinol and amarouciaxanthin A, rather than fucoxanthin itself, should be considered in mechanistic studies of the biological actions of dietary fucoxanthin. To clarify the nutritional and therapeutic effects of epoxy-xanthophylls, including fucoxanthin, further studies should be required on the tissue distributions and the biological actions of these metabolites.

References