Absorption, Storage and Distribution of β-Cryptoxanthin in Rat after Chronic Administration of Satsuma Mandarin (Citrus unshiu Marc.) Juice

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Fruits and vegetables contain numerous antioxidants, such as carotenoids. Recent epidemiologic studies have demonstrated that a high dietary consumption of fruit and vegetables rich in carotenoids or with high serum carotenoid concentrations results in lower risks of certain cancers, diabetes, and cardiovascular disease. These results indicate that absorbed carotenoids are stored in various organs. Previously, we found that β-cryptoxanthin, found especially in Satsuma mandarin (Citrus unshiu Marc.), is easily absorbed and can also survive for a relatively long time in the human body; however, little is known about the absorption, storage, and tissue distribution of β-cryptoxanthin. In this study, we measured serum and the content of β-cryptoxanthin in several rat tissues after chronic ingestion of Satsuma mandarin extract rich in β-cryptoxanthin. Rats were fed a standard commercial diet containing Satsuma mandarin extract (containing β-cryptoxanthin at 11.7 mg/kg diet) for eight weeks. After 3 h of fasting, serum, liver, spleen, kidney, lung, heart, testis, brain, and epididymal fat were collected. The concentrations of β-cryptoxanthin in serum and tissues were evaluated by high-performance liquid chromatography. There was a wide range in the tissue levels of β-cryptoxanthin; liver had the greatest value, with 1265.3 ng/g tissue, followed by spleen, kidney, lung, heart, brain, and testis. Epididymal fat had the lowest value, with 6.99 ng/g tissue. β-Cryptoxanthin was also detected in serum in a concentration of 5.76 ng/mL. These results indicate that β-cryptoxanthin is easily absorbed and accumulated in several organs.

Key words: carotenoid; beta-cryptoxanthin; Satsuma mandarin; tissue; absorption; distribution

β-Cryptoxanthin is a carotenoid pigment found especially in Satsuma mandarin (Citrus unshiu Marc.). Previously, we found that serum β-cryptoxanthin reflects the frequency of Satsuma mandarin consumption. β-Cryptoxanthin might be easily absorbed, and it can survive for relatively long periods in the human body. Furthermore, we found that individuals with high serum β-cryptoxanthin levels (that is, individuals who regularly ate Satsuma mandarin) had lower risks for liver diseases, arteriosclerosis, insulin resistance, metabolic syndrome, low bone mineral density, and oxidative stress from a nutritional epidemiologic survey targeting the residents of Mikkabi, a town located in the north ward of Hamamatsu City in Shizuoka Prefecture, which is known as the leading Satsuma mandarin producer in Japan. From these results, we assume that absorbed β-cryptoxanthin is accumulated in several organs and that β-cryptoxanthin may have a protective effect against oxidative stress in several tissues. However, little is known about the storage and tissue distribution of β-cryptoxanthin in the body after chronic ingestion.

In this study, we examined the serum concentration and tissue distribution of β-cryptoxanthin in rats after chronic administration of Satsuma mandarin extract.

MATERIALS AND METHODS

Animal and Materials The concentrated Satsuma mandarin juice was purchased from the Ehime Beverage, Inc. (Ehime, Japan). The concentrated Satsuma mandarin juice (SMJ) was freeze-dried and mixed with a standard commercial diet (CE-2 diet, CLEA Japan Inc., Tokyo, Japan) at 10% (w/w).

Twelve Wistar rats (4 weeks old and with a 75–85 g body weight) were purchased from CLEA Japan, Inc. (Tokyo, Japan). All animals were maintained in an environmentally controlled room under a 12:12-h light–dark cycle and fed a standard CE-2 diet for one week before use. Six rats were fed the SMJ diet, and six other rats in the age-matched normal group were fed the standard CE-2 diet for eight weeks. Food and water were allowed ad libitum. This study was conducted in accordance with the Guide for the Care and Use of Laboratory Animals adopted by the National Institute of Fruit Tree Science.

After the eight-week administration of standard and SMJ diets, blood samples were obtained from the abdominal vena cava under diethyl ether anesthesia. Serum was separated from blood cells by centrifugation. After blood collection, rats were euthanized by decapitation; then, liver, spleen, kidney, lung, heart, testis, brain, and epididymal fat tissue were removed and stored at −80°C until analyses. All rats were dissected under 3 h of fasting.

In this study, serum and tissue were collected after 3 h fasting because, in rodents, carotenoid might be eliminated rapidly and deposited in the liver.

Extraction Procedure and Carotenoid Analysis. Tissue Extraction An aliquot of tissues (200–500 mg) was homogenized in a centrifuge tube using a homogenizer (Ultra Turrax T25, Rose Scientific, Ltd., Edmonton, Canada) with 10 volumes of ethanol–H₂O (1:1) containing 50 µg/mL dibutylhydroxytoluene (BHT). The homogenized solution was saponified for 30 min at 60°C in 10% methanolic KOH containing 0.1% (w/w) BHT. After saponification, 3 mL of water and 4 mL of hexane containing 0.1% (w/w) BHT were added and mixed for 20 min using a vortex mixer. After centrifugation
(4500 rpm, 10 min), the organic phase was recovered, and the extraction procedure from the aqueous phase was repeated twice. The combined organic phase was concentrated at 30°C under stream of nitrogen gas. The residue was dissolved in 200 µL of ethanol–chloroform (19:1) with 0.1% BHT, filtered with a membrane (polytetrafluoroethylene (PTFE), 0.2 µm), and subjected to HPLC analysis.

**Serum Extraction** A 200 µL aliquot of serum was mixed with 5 mL of water and 0.5 mL of ethanol (containing 0.25 µg/mL BHT). A 2.5 mL of hexane containing 0.1% (w/w) BHT was added and mixed for 20 min using a vortex mixer. After centrifugation (4500 rpm, 10 min), the organic phase was recovered, and the extraction procedure from the aqueous phase was repeated twice. The combined organic phase was concentrated at 30°C under stream of nitrogen gas. The residue was dissolved in 200 µL of ethanol–chloroform (19:1) with 0.1% BHT, filtered with a membrane (PTFE, 0.2 µm), and subjected to HPLC analysis.

**HPLC Analyses** The HPLC analysis was carried out in the following conditions: column, YMC carotenoid column (5 µm, 4.6 mm i.d. x 250 mm); solvent system, A) methanol–acetonitrile–H₂O (7:1:2) and B) tert-butylmethylether (TBME)–methanol (9:1); elution schedule, initial 2.5 min of 25.5% of A in B followed by a linear gradient with increasing concentration of B from 25.5 to 80% in 22.5 min; column temperature, 42°C; flow rate, 1.0 mL/min; injection volume, 50 µL; detection, UV absorption at 440 nm. The carotenoid quantities were estimated by the absolute calibration method. The peak for each carotenoid was identified by its UV spectrum obtained by a diode-array detector.

**RESULTS**

There was no evidence of any influence of the chronic SMJ treatment on body weight (data not shown). Furthermore, diet intakes did not differ from those of the group receiving a normal diet and another receiving a SMJ diet during the experimental period (data not shown). The carotenoid contents in the SMJ extract and the control and SMJ diets are summarized in Table 1. The major carotenoids of the standard diet were lutein, zeaxanthin, and β-carotene. A small amount of β-cryptoxanthin was detected in the standard diet (0.066 mg/kg). The carotenoid composition except for β-cryptoxanthin in the SMJ diet was similar to that of the standard diet. The SMJ diet was particularly enriched with β-cryptoxanthin (11.734 mg/kg). The SMJ extract also included α-carotene, and it was less than one-tenth of β-carotene.

Table 2 shows the serum and tissue carotenoid concentrations of the control and SMJ diets fed rats. In the group of rats receiving the control diet, none of the five carotenoids was detected in serum and most tissues, with the exception of the liver. Although lutein and zeaxanthin were not detected in the liver of the group receiving the control diet, α- and β-carotene and β-cryptoxanthin were detected in small amounts. In the control diet, β-carotene content is about four times higher than that of β-cryptoxanthin, but the concentration of β-carotene in the liver was similar to that of β-cryptoxanthin. On the other hand, in the group of rats receiving the SMJ diet, there was a wide range in the tissue levels of β-cryptoxanthin; liver had the greatest value, with 1265.3 ng/g tissue. Spleen was the second, with 471.86 ng/g tissue, followed by kidney, lung, heart, brain, and testis. Epididymal fat had the lowest value, with 6.99 ng/g tissue. β-Cryptoxanthin was also detected in serum in a concentration of 5.76 ng/mL. Meanwhile, β-carotene was detected in spleen and testis, and α-carotene was also detected in spleen of SMJ diet fed group.

**DISCUSSION**

Previously, many studies using animals have focused on
the absorption, storage, and tissue distribution of carotenoids, such as β-carotene and/or lycopene. However, little is known about the bioavailability of β-cryptoxanthin. In this study, we tested the hypothesis that absorbed β-cryptoxanthin is stored in several organs after chronic administration of Satsuma mandarin juice, which is rich in β-cryptoxanthin. Our results show that absorbed β-cryptoxanthin is accumulated in various organs and that there was a wide range in tissue level. These results indicate that various physiological functions of β-cryptoxanthin seem to be related to higher bioavailability of β-cryptoxanthin.

Among dietary carotenoids, all-trans-β-carotene has the highest provitamin A activity. This compound is converted to vitamin A in the intestinal mucosa. The efficiency of conversion varies among different species. In humans, most dietary β-carotene is converted to vitamin A in the gut; however, a substantial amount of intact β-carotene is also absorbed into the lymph. In contrast, in rodents, very little or no β-carotene is absorbed intact; it is largely converted to vitamin A before absorption. On the other hand, the conversion of retinol from provitamin carotenoids by the human body is actively regulated by the amount of retinol available to the body. Therefore, it seems that a substantial amount of intact β-carotene, except for a requisite amount as provitamin A, is also absorbed and released into the blood circulation and then accumulated in several tissues. In fact, previously, Shapiro et al. reported that β-carotene is distributed in liver, adrenal, ovary, heart, kidney, lung, skin, brain, and muscle of rats after chronic ingestion of β-carotene (0.2% diet). On the other hand, β-cryptoxanthin is also provitamin A. The provitamin A activity of β-cryptoxanthin is less than half of β-carotene; therefore, it seems that the majority of ingested β-cryptoxanthin is absorbed directly and released into the blood circulation in its intact form.

In our studies, liver had the greatest value of β-cryptoxanthin, with 1265.3 ng/g tissue, as with other carotenoids, such as β-carotene and/or lycopene. Previously, we found that high serum β-cryptoxanthin is associated with a lower risk for liver diseases, such as alcoholic- and non-alcoholic-related liver dysfunction, from a cross-sectional Mikkabi study. Carotenoids are mainly accumulated in the liver and combined into lipoprotein for release into the blood circulation. Ingested carotenoids could participate in an antioxidant defense system when present in high concentrations of free radical species in the liver, and these physiological functions of carotenoids could inhibit the development of liver dysfunction. In fact, recently, it has been reported in many studies that carotenoids, such as β-carotene, lycopene, lutein, and β-cryptoxanthin, have antioxidant effects against lipid peroxidation in rat liver, HepG2 human liver cells, and multilamellar liposomes. Therefore, liver might be a valuable target organ for carotenoids.

We also found that spleen accumulated β-cryptoxanthin with a high level (471.86 ng/g tissue). This content of β-cryptoxanthin per weight was about 37.3% of that in liver. The amount of β-cryptoxanthin in the spleen seems to be higher than that of other carotenoids. We have no clear explanation for the reason that the spleen accumulates β-cryptoxanthin in a high concentration, but we concluded that β-cryptoxanthin may act on the immune system such as production of white blood cell lymphocytes. Alternatively, β-cryptoxanthin stored in erythrocytes might be removed from old erythrocyte and accumulated in spleen. In this point, further studies will be required.

In our study, β-cryptoxanthin was also found in the brain of rats fed the SMJ diet with 21.66 ng/g tissue. The content of β-cryptoxanthin per weight was about 1.71% of that in liver. The amount of β-cryptoxanthin in the brain seems also to be higher than that of other carotenoids. Previously, we found that β-cryptoxanthin was dose-dependently incorporated into the brain of senescence-accelerated mice (SAMP10) and that β-cryptoxanthin ameliorated the memory impairment of SAMP10 mice through refinement of oxidative stress in the brain by aging. β-Cryptoxanthin might be a more useful micronutrient for the prevention of age-related oxidative damage in the brain and cognitive dysfunction than other carotenoids.

On the other hand, in the SMJ diet fed group, liver concentration of β-carotene was five times higher than that in control diet fed group. Furthermore, β-carotene was accumulated in spleen and testis, and α-carotene was also accumulated in spleen of SMJ diet fed group. These were due to the increase of dietary β-carotene and α-carotene from SMJ diet. However, among two dietary groups, the difference of content ratio of β-cryptoxanthin/β-carotene in the liver did not consist with that in the diet. Although we have no clear explanation for this result, increased accumulation of β-carotene and β-cryptoxanthin in the liver seems to be independent of the increased amount of dietary intakes.

β-Cryptoxanthin is a type of carotenoid that has a hydroxyl (OH) residue attached to one of the rings at the end of the carbon chain (Fig. 1). Despite being a xanthophyll, it has provitamin A activity as well as the ability to bind to a carotene binding protein and a retinoic acid receptor. Moreover, due to the presence of the OH residue, β-cryptoxanthin has a higher polarity than β-carotene. However, its polarity is not quite as strong as lutein and zeaxanthin, which have an OH residue at both ends of their carbon chain. β-Cryptoxanthin possesses both hydrophilic side chain and hydrophobic one. Due to these physico-chemical properties specific to β-cryptoxanthin, the localization of β-cryptoxanthin in tissues or cells differs...
slightly from that of other types of carotenoids; this may be related to the diverse characteristics of β-cryptoxanthin. Further studies are necessary to provide more detailed information about the characteristics of β-cryptoxanthin.

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