

## Serum Concentration of $\beta$ -Cryptoxanthin in Japan Reflects the Frequency of Satsuma Mandarin (*Citrus unshiu* Marc.) Consumption

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Beta-cryptoxanthin ( $\beta$ -CRX) is a carotenoid pigment found in peach, papaya, and citrus fruits such as orange and tangerine. It is also found, especially, in Satsuma mandarin (*Citrus unshiu* Marc.), which is heavily produced in Japan and is mainly harvested from October to February. In this study, we investigated the relationship of serum concentration of  $\beta$ -CRX to the frequency of Satsuma mandarin consumption using 94 healthy nonsmoking female volunteers. In September, when Satsuma mandarin is not in season, even though the volunteers had not eaten citrus fruits or processed food including citrus for a two-month period, the serum concentration of  $\beta$ -CRX in the participants who ate more than four Satsuma mandarins daily was high enough to be statistically significant compared with the participants who rarely ate any ( $p < 0.05$ ). Furthermore, when the Satsuma mandarin was in season in January, the serum concentration of  $\beta$ -CRX increased remarkably depending on the frequency of Satsuma mandarin consumption. These results suggest that  $\beta$ -CRX is stored in some tissues for several months and that it is a useful biomarker for estimating the frequency of Satsuma mandarin consumption.

**Key words** — beta-cryptoxanthin, carotenoid, Satsuma mandarin, biomarker

## INTRODUCTION

Recent epidemiological studies provide strong support for the hypothesis that the daily consumption of fruits and vegetables is effective in the prevention of diseases such as cancer and coronary heart disease, in which oxidative damage to biological macromolecules plays an important role.<sup>1–3)</sup> Carotenoid pigments exist in abundance in fruits and vegetables and have been shown to have strong antioxidant and anti-tumor activities *in vitro* and *in vivo*.<sup>4,5)</sup> Although many studies regarding the absorption, metabolism, and distribution of carotenoids such as  $\beta$ -carotene and lycopene have been carried out in the past,<sup>6–10)</sup> less examined are the xanthophyll carotenoids, which are characterized by the presence of one or more functional groups containing oxygen. However, these carotenoids are also an important biological precursor of vitamin A.

Production of Satsuma mandarin, which is harvested from October to February, is heavy in Japan. Mandarins have been found to be an especially significant source of beta-cryptoxanthin ( $\beta$ -CRX).<sup>11,12)</sup> Recent *in vitro* and *in vivo* experiments have shown that  $\beta$ -CRX has cancer prevention attributes, making it a beneficial carotenoid for health.<sup>13–15)</sup> There have been some epidemiological studies on the serum concentration of  $\beta$ -CRX<sup>16–19)</sup>; however, we investigated here the relationship between the serum concentration of  $\beta$ -CRX and the frequency of Satsuma mandarin consumption. We recruited healthy nonsmoking female volunteers who live in Shizuoka, the Tokai district, which is an important producer of the Satsuma mandarin. Tokai residents consume larger quantities of Satsuma mandarin than residents elsewhere. The objective of this study was to evaluate how differences in consumption of this fruit influence serum concentration of  $\beta$ -CRX.

## MATERIALS AND METHODS

A total of 94 healthy nonsmoking female individuals, from 41 to 69 years of age (median, 56 years; SD, 6.81), who are residents of Shizuoka Prefecture in Japan, volunteered for the study. They understood the research objectives and agreed to blood tests to measure the concentration of serum  $\beta$ -CRX. The volunteers completed a self-administered questionnaire seeking information about their frequency of Satsuma mandarin consumption and other fruits and vegetables. In the survey, four levels were used to

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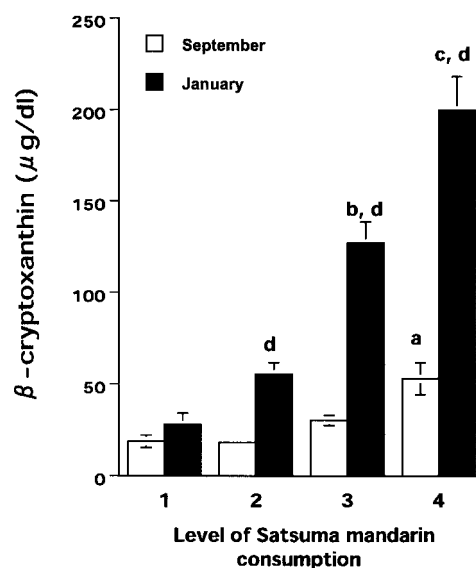
ascertain the level of consumption of Satsuma mandarin during the peak of the season from October to February. They were as follows: Level 1: "I rarely eat Satsuma mandarin." Level 2: "Although I sometimes eat Satsuma mandarin, I eat fewer than three pieces of this fruit a week." Level 3: "I eat one to three Satsuma mandarins daily." Level 4: "I eat more than four Satsuma mandarins daily." Furthermore, specific attention was given to the consumption of fruits and vegetables that contain  $\beta$ -CRX, such as apricot, chili, loquat, orange, peaches, pepper and papayas.

After completing a self-administered questionnaire in June, fasting blood samples were collected twice in September, when the Satsuma mandarin is not in season, and in January of the next year, when it is in season. Care was taken to ensure that volunteers had not consumed citrus fruits, juices, or related products for at least two months before the September blood test. After clotting for 30 min in the dark at room temperature, the blood samples were centrifuged for 10 min at 1800 *g* to obtain serum. Serum samples were stored at  $-80^{\circ}\text{C}$  until analysis. The concentration of serum  $\beta$ -CRX was analyzed by reverse-phase high-performance liquid chromatography according to the method previously reported.<sup>20)</sup>

All data are represented as means  $\pm$  S.E.M. The statistical significance of difference among each group or among the data in September and January in the same group were evaluated using a Kruskal-Wallis test followed by Scheffe's test or paired *t*-test, respectively. Significance was accepted if the null hypothesis was rejected at the  $p < 0.05$  level.

## RESULTS AND DISCUSSION

We examined the relationship of serum concentration of  $\beta$ -CRX with the frequency of Satsuma mandarin consumption using the 94 healthy non-smoking female volunteers. Even in September, when Satsuma mandarin is not in season, the concentration of serum  $\beta$ -CRX in Levels 4 was higher than it was in Level 1 (Fig. 1). Furthermore, in January, when Satsuma mandarin is in season, the concentration of serum  $\beta$ -CRX increased remarkably in a dose-dependent manner (Fig. 1). An increased amount of serum  $\beta$ -CRX in Levels 2, 3, and 4 was considered to be due to the consumption of this fruit because the frequency of consumption of other fruits or vegetables which contain  $\beta$ -CRX did not differ



**Fig. 1.** The Relationship of Serum Concentration of  $\beta$ -Cryptoxanthin with the Frequency of Satsuma Mandarin Consumption

The levels of Satsuma mandarin consumption are as follows: Level 1: "I rarely eat Satsuma mandarin." Level 2: "Although I sometimes eat Satsuma mandarin, I eat fewer than three pieces of this fruit a week." Level 3: "I eat one to three Satsuma mandarins daily." Level 4: "I eat more than four Satsuma mandarins daily." Open columns show the results in September, closed show those in January. All data are represented as means  $\pm$  S.E.M. Number of observations in group of levels 1, 2, 3, and 4 are 5, 11, 52, and 26 respectively. a;  $p < 0.05$  vs. data of September in group of level 1, b;  $p < 0.05$ , c;  $p < 0.01$  vs. data of January in group of level 1 in Scheffe's test. d;  $p < 0.001$  vs. data of September in the same group in paired *t*-test.

from level to level (data not shown). Two of these results are interesting. First, the amount of  $\beta$ -CRX absorbed from the intestine had probably accumulated in several kinds of tissue, such as skin and adipocyte, for several months. The  $\beta$ -CRX that accumulated in these tissues may be gradually released into the blood circulation. This would be demonstrated by the fact that the concentration of serum  $\beta$ -CRX in Level 4 in September was higher than it was in Level 1 even though none of the volunteers had eaten food considered to be a source of  $\beta$ -CRX, such as citrus fruits or related processed foods, including juice or canned foods for at least two months. Secondly, the serum  $\beta$ -CRX levels may reflect the frequency of consumption of Satsuma mandarin. Because one Satsuma mandarin (about 100 g) includes 1–2 mg of  $\beta$ -CRX in a juice sac and no other fruits or vegetables contain  $\beta$ -CRX in as large a quantity as this, we assume that the main source of  $\beta$ -CRX for Japanese is Satsuma mandarin. Although apricot, chili, loquat, papaya, peach, pepper, and tangerine contain  $\beta$ -CRX,<sup>12,21)</sup> they are not as large a

source of  $\beta$ -CRX as Satsuma mandarin because Japanese do not eat these foods as often. Actually, in this survey, the ingestion frequency of these foods was much lower in comparison with Satsuma mandarin and did not differ from level to level (data not shown).

Numerous studies on the absorption, metabolism, and distribution of carotenoids such as  $\beta$ -carotene and lycopene in several tissues have been reported<sup>6-10</sup>; however, little is known regarding the biokinetics of xanthophylls such as  $\beta$ -CRX. Xanthophylls such as  $\beta$ -CRX have a hydroxyl residue that exists as a xanthophyll ester with fatty acids in fruits or vegetables.<sup>21,22</sup> When a xanthophyll ester is absorbed from the intestine, it is cleaved by an endogenous enzyme to free xanthophylls.<sup>22</sup> Also, it is known that the blood concentration of  $\beta$ -CRX, in contrast to that of  $\beta$ -carotene or lycopene, is high even when the consumption of the substance is low.<sup>23,24</sup> For this reason, we assume that absorbed xanthophylls from the intestines accumulate easily in several tissues, such as, for example, skin or adipocyte, as xanthophyll esters form with fatty acid. Recently, it has been reported that xanthophyll esters are present in human skin,<sup>25</sup> these esters in human skin may be formed by reesterification of xanthophylls following absorption. From these facts, it can be concluded that xanthophylls are easily absorbed and accumulated in comparison with other carotenoids such as  $\beta$ -carotene. Therefore, it is conceivable that the serum concentration of xanthophylls is high in spite of their low consumption.

In conclusion, we investigated the relationship between serum  $\beta$ -CRX level and the frequency of Satsuma mandarin consumption. Our results clearly suggest that serum concentration of  $\beta$ -CRX in Japan reflects the frequency of Satsuma mandarin consumption. From this finding, it will be possible to estimate the relationship of the consumption of this fruit with chronic diseases by analyzing correlation of the serum concentration of  $\beta$ -CRX and a disease biomarker in urine or blood. Furthermore, results indicate that this substance can be stored in some tissues for several months. Whether or not absorbed  $\beta$ -CRX is primarily accumulated in a  $\beta$ -CRX ester form with fatty acid remains unclear. Another question that needs to be answered is which tissue it is in which  $\beta$ -CRX accumulates with the greatest ease. Further studies on the absorption and accumulation of  $\beta$ -CRX are needed.

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