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Beneficial effects of a beta-cryptoxanthin-containing beverage on body mass index and visceral fat in pre-obese men: Double-blind, placebo-controlled parallel trials

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ABSTRACT

Beta-cryptoxanthin (β-CX), a carotenoid abundant in Satsuma mandarin (*Citrus unshiu* Marc), is suggested to suppress visceral fat accumulation in animal models. Therefore, the aim of this study was to investigate whether daily intake of a beverage containing β-CX for 12 weeks reduces body mass index (BMI) and abdominal visceral fat. For this purpose, two double-blind, placebo-controlled parallel trials were conducted. In the first pilot trial, pre-obese and obese men who consumed a beverage containing 1 mg β-CX showed a smaller increase in BMI in the β-CX group than in the placebo group. In the second main trial, pre-obese men in the β-CX group who consumed a beverage containing 2 mg β-CX showed a larger decrease in BMI and visceral fat area compared with those in the placebo group, without notable side effects. These findings suggest that this β-CX-containing beverage may exert an anti-obesity effect in pre-obese men.

1. Introduction

The number of obese people is increasing around the world. The Non-Communicable Disease Risk Factor Collaboration estimated that the number of obese people reached 641 million in 2014 (Di Cesare et al., 2016). Because obesity confers a high risk of not only lifestyle-related disease such as diabetes and hypertension but also coronary heart disease (Guh et al., 2009), many anti-obesity drugs have been developed. However, these drugs often have severe side effects (Tschöp et al., 2016). Thus, new treatment strategies for preventing obesity without side effects are required.

Recently, certain foods have attracted attention to prevent obesity. Beta-cryptoxanthin (β-CX), a xanthophyll carotenoid, is abundant in vegetables and fruits such as Satsuma mandarin (*Citrus unshiu* Marc), papaya, and persimmon, which have a long dietary history. β-CX is absorbed in the small intestine, enters the circulation, and accumulates in the body in white adipose tissue, the liver, and other organs (Chung et al., 2009; Kaplan, Lau, & Stein, 1990). Several epidemiological studies have demonstrated that serum β-CX level and intake of β-CX are inversely correlated with BMI (Andersen et al., 2006; Ben Amara et al., 2015; Hirose et al., 2017; Sugiura et al., 2004). For example, a 7-year longitudinal study of Americans revealed that changes in serum β-CX level of non-smokers were inversely correlated with changes in BMI (Andersen et al., 2006). Moreover, a cross-sectional study investigating the relation between 98 nutritional factors and BMI in Japanese individuals showed that only intake of β-CX was inversely correlated with BMI (Hirose et al., 2017). These findings indicate that the increase in blood β-CX level through the intake of food containing β-CX is a new strategy with anti-obesity effects.

β-CX administration has been reported to suppress the accumulation of fat in adipose tissue of an obese mouse model (Takayanagi et al., 2011). However, the absorption of carotenoids and their accumulation in the body are markedly different between primates and rodents (Krinsky et al., 1990; Lee et al., 1999). Specifically, primates can accumulate absorbed carotenoids in abundance in organs, whereas rodents can accumulate only limited amounts, suggesting that an intervention study is necessary to evaluate the efficacy and absorption of β-CX in obese or pre-obese subjects. However, evidence of the effect and absorption of β-CX is limited; one clinical study has been reported in Japanese (Tsuchida, Mukai, Mizuno, Masuko, & Minagawa, 2008). In the previous study, subjects consumed two beverages containing β-CX per day only after meals. To make it easier for subjects to continue

Abbreviations: β-CX, beta-cryptoxanthin; VFA, visceral fat area; SFA, subcutaneous fat area; LDL, low-density lipoprotein; HDL, high-density lipoprotein; HbA1c, haemoglobin A1c; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; γ-GTP, γ-glutamyltranspeptidase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase; TB, total bilirubin; TP, total protein; UA, uric acid; UN, urea nitrogen; WBC, white blood cell count; RBC, red blood cell count; CEH-CDH, cholesterol ester hydrolase-cholesterol dehydrogenase; GPO, glycerol-3-phosphate oxidase; ACS-ACOD, acetyl coenzyme A synthetase and acetyl coenzyme A oxidase; JSCC, Japan Society of Clinical Chemistry

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Table 1
Composition of the test beverages for trial 1 and trial 2.

	Trial 1 (/90 mL)		Trial 2 (/65 mL)	
	Placebo	β-CX	Placebo	β-CX
Energy (kcal)	5.0	4.5	32.0	31.0
Protein (g)	0.1	0.1	0.5	0.5
Fat (g)	< 0.1	0.1	< 0.1	< 0.1
Carbohydrate (g)	0.9	0.5	7.5	7.3
Hesperidin (g)	0.08	0.08	0.11	0.12
β-CX (mg)	< 0.01	1.2	0.03	2.0

β-CX, beta-cryptoxanthin.

consuming β-CX, we considered evaluating β-CX efficacy when subjects consume a β-CX-containing beverage once a day and did not regulate their time of intake. Moreover, the previous study used emulsified β-CX, but we used natural, non-emulsified β-CX. Accordingly, we evaluated β-CX efficacy under different trial conditions.

Thus, to examine whether the daily consumption of a beverage containing β-CX (β-CX beverage) reduces BMI and visceral fat and increases the blood level of β-CX, we conducted two double-blind, placebo-controlled parallel trials in pre-obese men.

2. Materials and methods

2.1. Trial 1: Effect of 12 weeks' intake of the β-CX beverage on BMI in pre-obese and obese men

2.1.1. Test beverages

β-CX beverage and placebo beverage (90 mL, packed in foil pouches) were compared in this pilot trial. The beverages had similar colour and flavour. There were no differences in the energy and nutritional contents, except that β-CX beverage and placebo beverage contained 1.2 mg β-CX and < 0.01 mg β-CX, respectively (Table 1). In this trial, we used β-CX derived from Satsuma mandarin juice residue (ARKRAY, Inc., Kyoto, Japan). Because daily intake of hesperidin, which is a flavonoid present in high concentrations in Satsuma mandarin, has been reported to reduce BMI and serum triacylglycerol levels (Hanawa et al., 2008; Miwa et al., 2005; Park et al., 2001), the content of hesperidin in both beverages was adjusted so that it was approximately equal.

2.1.2. Subjects

Twenty-six Japanese men aged 27–60 years with BMI ranging from 25 to 32 kg/m^2 who did not use any regular medication were included in this trial. Throughout the trial, the subjects were instructed not to eat foods containing high levels of β-CX, such as Satsuma mandarin, papaya, persimmon, and loquat, and not to eat functional foods which may reduce body weight and adipose tissue including Food for Specified Health Uses, and not to change their lifestyle habits including exercise, sleeping, smoking, and alcohol consumption. Subjects who did not adhere to the instructions or whose intake rate of the test beverage was inadequate were excluded from the data analysis. This study was conducted in accordance with the guidelines of the Declaration of Helsinki. All procedures involving human subjects were approved (approval code: H21-079) by the Ethics Committee on Human Research of Yakult Central Institute (Tokyo, Japan). Written informed consent was obtained from all subjects before enrolment.

2.1.3. Study protocol

This pilot study was designed as a double-blind, placebo-controlled parallel trial consisting of a 1-week pre-treatment period and 12-week treatment period. During the pre-treatment period, subjects were randomly divided into the β-CX group and placebo group, which were matched for body weight and BMI. During the treatment period, each subject consumed a pack of β-CX beverage or placebo beverage per day after a meal from July to October 2009. Physical and clinical parameters were evaluated at the beginning of the pre-treatment period (baseline), the beginning of the treatment period (Week 0), and the last day of the treatment period (Week 12).

2.2. Trial 2: Effect of 12 weeks' intake of the β-CX beverage on BMI and visceral fat in pre-obese men

2.2.1. Test beverages

The effects of β-CX beverage and placebo beverage (65 mL), containing 2.0 mg and 0.03 mg β-CX, respectively, were compared in this main trial (Table 1). The two beverages were packed in cartons and had similar colour and flavour. There were no differences in the energy, nutritional, and hesperidin contents.

2.2.2. Subjects

The number of subjects was determined from the results of trial 1 using nQuery Advisor version 7.0® (Statistical solutions Ltd., Cork, Ireland). Overall, 92 Japanese men aged 25–63 years with BMI ranging from 25 to 30 kg/m^2, were included in this trial. The subjects did not use any regular medication or supplements, and did not have serious disease requiring urgent treatment. For all subjects, body weight was stable (within 2 kg) for 1 year before the study. As described above for trial 1, the subjects complied with dietary restrictions to avoid the intake of foods containing high amounts of β-CX and functional foods, and maintained their usual lifestyle habits to avoid changes during the trial. Subjects who did not comply or whose intake rate of the test beverage was low were excluded from the data analysis. This trial was conducted in accordance with the guidelines of the Declaration of Helsinki. All procedures involving human subjects were approved (approval code: H24-0118) by the institutional review board of Yokohama Tsuchida Medical Clinic (Kanagawa, Japan). Written informed consent was obtained from all subjects before enrolment.

2.2.3. Study protocol

This main trial was designed as a double-blind, placebo-controlled parallel trial consisting of a 4-week pre-treatment period and 12-week treatment period, and conducted at eight clinical centres in Japan (Ageo Kousei Hospital, Saitama; Kameido-minamiguchi Clinic, Tokyo; Koshigaya Seiwa Hospital, Saitama; Kodama Central Hospital, Saitama; Sakuragaoka Central Hospital, Kanagawa; Toyooka-daiichi Hospital, Saitama; Mizuno Internal Medical Clinic, Saitama; Yuki Clinic, Tokyo). During the pre-treatment period, subjects were randomly assigned to the β-CX group or placebo group by minimisation procedure; there were no differences in average body weight, BMI, and visceral fat area (VFA) between the two groups. During the treatment period from May to August 2012, each subject consumed a carton of β-CX beverage or placebo beverage per day (at any time). Subjects underwent evaluation of physical and clinical parameters every 4 weeks during the treatment period (Week 0, Week 4, Week 8, and Week 12; Week 0 represents the beginning of the treatment period). The primary endpoint was the change in BMI. Secondary endpoints were the changes in body weight, VFA, subcutaneous fat area (SFA), body fat ratio, waist circumference, and hip circumference.

2.3. Analysis

2.3.1. Physical and clinical parameters

Body weight and height were measured with a body fat scale (Tanita Co., Tokyo, Japan) and a stadiometer, respectively, to calculate the BMI in both trials. In trial 2, body fat ratio and waist and hip circumferences were measured with a body fat scale and a tape measure, respectively. Blood pressure and pulse rate were determined after a rest in the seated position for longer than 10 min. VFA and SFA were analysed (Week 0, Week 8, and Week 12) with a computed tomography (CT) scan,