ABSTRACT: For 44 wk, thirty male volunteers were given daily either 60 mg of synthesized all-trans β-carotene, a naturally-occurring β-carotene derived from Dunaliella bardawil, or a placebo. Basal levels of 9-cis β-carotene in plasma, platelets, and mononuclear cells were 10, 20, and 25% of those of the all-trans form, respectively. The plasma levels reached a maximum after two weeks of administration and plateaued thereafter in the subjects who took the β-carotene preparations. The all-trans β-carotene level in the subjects given the synthesized all-trans form was almost twice that for the Dunaliella preparation. The plasma 9-cis level was found to be higher in the all-trans β-carotene group than in the Dunaliella group, despite no intake of the 9-cis form in the all-trans group and the higher intake of the 9-cis form in the Dunaliella group. This finding suggests that isomerization of the all-trans form to the 9-cis form may occur in the body either during or after absorption.


Naturally-occurring carotenoids have recently attracted much attention, due to extensive epidemiological evidence and intervention studies which indicate their potential usefulness in cancer prevention (1,2). However, little is known about the metabolism of β-carotene, the most potent precursor of vitamin A, contained in significant amounts in fruit and vegetables. The halotolerant alga Dunaliella bardawil and the closely related species D. salina have the unique capacity to accumulate naturally-occurring β-carotenoids intracellularly, especially all-trans β-carotene and 9-cis β-carotene. With respect to bioavailability of the naturally-occurring β-carotenoids, a few investigations have shown that all-trans β-carotene is predominant in human plasma (3–5), whereas only a small amount of the geometrical isomers of all-trans β-carotene is found, even if a large amount of β-carotene isomer derived from alga has been taken orally (6–8). Little is known about the absorption of 9-cis β-carotene, which is the second most predominant form contained in the alga. It is not clear whether the 9-cis isomer is converted to the other stereoisomers during ingestion and absorption, or whether it is directly stored in specific tissues, but not in plasma. In this paper, we investigate changes in stereoisomers of β-carotene in plasma and in blood cells of healthy human subjects after ingesting either naturally-occurring β-carotene derived from D. bardawil or a synthesized all-trans β-carotene preparation.

MATERIALS AND METHODS

Subjects and β-carotene intake. Thirty male volunteers, aged 20–25 years old, were given either 60 mg of a synthesized all-trans β-carotene (kindly provided by Nippon Roche K.K., Tokyo, Japan), 60 mg of a naturally-occurring β-carotene derived from D. bardawil (Nikken Soohnsha Corporation, Gifu, Japan), or an identical placebo. The preparations were taken orally every morning immediately after breakfast for 44 wk. None of the subjects took any other vitamin supplements or medications before or during the study. The study was performed in a single blind manner. The study protocol was approved by the ethics committee of the hospital, and informed consent was obtained from the subjects themselves.

Blood collection. Blood was collected with EDTA-Na₂ after an overnight fast. The blood samples were centrifuged at 900 rpm for 10 min at room temperature to obtain platelet-rich plasma in the top layer and white blood cells containing red blood cells (RBC) in the bottom layer. The top layer was centrifuged again at 3000 rpm for 10 min to sediment the platelets, which were suspended in a sterile plastic tube with 10 mL of 0.9% NaCl and washed three times with 10 mL of 0.9% NaCl (2 × 5 min and 1 × 10 min). After the final washing, the platelet pellet was resuspended in 2.5 mL of 0.9% NaCl and sonicated for 1 min at 20 kc with a Handy Sonic (Ultrasonic Disruptor UD-201; Tomy Co. Ltd., Tokyo, Japan). RBC and mononuclear cells (MN) were separated by density-gradient centrifugation using a “Lymphoprep®” (Nycomed Pharma AS, Oslo, Norway). Three milliliters of the RBC–MN suspension were slowly placed on the 1.5 mL layer of the medium, and then centrifuged at 1,200 rpm for 30 min at 10°C. Thus, the MN layer was separated from the RBC layer. The RBC pellet was washed three times with 0.9% NaCl (2 × 5 min and 1 × 10 min), and after the final washing the cells were resuspended in an equivalent volume of 0.9% NaCl. The hematocrit of the RBC suspension was also measured for a final correction. The tubes containing MN were filled with 10 mL of 0.9% NaCl and centrifuged at 10°C for

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Abbreviations: HPLC, high-performance liquid chromatography; MN, mononuclear cell; RBC, red blood cell.
15 min at 1,000 rpm. To remove small amounts of RBC contaminated in the layer, the MN pellet was then washed for 40 s with 2 mL of hypotonic 0.2% NaCl solution, to which 2 mL of 1.6% NaCl was added after 40 s. After refilling the tubes with 10 mL of 0.9% NaCl, centrifugation and decantation at 1,000 rpm for 15 min at 10°C were repeated twice. After the final washing, the pellets were suspended in 2.5 mL of 0.9% NaCl, and the cells were counted with a leukocyte counter (Sysmex Platelet Counter, PL-110; Kobe, Japan). Then, the plasma, platelets, MN, and RBC were assayed for ß-carotene in this study.

Analysis of ß-carotene and retinol. Determination of ß-carotene has been reported previously (9). Briefly, 1 mL of ethanol containing 0.015% butylated hydroxytoluene was added to 0.2 mL of plasma or 0.4 mL of a cell suspension followed by vigorous shaking under nitrogen gas. Five mL of 0.9% NaCl was added to this mixture, which was then centrifuged at 3,000 rpm for 10 min, and 4 mL of the hexane layer was evaporated under a stream of nitrogen. The residue was dissolved in 50 µL of ethanol; a 20 µL aliquot was injected into the high-performance liquid chromatography (HPLC) column for determination of ß-carotene, and the 20 µL aliquot from the plasma extract sample was injected into the other HPLC column for retinol determination. The HPLC equipment for ß-carotene was an IRICA Σ 871 (IRICA Co. Ltd., Kyoto, Japan) with a Vydac (Hesperia, CA) reverse-phase C18 column (Cat. No. 201TP54; 250 × 4.6 mm), and the detector was an IRICA Σ 875 amperometric detector. The standard eluent was methanol/acetonitrile (95:5, vol/vol), including 50 mM NaClO4, and the flow rate was 1 mL/min. Authentic all-trans and 9-cis ß-carotenes were provided by Nippon Roche K.K., and were dissolved in methanol to produce the standard. The concentration of the authentic ß-carotene standard was spectrophotometrically determined using an absorption coefficient $E_{1%}^{1cm} = 2620$ at 450 nm for all-trans form and $E_{1%}^{1cm} = 2290$ at 446 nm for 9-cis form by a Hitachi U-2000 spectrophotometer (10) (Hitachi Co. Ltd., Tokyo, Japan). The HPLC for retinol measurement was a Shimadzu LC-5A (Shimadzu Co. Ltd., Kyoto, Japan) with an IRICA