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Determination of 9-*cis* β-carotene and ζ-carotene in biological samples☆

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Abstract

Concentrations of 9-*cis* β-carotene (9-*cis* βC) and ζ-carotene (ζC) in biological samples may provide crucial information on the biological activities of these carotenoids. However, in high-performance liquid chromatography (HPLC) these carotenoids are often co-eluted. Therefore, there is an urgent need to develop a method for 9-*cis* βC and ζC quantitation. Both 9-*cis* βC and ζC have peak absorbance at 400 and 450 nm, respectively, whereas only 9-*cis* βC has peak absorbance at 475 nm. We developed a HPLC method to quantitate 9-*cis* βC and ζC by using peak absorbance ratios. The 9-*cis* βC/ζC peak area was monitored at 475, 450 and 400 nm. The 9-*cis* βC was quantified by using absorbance value at 475 nm; ζC was then calculated from the 9-*cis* βC/ζC peak at 400 nm by subtracting 9-*cis* βC contribution at 400 nm using the 400-nm/475-nm peak absorbance ratio of 9-*cis* βC (0.39). This method was applied to determine 9-*cis* βC and ζC concentrations in serum and breast milk samples (n=12) from American lactating women and serum and breast adipose tissue samples (n=16) from Korean women with either benign or malignant breast tumors. 9-*cis* βC concentrations in serum and breast milk of American women, and serum and adipose tissue of Korean women were 7.1±0.8 and 1.1±0.2 nM, and 15.6±1.1 nM and 0.2±0.1 nmol/g, respectively. ζC concentrations in the above samples were 54.2±7.2 and 8.3±1.8 nM, and 49.0±3.9 nM and 0.3±0.1 nmol/g, respectively.

Keywords: 9-*cis* β-carotene; ζ-carotene; Serum; Breast milk; Adipose tissue

1. Introduction

Carotenoids represent a large group of phytochemicals that may contribute to health and disease prevention. It is generally accepted that serum carotenoid concentrations are markers of recent fruit and vegetable intake [1], whereas tissue carotenoid levels are indicators of longer-term carotenoid consumption patterns [2]. The major β-carotene (βC) isomer in human serum is all-*trans* βC (tβC; Fig. 1A), with small or negligible amounts of 13-*cis* βC and 9-*cis* βC (Fig. 1B, C). However, there are considerable amounts of 9-*cis* βC and 13-*cis* βC present in various human tissues [3]. It has been suggested that the distribution pattern of βC isomers in various organs may explain, in part, tissue-specific functions of βC [4].

ζ-Carotene (ζC) is a precursor of lycopene (Fig. 1F), which is widely distributed in fruits and vegetables such as tomatoes and tomato-based products, apricot, cantaloupe and oranges [5–9], and in human biological samples such as blood and milk [10,11]. Supplementation with ζC-containing foods increases ζC levels in human serum [12] and human breast tissue [13]. Like other carotenoids, ζC has several isomers such as 7,8,7′,8′ tetrahydrolycopene and 7,8,11,12 tetrahydrolycopene (Fig. 1D and E) [14,15]. Although the biological importance of ζC and its isomers has not been clearly studied, some studies have suggested that ζC may be an important carotenoid that contributes to the overall health benefit resulting from the consumption of fruits and vegetables [16].

High-performance liquid chromatography (HPLC) provides an effective method for separation and quantitation of carotenoids in biological samples. However, under current

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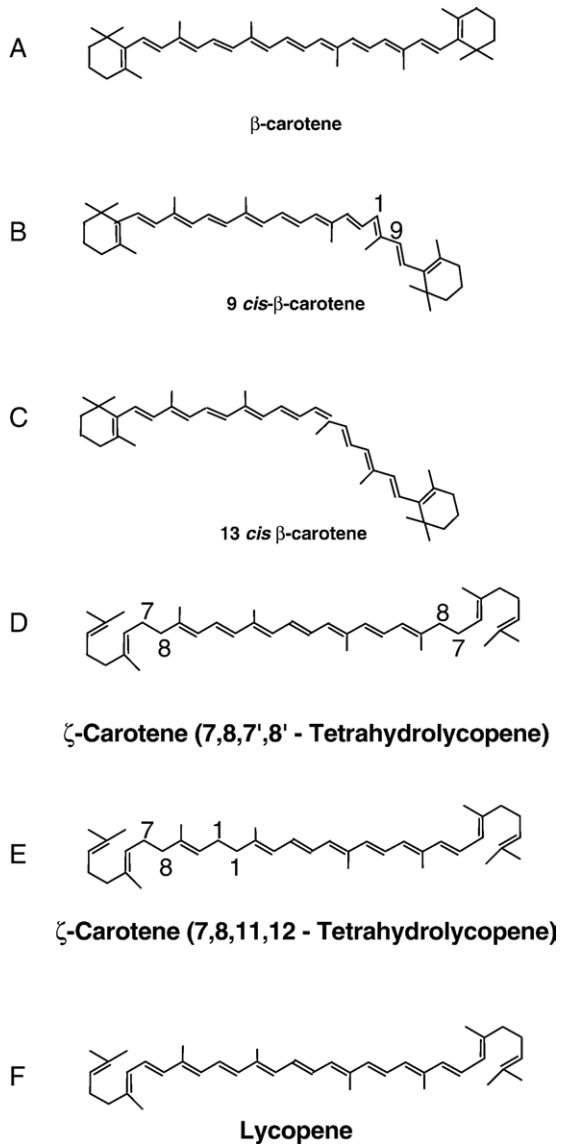

Fig. 1. Structures of (A) tβC; (B) 9-*cis* βC; (C) 13-*cis* βC; (D) ζC (7,8,7′,8′ tetrahydrolycopene); (E) ζC (7,8,11,12 tetrahydrolycopene); (F) lycopene.

HPLC conditions using a C30 column with a mobile phase of methanol/*tert*-butyl methyl ether/water, 9-*cis* βC and one of the ζC isomers elute at the same time [7,17]. A specific calculation method for quantitating both 9-*cis* βC and ζC in this mixture is needed. In this paper, we report a method to selectively calculate the concentrations of both 9-*cis* βC and ζC in biological samples, including human serum, milk and adipose tissue.

2. Materials and methods

2.1. Chemical products

HPLC-grade acetonitrile, tetrahydrofuran (stabilized with 0.01% butylated hydroxytoluene), methanol and water were purchased from J. T. Baker Chemical (Philipsburg, NJ, USA). *tert*-Butyl methyl ether was purchased from Sigma-Aldrich (St. Louis, MO, USA). Solvents were filtered through a 0.45-μm membrane filter and degassed prior to use. tβC (type IV) and lycopene were purchased from Sigma (St. Louis, MO, USA). Echinenone and *trans*-ζC were gifts from Hoffmann-La Roche (Nutley, NJ, USA).

2.2. Sample collection

Ten milliliters of fasting blood was collected in no additive vacutainer from each of 12 healthy lactating American women (1–8 months postpartum, 18–40 years old) participating in a previously conducted study examining carotenoid concentrations in serum and breast milk [17]. Foremilk (10 ml) was also collected by hand expression at the same time as blood collection. Blood samples were centrifuged at 800×*g* for 15 min at 4°C within 1 h of sample collection. Both serum and milk aliquots were stored at −70°C for carotenoid analysis which was performed within 5 years.

Breast adipose tissues were obtained from women who were either diagnosed with a benign breast tumor (n=8) during surgical resection or breast cancer (n=8) during mastectomy or breast quadrantectomy in Korea, who were participants of a previously published study [18]. Overnight fasting blood samples were also collected. Serum was centrifuged at 4°C for 15 min at 800×*g*. Both tissue and serum samples were stored at −70°C for analysis which was performed within 5 years.

The study protocols from which these samples were obtained were approved by the Institutional Review Board of Tufts-New England Medical Center and the Asian Medical Center, Seoul, Korea. Informed consents were obtained from all subjects.

2.3. Extraction of carotenoids in serum, milk and adipose tissue samples

One hundred fifty microliters of serum was analyzed using a modified extraction method reported previously [19]. The samples with an internal standard (known amount echinenone in ethanol) were extracted with 3 ml of chloroform/methanol (2:1) followed by 2 ml of hexane. The two extracts were combined and evaporated to dryness under nitrogen. The residue was dissolved in 150 μl of ethanol, and a 50-μl aliquot was injected onto an HPLC system.

Carotenoids in milk were extracted following the procedures described previously [17] with minor modifications. To the 2 ml of milk sample was added 100 μl of 12% pyrogallol in ethanol, 3 ml of KOH in water (1:1, wt/wt) and 3 ml of ethanol. The mixture was vortexed and sonicated for 1 min, then incubated at 37°C for 2 h. After incubation, the sample was cooled down to room temperature and 100 μl of a known amount of echinenone in ethanol was added as an internal standard, followed by the addition of 4 ml of hexane. The mixture was vortexed and sonicated for 1 min, and centrifuged at 800×*g* at 4°C for 5 min. The upper layer was removed. The extraction with 2 ml of hexane was repeated and the upper layers combined. Three milliliters of water and 3 ml of ethanol

were added to the extract. After centrifugation, the upper layer was collected and evaporated to dryness under nitrogen. The residue was dissolved in 100 μl of ethanol, and a 50-μl aliquot was injected onto an HPLC system.

Approximately 40 mg of adipose tissue was used for the extraction following the procedures described previously [18] with minor modifications. To the sample was added 100 μl of 12% pyrogallol in ethanol, 200 μl of 30% KOH in water and 1 ml of ethanol. The mixture was incubated at 37°C for 2 h. After incubation, the sample was cooled down to room temperature and 100 μl of known amount of echinenone in ethanol was added as an internal standard. The mixture was extracted with 3 ml of ether–hexane (2:1, v/v). The mixture was vortexed and then centrifuged at 800×*g* at 4°C for 5 min. The upper layer was removed. The extraction with 2 ml of ether–hexane was repeated and the upper layers combined. To the extract was added 1 ml of water and 1 ml of ethanol. The mixture was vortexed and centrifuged at 800×*g* for 5 min. The upper layer was collected and evaporated to dryness under nitrogen. The residue was dissolved in 100 μl of ethanol, and a 50-μl aliquot was used for HPLC analysis.

2.4. HPLC analysis

The extracted samples were analyzed for carotenoids using a reverse-phase, gradient HPLC method as previously described [18]. The HPLC system consisted of a series 410 LC pump (Perkin-Elmer, Norwalk, CT, USA), a Waters 717 plus autosampler (Waters Co., Milford, MA, USA), a C18 guard column (3 μm, 33×4.6 mm, Perkin-Elmer), a C30 carotenoid column (3 μm, 150×4.6 mm, YMC, Wilmington, NC, USA), an HPLC column temperature controller at 16°C (Model 7950 Column Heater/Chiller, Jones Chromatography, Lakewood, CO, USA) and a Waters 840 digital 350 data station. The Waters 994 programmable photodiode array detector was set at 400, 450 and 475 nm for carotenoids. The HPLC mobile phase Solvent A was methanol/*tert*-butyl methyl ether/water (83:15:2, v/v/v, with 1.5% ammonium acetate in the water), and the mobile phase Solvent B was methanol/*tert*-butyl methyl ether/water (8:90:2, v/v/v, with 1% ammonium acetate in the water). The gradient procedure at a flow rate of 1 ml/min began at 100% Solvent A for 2 min and went to 70% Solvent A over a 6-min linear gradient and held at 70% A for 3 min. This was followed by a 10-min linear gradient to 45% Solvent A and a 2-min hold at 45% Solvent A, then a 10-min linear gradient to 5% Solvent A, a 4-min hold at 5% Solvent A and, finally, a 2-min linear gradient back to 100% Solvent A. The system was held at 100% Solvent A for 10 min for equilibration back to initial conditions.

2.5. Quantitation of 9-cis βc and ζc

As shown in Fig. 2, both 9-*cis* βC and ζC absorb at 400 nm and 450 nm, whereas only 9-*cis* βC absorbs light at 475 nm. The amount of 9-*cis* βC was then quantified by determining peak areas at 475 nm in the HPLC chromatograms, calibrated against known amounts of standard. The detection limit of 9-*cis* βC was 0.2 pmol. Standard 9-*cis* βC had an absorbance ratio (400/475 nm) of 0.39. Therefore, for a mixture of 9-*cis* βC and ζC, the actual absorbance of ζC was determined using the following steps:

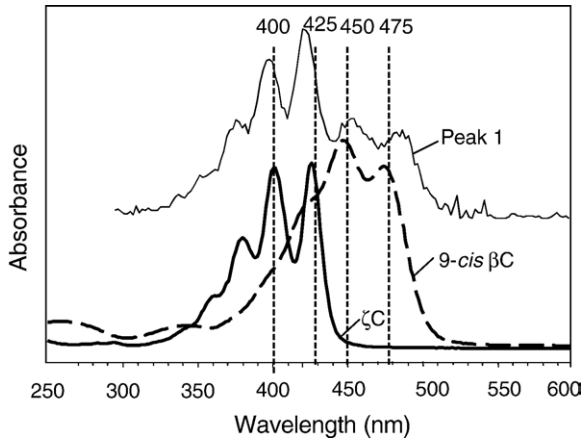

Fig. 2. Spectra of ζC, 9-*cis* βC and their co-eluted fraction (Peak 1).

(1) Peak area of 9-*cis* βC (400 nm)=peak area of 9-*cis* βC (475 nm)×0.39
(2) Total peak area (400 nm)=peak area of 9-*cis* βC (400 nm)+peak area of ζC (400 nm)
(3) Peak area of ζC (400 nm)=total peak area (400 nm) −peak area of 9-*cis* βC (400 nm)
(4) ζC (ng)=peak area of ζC (400 nm)×ng/area[1]

2.6. LC/APCI-MS analysis

Two milliliters of serum was extracted as above, dissolved in 60 μl of ethanol, and a 50 μl aliquot was injected in the HPLC to collect the 9-*cis* βC/ζC peak for LC/MS analysis using atmospheric pressure chemical ionization (APCI). The procedure to collect this fraction is described below.

(1) The extract was injected onto the same HPLC system described above, except that the system used a C18 guard column (3 μm, 4.6×33 mm; Perkin-Elmer) and a C18 analytical column (3 μm, 4.6×83 mm; Perkin-Elmer) [19]. The one-step-wash isocratic procedure at a flow rate of 1 ml/min employed solvents based on combinations of acetonitrile/tetrahydrofuran/water. Solvent A was 50:20:30 (v/v/v), and Solvent B was 50:44:6 (v/v/v). Twenty-five percent Solvent A was used for 25 min, followed by a 2-min linear gradient to 100% Solvent B and held at 100% B for 9 min to wash out the residue in the column. Then, a 2-min linear gradient back to 25% Solvent A. 9-*cis* βC and ζC co-eluted using this system and the mixture was collected and evaporated to dryness under nitrogen. The residue was redissolved in 60 μl of ethanol for further separation.

[1] ng/area was as determined by curves using standard ζC at 400 nm.

(2) The collected elute from Step 1 was injected onto the same HPLC system as described above using a YMC C30 column. The ζC peak, which contains small amounts of 9-*cis* βC, was collected to obtain a purer fraction and evaporated to dryness under nitrogen. The residue was resuspened in 60 μl of ethanol for LC/APCI-MS analysis.

LC/MS analysis was carried out using an Agilent 1100 series HPLC system (Palo Alto, CA, USA)/Esquire-LC (Wilmington, DE, USA) mass spectrometer (Bruker Daltonic, Inc., Bremen, Germany). The Agilent 1100 series HPLC system was equipped with an autosampler, a diode-array UV/visible diode array detector, a C18 guard column and a C18 analytical column (Perkin-Elmer). The LC mobile phases consisted of acetonitrile/tetrahydrofuran/water, with solvent A being 50:20:30 (v/v/v) and Solvent B being 50:44:6 (v/v/v). The mobile phases were pumped through a 0.005-in. ID PEEK with 15% Solvent A and 85% Solvent B at a flow rate of 1 ml/min. The LC/MS with APCI source and ion trap was used for analysis. APCI was performed in a positive mode [20]. The instrumental parameters were optimized with regard to maximum signal intensity of molecular ions, which included nitrogen nebulizer pressure of 60 psi, the counter-current drying gas temperature of 300°C at a flow rate of 5 L/min that was supplied from a liquid nitrogen cylinder and vaporizer temperature of 300 °C. The APCI source was a corona discharge from a

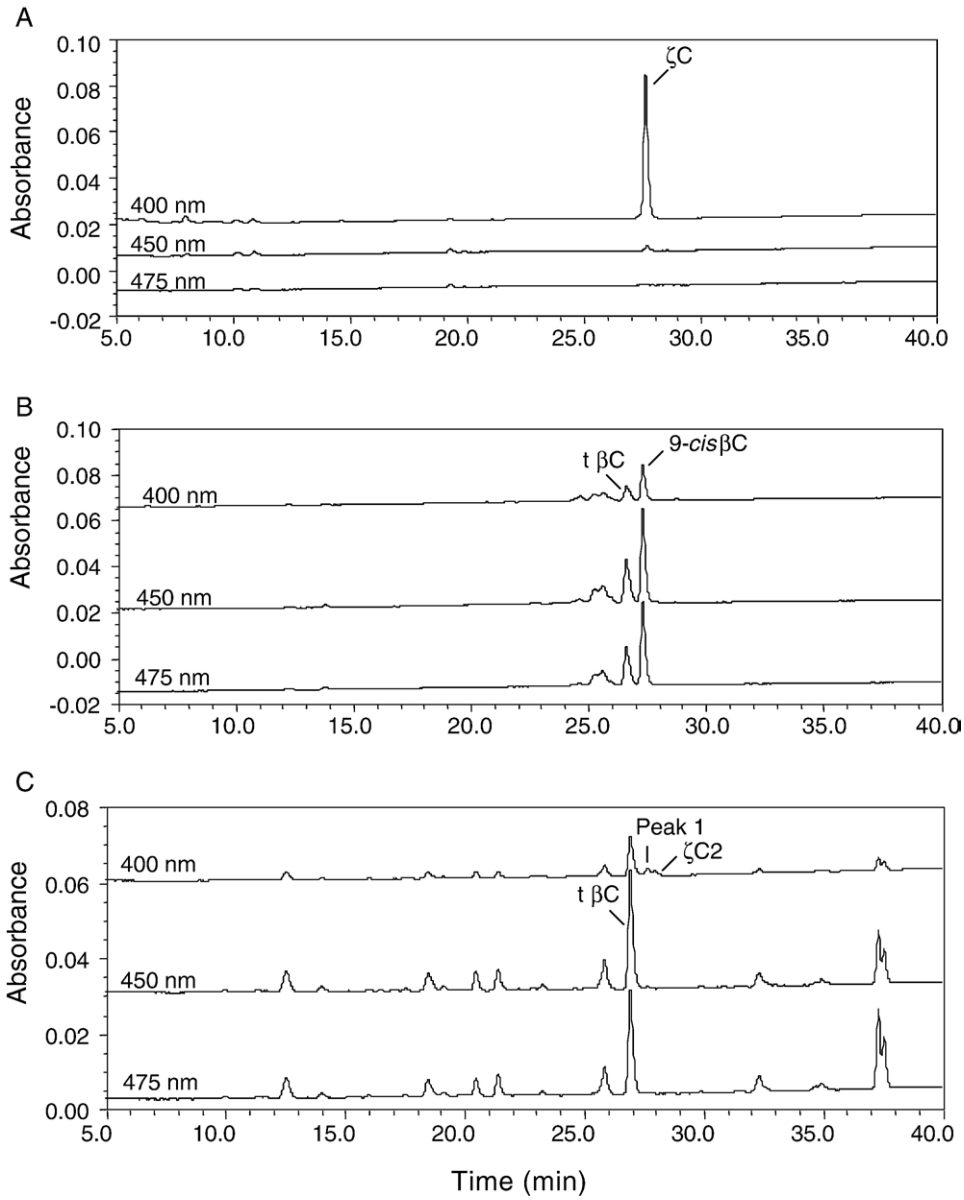

Fig. 3. HPLC chromatograms of ζC isomers and 9-*cis* βC. (A) Chromatogram of ζC standard in ethanol; (B) chromatogram of 9-*cis* βC standard in ethanol; and (C) chromatogram of an extract of breast adipose tissue.

needle generated by a corona needle voltage of 1800 V, capillary current of 2000–3000 and endplate offset of −500 V. A positive iron scan mode was used to record the abundance of the collected ζC at *m*/*z* 530–560.

3. Results

3.1. Verification of co-elution and identification of 9-cis βc and ζc

As shown in Fig. 3, standards of both ζC and 9-*cis* βC had the same retention time (Fig. 3A, B, respectively). In the biological samples, such as adipose tissue (Fig. 3C), and serum and milk, a mixed peak (Peak 1 in Fig. 3C) exists at the same retention time as that of ζC and 9-*cis* βC. Peak 1 had absorbance at 400, 450 and 475 nm. Spectral analysis showed that Peak 1 was a combination of the spectra of ζC and 9-*cis* βC (Fig. 2). This demonstrates that ζC and 9-*cis* βC are present in the biological samples.

In the serum analysis it was noted that another peak eluted just after the 9-*cis* βC/ζC peak and had 400 nm absorbance but none at 475 nm (Fig. 3C), suggesting that it may be a ζC isomer. UV/Vis analysis showed that it is identical with the ζC standard and was designated as ζC2. ζC2 was found in all tissues evaluated in our study (serum, milk and adipose tissue).

The LC/MS method was used to verify the identity of Peak 1. As shown in Fig. 4, LC/APCI-MS analysis indicated that Peak 1 (Fig. 4B) contained a compound that had the same MS spectrum as that of ζC (Fig. 4A) [*M*/*Z*=540, $(M+H)^+$=541], providing further evidence that ζC co-eluted with 9-*cis* βC. The 9-*cis* βC [*M*/*Z*=536, $(M+H)^+$=537] level in this sample was below the detection limit of MS. Therefore, its mass could not be detected.

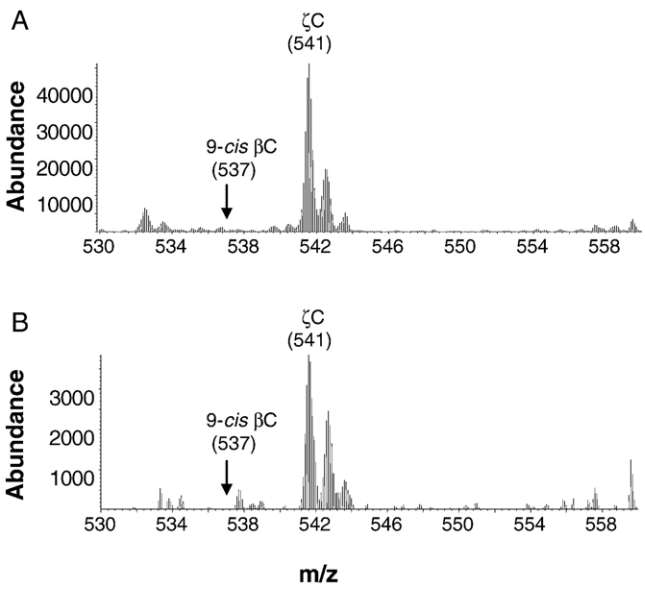

Fig. 4. LC/APCI-MS analyses of (A) ζC standard and (B) a collected fraction of the Peak 1 mixture.

Table 1
Analytical recovery of tβC (208.8 ng) and ζC (168.0 ng) in pooled serum

Compound	*n*	Recovery (%)	S.D.	CV (%)	Range (%)
tβC	6	95.9	2.7	2.8	91–98
ζC	6	103.4	1.5	1.4	101–105

3.2. Determination of the recovery of the extraction process, precision, accuracy and detection limits of tβc and ζc

The analytical recoveries from pooled serum added known amounts of tβC and ζC were 95.9±2.7% and 103.4±1.5%, respectively, as shown in Table 1. The precision (within day and between days) and the accuracy of the method are given in Tables 2 and 3. The detection limits of tβC and ζC, which were judged using three times noise of baseline as the criterion, are 0.1 ng for both of these carotenoids.

3.3. Quantitation of 9-cis βc and ζc isomers in biological samples

9-*cis* βC and the ζC isomers (ζC1 and ζC2) in serum, milk and adipose tissues in the 12 healthy lactating American women were calculated using the method described above in Section 2.5. In addition, other carotenoids, such as tβC and all-*trans* lycopene (*t*-Lycopene), were also quantitated. The results are shown in Table 4. The carotenoid concentrations in serum and adipose tissues in Korean women are calculated and shown in Table 5. tβC was found to be the major carotenoid in serum, breast milk and adipose tissue. ζC also presented a substantial amount in these samples. The ratio of total ζC (ζC1 and ζC2) to lycopene was 1.2:1 in serum and 3:1 in milk in the healthy lactating American women. It was 1.2:1 in serum and 6:1 in adipose tissues of Korean women with either a benign breast tumor or breast cancer in our study, respectively.

4. Discussion

There was limited information on concentration of isomers of βC in serum. In the report by Stahl et al. [3], 9-*cis* βC was not found in serum samples. Also, few data on tβC in breast fat tissue were reported [21], where the data were on dried (lyophenized) fat tissue (human fat usually contains less than 20% water). With correction on water

Table 2
Analytical precision of tβC and ζC standards by HPLC analysis

	n	Mean (ng)	S.D.	CV (%)
Within day				
tβC	6	57.6	0.7	1.3
ζC	9	20.1	0.2	1.2
Between days				
tβC	3	58.7	1.8	3.0
ζC	3	20.1	0.2	1.0

Table 3
Analytical accuracy of tBC (208.8 ng) and zc (168.0 ng) in pooled serum

Compound	*n*	Relative error (%)	S.D.	Range (%)
tBC	6	−4.1	2.7	−9.1 to −1.9
zc	6	3.4	1.5	1.5 to 4.2

content, the reported concentration of tβC in the American breast tissue of cancer cases (0.47–1.23 μg/g dry wet) and benign cases (0.98–1.52 μg/g dry wet) was comparable with the cancer/benign cases observed in this report. In breast milk of the US lactating women (n=12), the βC concentration was reported as 36.1–50.4 nmol/L [22], which is comparable with our data of 35.4 nM of tβC of the healthy American lactating women.

Given that ζC and 9-*cis* βC cannot be separated by HPLC with a C30 column using currently available methods, the existence of ζC in human samples has never been reported. Now, we developed an indirect method to distinguish the concentrations of ζC and 9-*cis* βC in blood, milk and adipose tissue. Our results indicate that ζC concentrations in serum were 12- and 5-fold higher than that of 9-*cis* βC in American and Korean women, respectively. Interestingly, the ratio of ζC1+ζC2 to lycopene and their concentrations is remarkably similar between these two groups of women. This may be due to the fact that ζC isomers and lycopene are found in the same type of foods, i.e., tomatoes and tomato products. These quantities of ζC isomers in biological tissues warrant further study of their possible role in human health. Furthermore, the ability to accurately and quantitatively determine 9-*cis* βC may be of importance given that its metabolite, 9-*cis* retinoic acid, is active in the regulation of gene expression [23,24].

ζC is an acyclic carotenoid that is present in many fruits and vegetables such as tomatoes and tomato-based products, apricot, cantaloupe and oranges. Several ζC isomers are found in plants [15]; both 7,8,7′,8′ tetrahydrolycopene (Fig. 1C) and 7,8,11,12 tetrahydrolycopene (Fig. 1D) have been reported [14]. However, very little attention has been paid to the ζC isomers in biological samples. We found two major ζC isomers in these biological samples. We identified components in this mixture peak by using UV spectra against standards of 9-*cis* βC and ζC and by using APCI-MS. The results were not confirmed by an NMR analysis due to the limited amount of samples. Because of the lack of standards for all but one ζC isomer, we were unable to identify the specific ζC isomers. Further research is needed in this regard.

To date, the separation of 9-*cis* βC from ζC isomers has been difficult [7]. In this study, we report a method to quantify both 9-*cis* βC and ζC in a co-eluted fraction. Our results demonstrate that 9-*cis* βC can be quantitated at 475 nm, a wavelength at which the absorbance of ζC is minimal. ζC isomers are present in human biological samples and can be quantified by a HPLC system using C30 and an absorbance of 400 nm, along with the proper calculation. Considering the significant amount of ζC in circulation and tissues of humans and their responses to dietary intake [12], accurate quantitation of this carotenoid is important in determining its possible health benefits.

Table 4
Concentration of major carotenoids including tβC, 9-*cis* βC, ζC Peak 1 (ζC1), ζC Peak 2 (ζC2) and *t*-Lycopene in serum and milk samples of healthy American lactating women (mean±S.E.M.)

	tβC	9-*cis* βC	ζC1	ζC2	*t*-Lycopene
Serum (nM) (n=12)	258.0±55.5	7.1±0.8	54.2±7.2	33.6±4.8	73.9±9.1
Milk (nM) (n=12)	35.4±8.7	1.1±0.2	8.3±1.8	13.7±3.1	7.2±0.6

Table 5
Concentration of major carotenoids including tβC, 9-*cis* βC, ζC1, ζC2 and *t*-Lycopene in serum and adipose tissue of Korean women with either a benign breast tumor or breast cancer (mean±S.E.M.)

	tβC	9-*cis* βC	ζC1	ζC2	*t*-Lycopene
Serum (nM) (n=16)	667.7±56.3	15.6±1.1	49.0±3.9	35.7±4.8	70.0±14.0
Adipose tissue (nmol/g) (n=16)	1.2±0.2	0.2±0.1	0.3±0.1	0.3±0.1	0.1±0.02

References

[1] van Kappel AL, Steghens JP, Zeleniuch-Jacquotte A, Chajes V, Toniolo P, Riboli E. Serum carotenoids as biomarkers of fruit and vegetable consumption in the New York Women's Health Study. Public Health Nutr 2001;4:829–35.

[2] El-Sohemy A, Baylin A, Kabagambe E, Ascherio A, Spiegelman D, Campos H. Individual carotenoid concentrations in adipose tissue and plasma as biomarkers of dietary intake. Am J Clin Nutr 2002; 76:172–9.

[3] Stahl W, Schwarz W, Sundquist AR, Sies H. *Cis-trans* isomers of lycopene and beta-carotene in human serum and tissues. Arch Biochem Biophys 1992;294:173–7.

[4] Clinton SK, Emenhiser C, Schwartz SJ, Bostwick DG, Williams AW, Moore BJ, et al. *Cis-trans* lycopene isomers, carotenoids, and retinol in the human prostate. Cancer Epidemiol Biomarkers Prev 1996;5: 823–33.

[5] Khachik F, Beecher GR, R LW. Separation, identification, and quantification of the major carotenoids in extracts of apricots, peaches, cantaloupe, and pink grapefruit by liquid chromatography. J Agric Food Chem 1989;37:1465–73.

[6] Khachik F, Beecher GR, Smith Jr JC. Lutein, lycopene, and their oxidative metabolites in chemoprevention of cancer. J Cell Biochem Suppl 1995;22:236–46.

[7] Rouseff R, Raley L. Application of diode array detection with a C30 reversed phaseolumn for the separation and identification of saponified orange juice carotenoids. J Agric Food Chem 1996;44: 2176–81.

[8] Lee HS. Objective measurement of red grapefruit juice color. J Agric Food Chem 2000;48(5):1507–11.

[9] Khachik F, Goli MB, Beecher GR, Holden JM, Lusby WR, Tenorio MD, et al. Effect of food preparation on qualitative and quantitative distribution of major carotenoid constituents of tomatoes and several green vegetables. J Agric Food Chem 1992;40:390–8.

[10] Khachik F, Beecher GR, Goli MB, Lusby WR, Smith Jr JC. Separation and identification of carotenoids and their oxidation products in the extracts of human plasma. Anal Chem 1992;64:2111–22.
[11] Khachik F, Spangler CJ, Smith Jr JC, Canfield LM, Steck A, Pfander H. Identification, quantification, and relative concentrations of carotenoids and their metabolites in human milk and serum. Anal Chem 1997;69:1873–81.
[12] Paetau I, Khachik F, Brown ED, Beecher GR, Kramer TR, Chittams J, et al. Chronic ingestion of lycopene-rich tomato juice or lycopene supplements significantly increases plasma concentrations of lycopene and related tomato carotenoids in humans. Am J Clin Nutr 1998;68: 1187–95.
[13] Zhao Z, Khachik F, Richie Jr JP, Cohen LA. Lycopene uptake and tissue disposition in male and female rats. Proc Soc Exp Biol Med 1998;218:109–14.
[14] Pfander H, Gerspacher M, Rychener M, Schwabe R. Key to carotenoids. 2nd ed. Basel: Birkhauser Verlag; 1987. p. 25–6.
[15] Bonora A, Pancaldi S, Gualandri R, Fasulo MP. Carotenoid and ultrastructure variations in plastids of *Arum italicum* Miller fruit during maturation and ripening. J Exp Bot 2000;51:873–84.
[16] Beecher GR, Khachik F. Evaluation of vitamin A and carotenoid data in food composition tables. J Natl Cancer Inst 1984;73:1397–404.
[17] Johnson EJ, Qin J, Krinsky NI, Russell RM. Beta-carotene isomers in human serum, breast milk and buccal mucosa cells after continuous oral doses of all-*trans* and 9-*cis* beta-carotene. J Nutr 1997;127: 1993–9.
[18] Yeum KJ, Ahn SH, Rupp de Paiva SA, Lee-Kim YC, Krinsky NI, Russell RM. Correlation between carotenoid concentrations in serum and normal breast adipose tissue of women with benign breast tumor or breast cancer. J Nutr 1998;128:1920–6.
[19] Tang G, Krinsky NI. Differentiation between central and eccentric cleavage of beta-carotene. Methods Enzymol 1993;214:69–74.
[20] Ferreira AL, Yeum KJ, Russell RM, Krinsky NI, Tang G. Enzymatic and oxidative metabolites of lycopene. J Nutr Biochem 2003;14: 531–40.
[21] Zhang S, Tang G, Russell RM, Mayzel KA, Stampfer MJ, Willett WC, et al. Measurement of retinoids and carotenoids in breast adipose tissue and a comparison of concentrations in breast cancer cases and control subjects. Am J Clin Nutr 1997;66(3):626–32.
[22] Canfield LM, Giuliano AR, Neilson EM, Yap HH, Graver EJ, Cui HA, et al. Beta carotene in breast milk and serum is increased after a single beta-carotene dose. Am J Clin Nutr 1997;66(1):52–61.
[23] Heyman RA, Mangelsdorf DJ, Dyck JA, Stein RB, Eichele G, Evans RM, et al. 9-*cis* retinoic acid is a high affinity ligand for the retinoid X receptor. Cell 1992;68:397–406.
[24] Levin AA, Sturzenbecker LJ, Kazmer S, Bosakowski T, Huselton C, Allenby G, et al. 9-*cis* retinoic acid stereoisomer binds and activates the nuclear receptor RXR alpha. Nature 1992;355:359–61.