

Plasma appearance of labeled β -carotene, lutein, and retinol in humans after consumption of isotopically labeled kale

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Abstract The bioavailability of carotenoids from kale was investigated by labeling nutrients in kale with ^{13}C , feeding the kale to seven adult volunteers, and analyzing serial plasma samples for labeled lutein, β -carotene, and retinol. Ingested doses of labeled carotenoids were 34 μmol for β -carotene and 33 μmol for lutein. Peak plasma concentrations, areas under the plasma concentration-time curves (AUCs), and percentages of dose recovered at peak plasma concentrations were calculated. Average peak plasma concentrations were 0.38, 0.068, and 0.079 μM for [^{13}C]lutein, [^{13}C] β -carotene, and [^{13}C]retinol, respectively. Average AUC values (over 28 days) were 42.8, 13.6, 13.2 $\mu\text{M h}$ for [^{13}C]lutein, [^{13}C] β -carotene, and [^{13}C]retinol, respectively. Percentages of dose recovered at peak plasma concentrations were 3.6, 0.7, and 0.7% for [^{13}C]lutein, [^{13}C] β -carotene, and [^{13}C]retinol, respectively. A positive relationship was observed between baseline plasma retinol levels and [^{13}C]retinol plasma response. It is possible that this relationship was mediated either through some aspect of β -carotene absorption or via the common pathways of metabolism for postdose and endogenous retinoid.—Novotny, J. A., A. C. Kurilich, S. J. Britz, and B. A. Clevidence. Plasma appearance of labeled β -carotene, lutein, and retinol in humans after consumption of isotopically labeled kale. *J. Lipid Res.* 2005. 46: 1896–1903.

Supplementary key words bioavailability • vitamin A • liquid chromatography-mass spectrometry • *Brassica oleracea*

Carotenoids are a large class of compounds that may contribute to the health-promoting effects of plant-based foods by acting as antioxidants in the defense against oxidative stress and free radicals (1–3). Some carotenoids, such as β -carotene, are also precursors to vitamin A and therefore make plant-based foods a potentially important source of vitamin A for humans. Diets high in the carotenoids lutein and zeaxanthin seem to prevent the onset of age-related macular degeneration (4).

For health professionals to make recommendations for carotenoid intakes to provide these health benefits, it is necessary to have a clear understanding of carotenoid bioavailability from food sources. Because carotenoids are present in fasting plasma, the mixing of endogenous carotenoid with newly ingested carotenoid complicates bioavailability studies. Labeling compounds with isotopes allows the discernment of newly ingested nutrients from endogenous nutrients. It is additionally helpful if the labeled nutrient can be administered within the food matrix, because absorption of pure carotenoids is much higher than that of carotenoids in foods (5–9).

In the present study, we used kale containing β -carotene and lutein labeled with ^{13}C to study their bioavailability. The kale was fed to volunteers, and plasma responses of the labeled compounds were used to investigate the bioavailability of lutein and β -carotene from kale.

EXPERIMENTAL PROCEDURES

Intrinsic labeling of kale

The method for labeling kale nutrients with ^{13}C has been described previously (10). Briefly, kale (*Brassica oleracea* variety *acephala* cultivar Vates) was grown in a controlled-environment chamber with the only source of carbon being $^{13}\text{CO}_2$ to achieve complete ^{13}C labeling of the kale tissue. The labeling chamber consisted of a clear acrylic box (Plexiglas G; Rohm and Haas, Wilmington, DE) with a separate base. The top fit into a water-filled trough in the base to provide a leak-proof seal to prevent the exchange of $^{13}\text{CO}_2$ with atmospheric CO_2 . Plants were grown in Nalgene bins inside the acrylic box and were subirrigated. The partial pressure of CO_2 inside the chamber was continuously monitored via a WMA-3 PP System and a WMA-3 PID controller (PP Systems, Haverhill, MA), which injected $^{13}\text{CO}_2$ (>99% ^{13}C ; Isotec, Inc., Miamisburg, OH) if levels decreased to <40 Pa. The acrylic box was placed inside a controlled-environment chamber for maintenance of temperature (24°C), humidity (90%), and

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light (24 h photoperiod, 900 $\mu\text{mol}/\text{m}^2/\text{s}^1$ photosynthetically active radiation incident on the labeling chamber).

The kale was produced in two growth cycles. Kale plants were harvested at leaf canopy closure. Harvest was performed rapidly in dim light after opening the acrylic growth box. Once roots and primary leaves were removed, material for the feeding study was cleaned, weighed, and blanched. The blanched kale was chopped into 1 inch \times 1 inch pieces, mixed for batch uniformity, weighed into 50 g portions, and frozen at -80°C until preparation for consumption. Each batch of kale was processed and frozen immediately after harvest.

Subjects

Seven healthy, nonsmoking volunteers (four men, three women) from the Beltsville, MD, area participated in this study. All procedures were approved by the Johns Hopkins University Bloomberg School of Public Health Committee on Human Research, and subjects gave written, informed consent before participation. Subjects' characteristics (mean \pm SD) were as follows: aged 46 ± 14 years, body weight of 71 ± 8 kg, and body mass index (BMI) of 25 ± 3 . Average baseline plasma concentrations of key analytes (mean \pm SD) were $0.34 \pm 0.21 \mu\text{M}$ for β -carotene, $0.35 \pm 0.09 \mu\text{M}$ for lutein, and $2.88 \pm 0.43 \mu\text{M}$ for retinol.

Study design, diet, and sample collection

Each subject consumed a single treatment of ^{13}C -labeled kale. The 400 g (3 cup) treatments of frozen kale were transferred to a refrigerator (6°C) for 12 h, then warmed in a microwave oven (900 W) for 2 min before consumption. The treatment was served with 30 g of safflower oil ($n = 6$) or peanut oil for our pilot subject ($n = 1$). This dose provided 34 μmol of [^{13}C] β -carotene (20 mg) and 33 μmol of lutein (20 mg). Each subject received three 50 g portions from kale growth batch 1 and five 50 g portions from kale growth batch 2.

Blood was collected according to the following schedule: 10 times on the dose day (0, 2, 3, 4, 5, 6, 8, 10, 12, and 14 h), 2 times on the following day (one fasting morning sample and one afternoon sample), each morning for the next 3 days (fasting), and semiweekly for the next 3 weeks (fasting). Sampling for the pilot subject included two additional weeks, but those time points were not included in the data analysis presented here, because when comparing kinetics curves, it is important that the time points be the same for all subjects. Blood samples were collected into vacutainers containing EDTA and centrifuged at 2,560 g for 10 min. Plasma aliquots of 1 ml were stored in cryo-vials at -80°C until analysis.

Subjects consumed a controlled diet for 1 week before the kale dose and throughout the entire collection period. Subjects were instructed to consume all foods and only foods provided by the Beltsville Human Nutrition Research Center. The diet contained 15% of energy from protein, 32% from fat, and the balance from carbohydrate. The diet provided 2 mg/day β -carotene and 2 mg/day lutein for all subjects. The vitamin A content of the diet depended on the calorie level of the subject; the diets contained 730 retinol equivalents per 2,000 kcal. During the day of kale ingestion, the subjects consumed foods free of lutein, β -carotene, and vitamin A, and lunch and dinner were provided at 5 and 10 h, respectively, after kale consumption on this day. Vitamins and supplements were prohibited throughout the study.

Reagents

Hexane, ethanol, chloroform, isopropanol, methanol (MeOH), and methyl tertiary-butyl ether (MTBE) were purchased from Fisher Scientific. β -Apo-8'-carotenal, β -carotene, retinol, retinyl acetate, retinyl palmitate, and butylated hydroxytoluene were purchased from Sigma Chemical Co. (St. Louis, MO). β -Caro-

tene-d8 (10,10',19,19,19,19',19',19'-[$^2\text{H}_8$] β -carotene) and retinyl acetate-d8 (10,19,19,19,14,20,20,20-[$^2\text{H}_8$]retinyl acetate) were purchased from Cambridge Isotope Laboratories (Woburn, MA). Lutein was purchased from Extrasynthase (Genay, France).

Kale extraction

Under subdued light, kale was ground into a fine powder with liquid nitrogen using a mortar and pestle. Duplicate 3 g samples of powder were weighed into each of two 25 ml test tubes for extraction, and an internal standard mix was added (50 μl of solution containing 0.5 $\mu\text{g}/\text{ml}$ β -carotene-d8 and 0.356 $\mu\text{g}/\text{ml}$ β -apo-8'-carotenal). Nine milliliters of 0.1% butylated hydroxytoluene in ethanol was added to each sample, and samples were vortex-mixed for 2 min. Next, 9 ml of hexane-toluene (5:4, v/v) was added, and samples were vortex-mixed and placed in a 70°C water bath. After 10 min, samples were removed from the water bath and 180 μl of 80% KOH was added; samples were then vortex-mixed and returned to the water bath for an additional 15 min, with samples vortex-mixed at 8 min. Samples were removed from the water bath and placed on ice, and then 3 ml of distilled, deionized water was added before vortex mixing. Samples were then centrifuged at 1,140 g for 10 min, and the organic layer was collected to a separate test tube. The extraction was repeated two more times with 6 ml of hexane-toluene (10:8, v/v). A 100 μl aliquot was taken from the combined organic layer. The samples were then dried under N_2 and reconstituted in 200 μl of MTBE/MeOH (1:1, v/v) before injection onto the LC-MS system.

Plasma sample preparation for lutein, retinol, and retinyl ester

Sample preparation and analysis were described in detail previously (10). Briefly, labeled and unlabeled lutein, retinol, and retinyl ester were extracted from 0.5 ml of plasma under subdued lighting. Plasma was thawed, and internal standards (β -apo-8'-carotenal, retinol-d8, and retinyl acetate) were added immediately before the addition of 0.5 ml of ethanol. Samples were extracted twice with 1.5 ml of hexane. The combined hexane extracts were dried under N_2 and reconstituted in 200 μl of MTBE/MeOH (1:1, v/v) before injection onto the LC-MS system.

Plasma sample preparation for β -carotene

An additional solid-phase extraction (SPE) step was required before β -carotene analysis because of coelution of [$^{13}\text{C}_{40}$] β -carotene (m/z 577) with an unknown metabolite (m/z 577) after the extraction procedure described above. Thus, an additional plasma aliquot (0.5 ml) was extracted and subjected to SPE (11). The initial portion of the β -carotene extraction was the same as described above, except that β -carotene-d8 was added as the internal standard. After drying, the samples were reconstituted in 200 μl of chloroform and applied to Strata NH_2 SPE cartridges (Phenomenex, Torrance, CA) preconditioned with 2 ml of hexane. The samples were drawn into the column and eluted with 3 ml of chloroform-isopropanol (2:1, v/v). After drying under N_2 , samples were reconstituted in 200 μl of hexane and applied to another Strata NH_2 SPE cartridge preconditioned with 2 ml of hexane. β -Carotene was eluted with 4 ml of hexane, dried under N_2 , and reconstituted in 200 μl of MTBE/MeOH (1:1, v/v) before injection on the LC-MS system.

LC-MS conditions

The liquid chromatograph used was an Agilent 1100 series instrument with a cooled autosampler, automatic solvent degasser, binary pump, cooled column compartment with a YMC C_{30} column (3 μm , 250×4.6 mm) and C_{30} guard cartridge, and a G1315A diode array detector. The LC system was connected to

an Agilent G1946A mass spectrum detector with an atmospheric pressure chemical ionization source.

The solvent system consisted of solvent A, which was 1 mM ammonium acetate in MeOH, and solvent B, which was MTBE (12). A solvent gradient was used in which the mobile phase consisted of 15% solvent B at 0 min and was increased linearly to 30% solvent B at 12 min. The mobile phase was held at 30% solvent B until 18 min, when solvent B was increased to 100% and held until 23 min, then decreased back to 15% for 10 min for column re-equilibration. The injection volume was 50 μ l, and the flow rate was 1 ml/min. The diode array detector was set to collect signal at 450 and 325 nm.

The mass spectrum detector source conditions were as follows: spray chamber gas temperature set at 350°C, vaporizer temperature set at 400°C, nitrogen nebulizer pressure set at 45 psi, and corona current set at 5 μ A. Initially, the capillary voltage was set to 3,800 V and the drying gas (nitrogen) was 7 l/min; at 7.5 min, the capillary voltage changed to 3,600 V and the drying gas to 6 l/min.

Selected ion monitoring was used to detect the following protonated molecules: unlabeled lutein and [$^{13}\text{C}_{40}$]lutein (m/z 551 and 591, respectively; after loss of water); unlabeled β -carotene, β -carotene-d8, and [$^{13}\text{C}_{40}$] β -carotene (m/z 537, 545, and 577); unlabeled retinol, retinol-d8, and [$^{13}\text{C}_{20}$]retinol (m/z 269, 276, and 289); and unlabeled β -apo-8'-carotenal (m/z 417). Data were collected using Agilent Chemstation software. Molar concentrations of labeled and unlabeled lutein were calculated based on a response curve for internal standard β -apo-8'-carotenal versus lutein, labeled and unlabeled retinol concentrations were calculated based on a response curve for internal standard retinol-d8 versus retinol, and labeled and unlabeled β -carotene concentrations were calculated based on a response curve for internal standard β -carotene-d8 versus β -carotene. The method was validated for β -carotene, lutein, and retinol against a National Institute of Standards and Technology standard reference material (SRM 968C; Gaithersburg, MD).

Retinyl ester concentrations were determined by diode array detection against an external standard curve of retinyl palmitate under the assumption that most retinyl ester present in the samples would be formed from the dose (no other vitamin A source was ingested by subjects), yet acknowledging that fasting circulating retinyl ester is low but greater than zero.

Calculations and statistics

Area under the plasma concentration-time curve (AUC) for the 28 day sampling was calculated by the trapezoidal method using Microsoft Excel 2000 version 9. Peak plasma mass was calculated by multiplying the peak plasma concentration by estimated plasma volume (45 ml plasma/kg body weight) (13). A paired t -test was used to compare the [^{13}C]lutein AUC value with the [^{13}C] β -carotene AUC value and with the [^{13}C] β -carotene AUC plus [^{13}C]retinol AUC, with $P < 0.05$ considered significant. Pearson product moment correlation analysis was used to identify associations among response variables. Statistical analysis was performed using SigmaStat 3.0 (SPSS, Inc.).

RESULTS

Concentrations of labeled β -carotene, lutein, and retinol in plasma increased markedly after ingestion of the isotopically labeled kale. Lutein was first detected between 3 and 6 h after the dose, β -carotene was first detected at 4 or 5 h after the dose, and retinol was first detected between 4 and 6 h after the dose. The time of peak plasma concentration ranged between 10 and 36 h for labeled lutein and

between 12 and 24 h for labeled retinol. Labeled β -carotene exhibited a double peak in plasma, with the first peak occurring between 8 and 10 h and the second peak occurring between 24 and 36 h. [^{13}C]lutein was detected in plasma of all subjects throughout the 28 day sampling period, [^{13}C] β -carotene was detected throughout the 28 day sampling in three subjects (including the pilot subject, for whom [^{13}C] β -carotene could be detected for 46 days) and for 24 days in plasma of four subjects, and [^{13}C]retinol was detected throughout the 28 day sampling in six subjects (including the pilot subject, for whom [^{13}C]retinol could be detected for 46 days) and for 22 days in plasma of one subject. Plasma analyte concentrations are shown as a function of time for [^{13}C]lutein in Fig. 1, for [^{13}C] β -carotene in Fig. 2, for [^{13}C]retinol in Fig. 3, and for retinyl ester in Fig. 4.

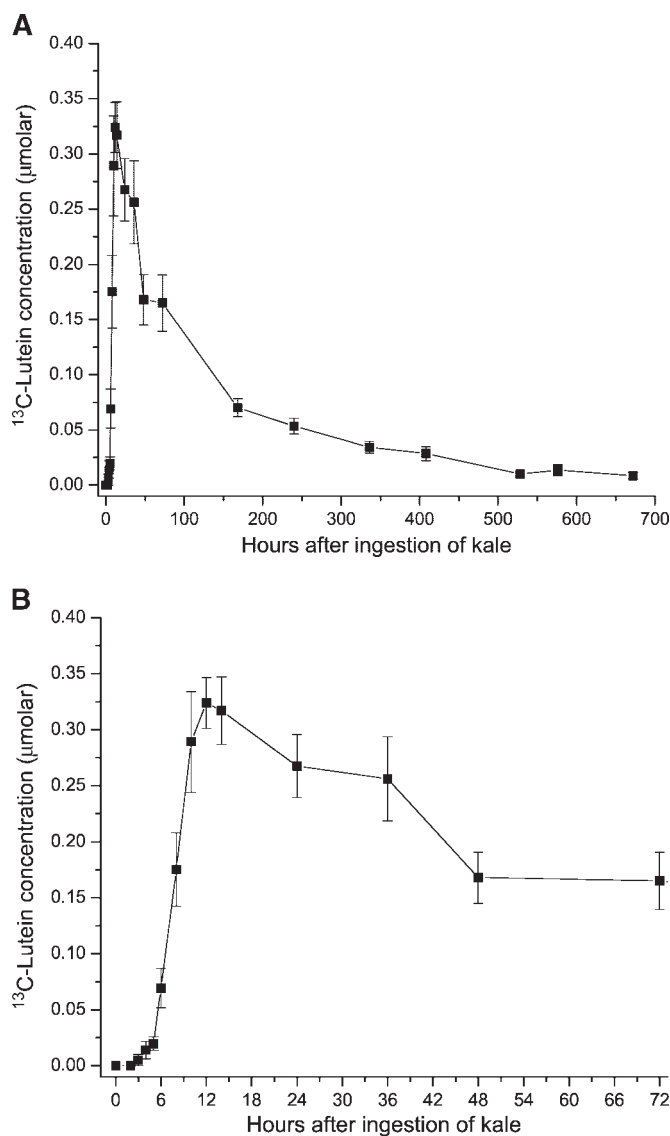


Fig. 1. Plasma [^{13}C]lutein concentration as a function of time after ingestion of [^{13}C]labeled kale. Each point represents the average value for the study population. Error bars represent SEM. Panel A shows data across the entire plasma collection period, and panel B shows data for the first 72 h.

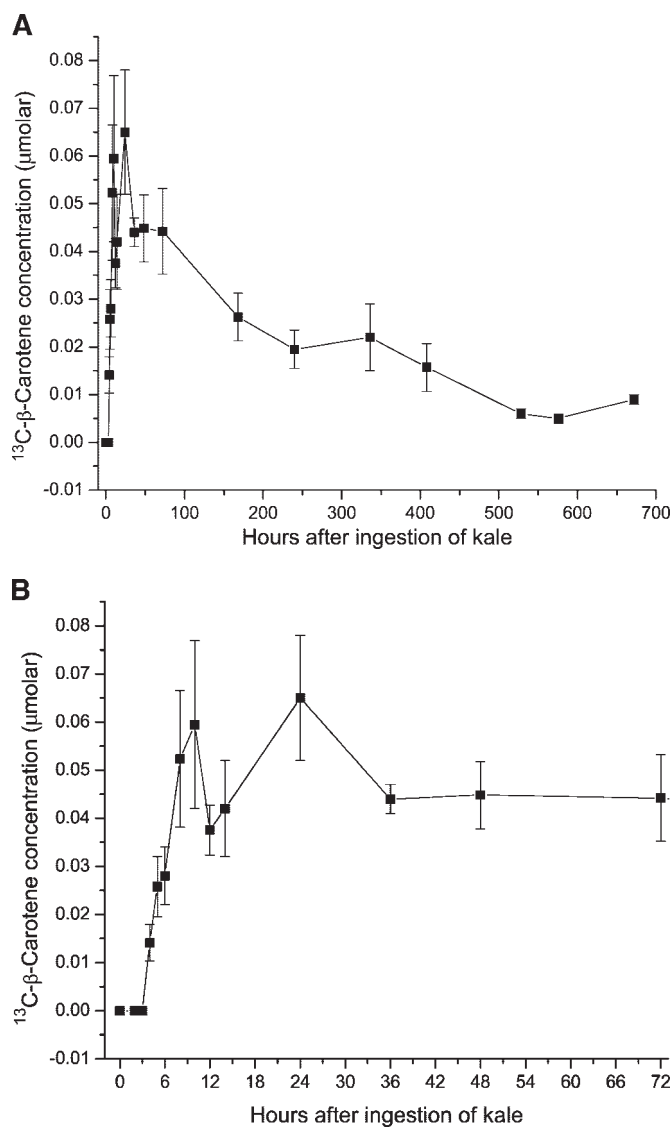


Fig. 2. Plasma [^{13}C] β -carotene concentration as a function of time after ingestion of ^{13}C -labeled kale. Each point represents the average value for the study population. Error bars represent SEM. Panel A shows data across the entire plasma collection period, and panel B shows data for the first 72 h.

Plasma response variables for labeled nutrients are shown in **Table 1**. The average peak plasma concentrations for the seven subjects were $0.38 \mu\text{M}$ for labeled lutein (range 0.26 – 0.51), $0.068 \mu\text{M}$ for labeled β -carotene (range 0.041 – 0.12), and $0.079 \mu\text{M}$ for labeled retinol (range 0.042 – 0.12). Peak plasma concentrations of labeled compounds in terms of percentage of dose were as follows: 3.6% for lutein, 0.7% for β -carotene, and 0.7% for retinol (on a molar basis).

The doses of lutein and β -carotene in the kale were nearly matched, being $34 \mu\text{mol}$ for β -carotene and $33 \mu\text{mol}$ for lutein. However, AUC values for the two carotenoids were significantly different. Lutein AUC ($42.8 \mu\text{M h}$) was significantly greater than AUC values for [^{13}C] β -carotene ($13.6 \mu\text{M h}$) and [^{13}C] β -carotene plus [^{13}C]retinol ($26.8 \mu\text{M h}$).

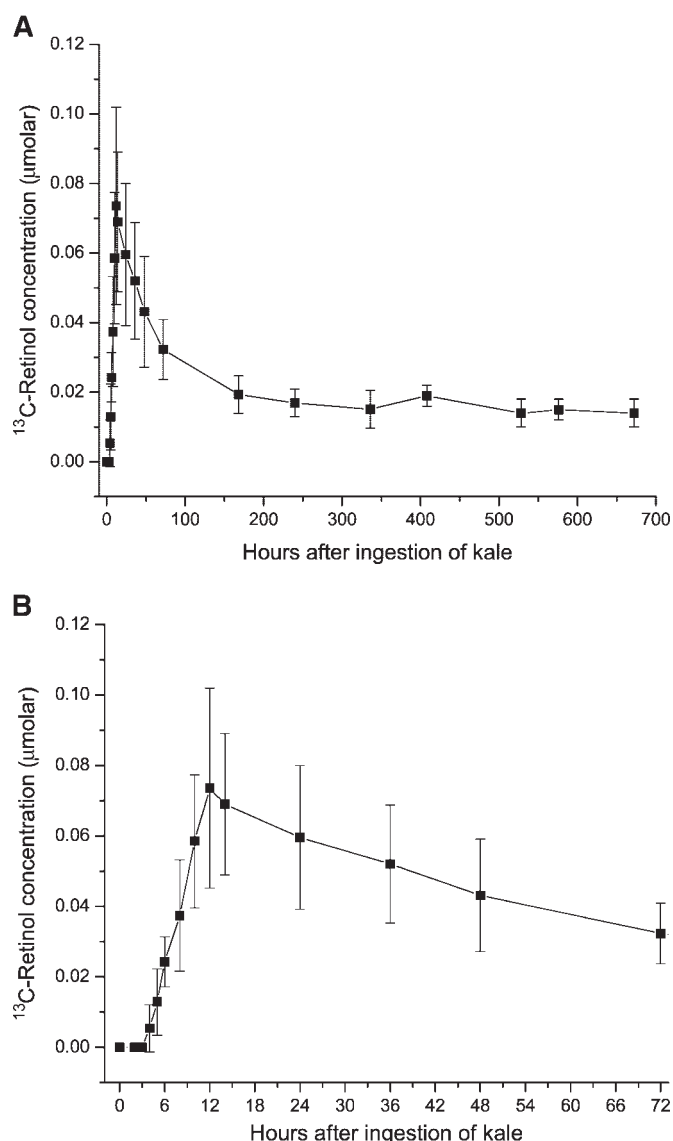


Fig. 3. Plasma [^{13}C]retinol concentration as a function of time after ingestion of ^{13}C -labeled kale. Each point represents the average value for the study population. Error bars represent SEM. Panel A shows data across the entire plasma collection period, and panel B shows data for the first 72 h.

No relationship was observed between [^{13}C]lutein AUC and [^{13}C] β -carotene AUC, but when the AUC values for [^{13}C] β -carotene and [^{13}C]retinol were summed, the correlation between that sum and [^{13}C]lutein AUC produced an R value of 0.73. This relationship is shown graphically in **Fig. 5**. The graph shows that the subjects were clustered into two groups: those with lower [^{13}C]lutein AUC also exhibited a lower summed AUC for [^{13}C] β -carotene plus [^{13}C]retinol, and those with a higher [^{13}C]lutein AUC exhibited a higher summed AUC for [^{13}C] β -carotene plus [^{13}C]retinol. The two subjects with the higher AUC values had other characteristics fairly close to the mean values for this population. One subject was a 55 year old male, weight of 85 kg, BMI of 25.4, baseline β -carotene of $0.29 \mu\text{M}$, and baseline lutein of $0.32 \mu\text{M}$; the other subject was

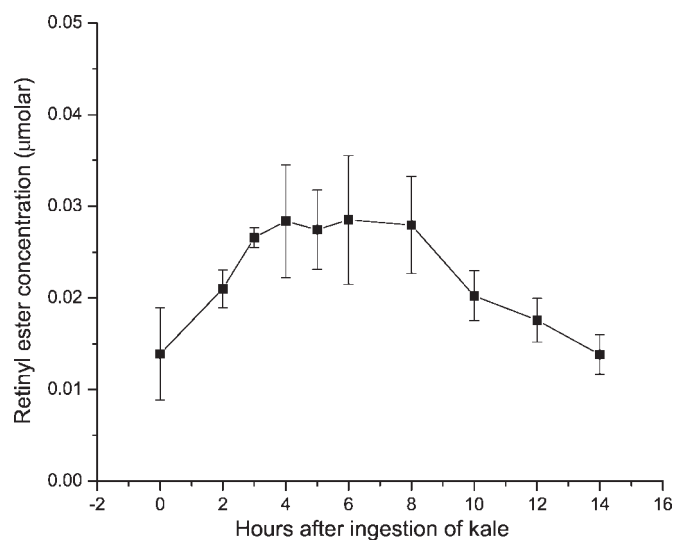


Fig. 4. Plasma retinyl ester concentration as a function of time after ingestion of ^{13}C -labeled kale. Each point represents the average value for the study population. Error bars represent SEM.

a 53 year old female, weight of 68 kg, BMI of 25.7, baseline β -carotene of $0.13 \mu\text{M}$, and baseline lutein of $0.35 \mu\text{M}$.

Correlation analysis suggested an association between baseline plasma retinol concentration and ^{13}C retinol plasma response. Baseline plasma retinol concentration was associated with ^{13}C retinol AUC ($R = 0.75$) and ^{13}C retinol peak plasma concentration ($R = 0.73$). The association between baseline plasma retinol concentration and ^{13}C β -carotene peak 1 plasma concentration was weaker ($R = 0.60$). These relationships are expressed graphically in **Fig. 6**. These relationships were not related to body weight or BMI.

DISCUSSION

Isotopes have been used previously to study the bioavailability and metabolism of carotenoids (14–32). Previous reports of clinical studies have involved administration of pure compounds labeled with deuterium (17, 21, 22, 24, 26, 27, 30), pure compounds labeled with ^{13}C (15, 16, 19, 32), pure compounds labeled with ^{14}C (28, 29), plant foods intrinsically labeled with deuterium (30), and plant foods intrinsically labeled with ^{13}C (10). Other than the report of our pilot study (10), this study is the only one in which

TABLE 1. Plasma nutrient concentrations

Nutrient	Baseline Concentration	Peak ^{13}C Concentration	Percentage of Dose at Peak	Area under the Plasma Concentration-Time Curve
	μM			$\mu\text{M h}$
β -Carotene	0.34 ± 0.21	0.068 ± 0.032	0.7 ± 0.3	13.6 ± 5.8
Lutein	0.35 ± 0.093	0.38 ± 0.080	3.6 ± 0.7	42.8 ± 15.1
Retinol	2.88 ± 0.43	0.079 ± 0.027	0.7 ± 0.2	13.2 ± 3.7

Values are expressed as means \pm SD.

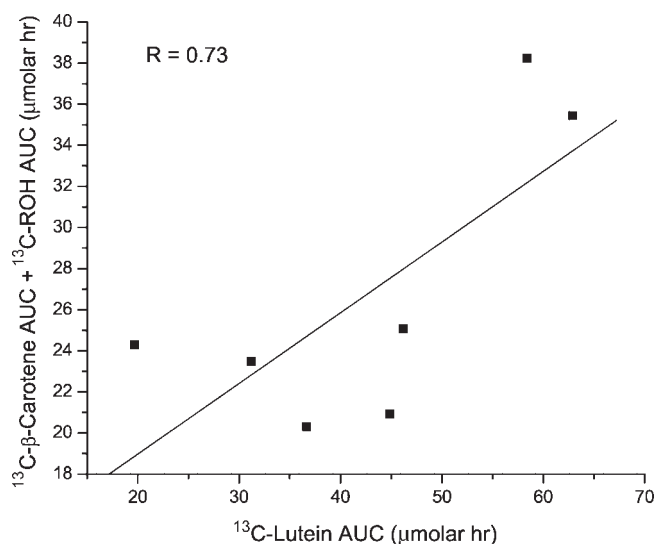


Fig. 5. Relationship between summed area under the plasma concentration-time curve (AUC) for ^{13}C β -carotene plus ^{13}C retinol (ROH) versus AUC for ^{13}C lutein. The line was obtained through linear regression of the data.

the nutrients were completely labeled with ^{13}C in a plant matrix and in which two isotopically labeled carotenoids were fed simultaneously. Our study design allowed us to compare the body's assimilation of lutein and β -carotene and its metabolite retinol from a plant food, and this design facilitated the investigation of relationships that might exist between the absorption and metabolism of these carotenoids. Note that there were no other carotenoids present in measurable quantities in the kale, in accord with previously published analyses (33).

Our kale preparation was served blanched and chopped into 1 inch \times 1 inch squares. Although pureeing the kale would likely have made the nutrients more bioavailable (8, 34), we attempted to mimic a standard kale preparation. However, our dose was large (3 cups of kale) to increase the likelihood of a measurable plasma response, and intakes of 20 mg of each carotenoid are much larger than estimates of habitual daily intakes, which are 2 mg/day for β -carotene and 2 mg/day for lutein plus zeaxanthin (35).

The doses of ^{13}C β -carotene and ^{13}C lutein were very close in mass, allowing the comparison of plasma response between the two carotenoids. ^{13}C lutein AUC was three times greater than ^{13}C β -carotene AUC and 1.6 times greater than ^{13}C β -carotene plus ^{13}C retinol AUC. This suggests that lutein is substantially more bioavailable from kale than β -carotene. Similar results have been observed in other studies, both in vivo and in vitro (8, 36, 37), although the opposite findings have also been observed in vitro (38, 39). Monitoring plasma carotenoid response after a dose reflects a compilation of many processes, including release from the plant matrix, solubilization into oil, incorporation into micelles, transfer into the mucosal cell, incorporation into chylomicron particles, and transport to liver and extrahepatic tissues through the systemic

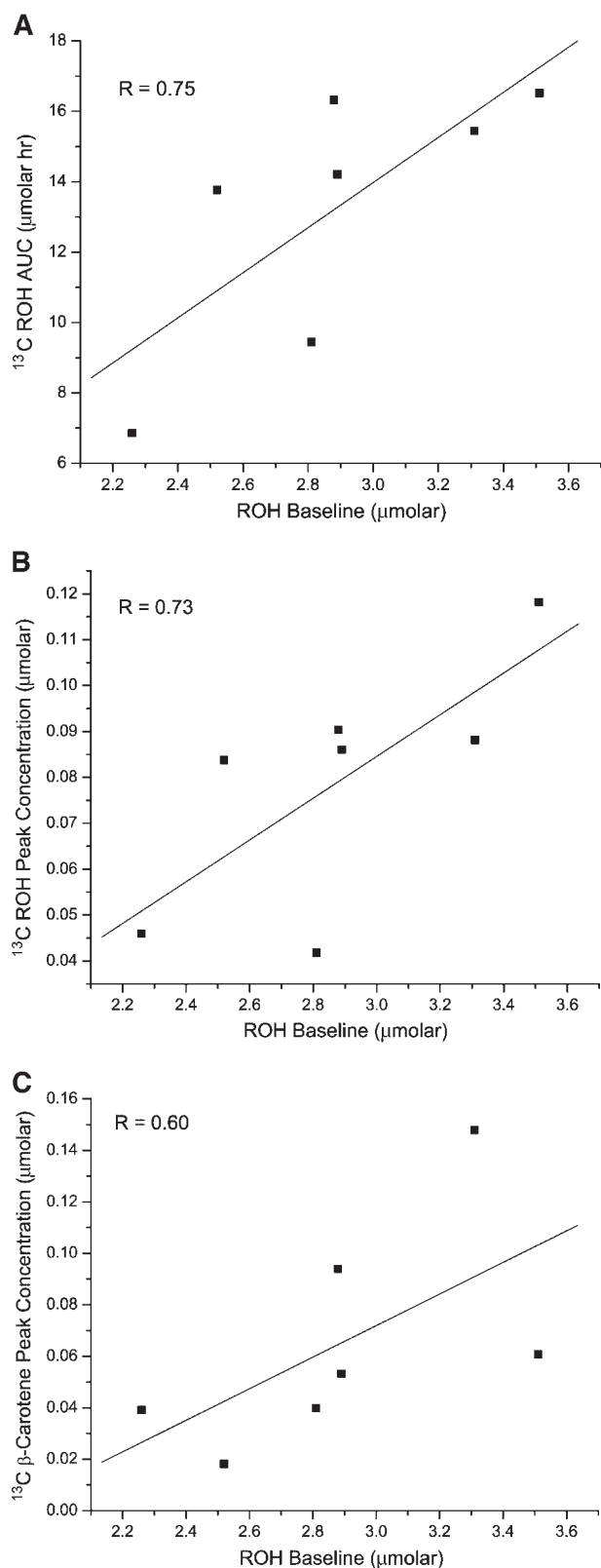


Fig. 6. Relationships among plasma response variables for individual subjects. Top, [¹³C]retinol (ROH) AUC versus baseline plasma retinol concentration. Middle, [¹³C]retinol peak plasma concentration versus baseline plasma retinol concentration. Bottom, [¹³C]β-carotene peak plasma concentrations versus baseline plasma retinol concentration. The lines on each graph were obtained through linear regression of the data.

circulation. Differences between β-carotene and lutein have already been found for some of these steps, such as release from the plant matrix (8) and micellarization (36). In vitro studies have shown that lutein partitions into micelles more effectively than β-carotene (36), and this phenomenon would allow better movement of lutein into intestinal epithelial cells. It has also been suggested that β-carotene may be more deeply embedded in the matrix of a green, leafy vegetable than lutein (8), which would also account for these results.

If a subject is assumed to have 45 ml plasma/kg body weight (13), then one can calculate the portion of the dose of each nutrient that is found in plasma at a given time point. This calculation showed that the peak [¹³C]lutein concentration accounted for 3.6% of the dose, the peak [¹³C]β-carotene concentration accounted for 0.7% of the β-carotene dose, and peak [¹³C]retinol concentration accounted for 0.7% of the β-carotene dose (on a molar basis). Therefore, this measure also supports the prospect of greater bioavailability of lutein from kale compared with β-carotene.

Although lutein bioavailability seems to be greater than that for β-carotene, correlation analysis suggests that the absorption of these compounds is related. And although the plasma response variables for [¹³C]lutein and [¹³C]β-carotene alone did not appear correlated, a relationship did arise between the sum of AUC values of [¹³C]β-carotene plus [¹³C]retinol and [¹³C]lutein AUC. This suggests that the variability of β-carotene conversion to retinol may have made the association between [¹³C]lutein AUC and [¹³C]β-carotene AUC difficult to detect with this small number of subjects.

Carotenoids have been found to interact during absorption, and this may have influenced our results. Kostic, White, and Olson (40) found that plasma lutein response was reduced when lutein and β-carotene were administered simultaneously. The influence of lutein on β-carotene absorption was less clear, with lutein resulting in an increased plasma β-carotene response for three subjects and a reduced plasma β-carotene response for five others.

It is important to understand the absorption of β-carotene from plant foods, because plant foods are the major source of vitamin A nutriment in many parts of the world (41). Among children younger than 5 years of age, vitamin A deficiency is estimated to affect 127 million (42). The potential of β-carotene to supply vitamin A is controversial (43, 44), and the bioefficacy of β-carotene as a source of vitamin A has been under scrutiny (44, 45). A difficulty in studying the bioefficacy of β-carotene as vitamin A rests in the fact that retinol obtained from newly ingested sources is difficult to discern from endogenous retinol, because serum retinol levels are homeostatically controlled by the liver (46). Thus, unless the retinol is tagged to be discernible from endogenous retinol, the newly ingested retinol is lost in the endogenous pool. Our method of labeling plants circumvents that problem.

A relationship between fasting baseline retinol and postdose plasma [¹³C]retinol response was suggested by

these data. Some of the processes that may be responsible for such a relationship include improved β -carotene absorption (either at the mucosal apical surface or in the incorporation of β -carotene into chylomicron particles), increased conversion of β -carotene to retinoid, greater release of retinol from the liver, or slower retinol uptake by the liver. A previous study supports the possibility that retinol may increase β -carotene absorption. Lemke et al. (29) found that β -carotene was more efficiently absorbed after a vitamin A supplementation period compared with a period of lower vitamin A intake. These researchers hypothesized that the vitamin A supplementation either improved gut epithelial health or increased biliary β -carotene output (which would alter the isotope ratio measured in that study, thus giving the appearance of increased β -carotene absorption). We investigated the relationship between baseline plasma retinol concentration and [^{13}C] β -carotene peak I concentration (expected to represent newly absorbed β -carotene in chylomicron particles) to find that the R value was 0.60, which does not suggest a strong relationship but is nonetheless notable for this small number of subjects. If β -carotene movement across the apical surface of the intestinal mucosa had improved, one would expect an increase in the peak concentration for the first β -carotene peak and a larger AUC value for retinyl ester. However, the retinyl ester AUC value showed no relationship to baseline plasma retinol ($R = 0.2$). Improved β -carotene incorporation into chylomicron particles would be in accord with these results, because that would likely increase [^{13}C]retinol response and [^{13}C] β -carotene peak I concentration and have no apparent direct effect on retinyl ester AUC. In vitro studies of β -carotene transfer across Caco-2 monolayers with retinoid-enriched media would help to clarify this issue. An alternative explanation would be related to the release and uptake of retinol by liver. Serum retinol levels, which are not influenced by diet for vitamin A-adequate individuals because serum retinol is homeostatically maintained by the liver (46), are determined by a complex balance of processes (47). Clearly, the [^{13}C]retinol from the kale and the endogenous retinol would share pathways of metabolism; thus, the observation of a relationship between post-dose labeled retinol and endogenous retinol may simply reflect the common pathways.

In conclusion, isotopic labeling of kale has provided the opportunity to investigate the absorption of lutein and β -carotene from kale without the complication of the dose compounds being lost in the endogenous pool. This is particularly useful for retinol, whose plasma levels are well controlled by the liver, thus making it especially difficult to observe newly ingested retinol after ingestion of an unlabeled vitamin A or provitamin A dose. ■

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