ELSEVIER

Journal of Nutritional Biochemistry 11 (2003) 663–670

THE JOURNAL OF Nutritional Biochemistry

Bioavailability of lutein in humans from intrinsically labeled vegetables determined by LC-APCI-MS[1]

Annette Lienau[a], Tobias Glaser[b], Guangwen Tang[b,*], Gregory G. Dolnikowski[b], Michael A. Grusak[c], Klaus Albert[a]

[a]*Institute of Organic Chemistry, University of Tuebingen, Tuebingen, Germany*
[b]*Jean Mayer USDA Human Nutrition Research Center on Aging, Tufts University, Boston, MA, USA*
[c]*USDA-ARS Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine, Houston, TX, USA*

Abstract

The aim of the investigation was to assess a stable isotope method for determining the relative bioavailability of food-derived lutein in humans. Subjects were administered a single dose of deuterium-labeled carotenoids from intrinsically labeled spinach or collard green; 10 mL blood samples were drawn at various time points over a 34 days period. The vegetables had been hydroponically grown using 25 atom-% deuterated water. Lutein molecules in the vegetables were partially deuterated with a highest abundance isotopomer at $M_0 + 8$ (unlabeled molecular mass, M_0, plus 8 additional mass units from 8 deuterium atoms in the molecules). This allowed labeled lutein to be distinguished from endogenous lutein in serum samples after consuming the labeled meal. The presence of labeled lutein in the circulation was determined by liquid chromatography-mass spectrometry (LC/MS) equipped with an atmospheric pressure chemical ionization (APCI) interface. The quantification of the labeled lutein in serum samples enabled the calculation of the enrichment for each time point after the dose; these values were plotted vs. time to generate absorption-clearance curves for each of the subjects. Area under the curve analyses of four different subjects (integrated over 29 days) yielded serum lutein responses of 128, 145, 149, and 262 μg-day/mg dietary lutein, following an acute dose of spinach containing 15.4, 18.8, 18.8 and 9.8 mg labeled lutein, respectively. This technique will facilitate the study of lutein bioavailability from different foods of diverse carotenoid composition and/or following various food preparation procedures.

Keywords: Lutein; Carotenoids; Deuterated vegetables; Bioavailability; LC/APCI-MS

1. Introduction

The xanthophyll lutein is an important carotenoid. It is abundant in the human body in serum and several organs, such as liver, adipose tissues, and retina. Predominant sources of lutein are dark green, leafy vegetables, such as spinach, broccoli, and collard green [1].

Lutein, just like the other carotenoids, has antioxidant properties, i.e., it protects cells and organisms against photo-oxidative damages. It interferes with free radical formation, acting as a radical scavenger, and it quenches the very reactive singlet molecular oxygen [2–5].

Several clinical and epidemiological studies have indicated that a high intake of lutein has beneficial effects on human health [6]. The predominant concentration of lutein and its accompanying carotenoid zeaxanthin in the human retina, particularly in the area of sharp and detailed vision called macula lutea, leads to the conclusion that these compounds are involved in the protection of the eye from blue light damage. Clinical studies have shown that a high intake of lutein and zeaxanthin is inversely correlated with the pathogenesis of age-related macular degeneration (AMD) and cataracts [7–11]. Furthermore, several epidemiological studies suggest that higher levels of lutein in the serum were associated with lower risk for developing coronary heart disease, apoplexy [12,13], and certain types of malignancies, such as breast cancer, lung cancer, colon cancer, and prostate cancer [14–18].

* Corresponding author. Tel.: +1-617-556-3236; fax: +1-617-556-3344

E-mail address: guangwen.tang@tufts.edu (G. Tang).

[1] Financial Support: NATO Collaborative Linkage Grant "Determination of Carotenoid Biometabolites Using Advanced HPLC, NMR and MS" (No. 978601), the USDA-CSREES-NRI (99-35200-7564), and USDA ARS Nos. 581950-9-001 and 58-6250-6-001. Any opinions, findings, conclusion, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the US Dept. of Agriculture.

0955-2863/03/$ – see front matter
doi:10.1016/j.nutbio.2003.08.004

Nevertheless, it is not yet possible to suggest wide use of lutein supplements, because the proper dosage is still unknown. Vigorous supplementation with lutein might even be harmful, due to a possibly pro-oxidant behavior of carotenoids at high concentrations, as in the case of β-carotene supplementation in smokers [19]. Moreover, the absolute bioavailability of lutein in humans has not been determined, either from supplements or from natural sources [20–22].

To determine the enrichment and bioavailability of lutein, changes of the lutein concentration in the circulation after the intake of foods are compared to baseline concentration of lutein. Until now, studies conducted to determine blood concentration changes of different carotenoids consisted of chronic supplementations, usually of two to four weeks [23–27]. This was necessary to obtain an increase in the serum carotenoid concentration that was sufficient for quantification by HPLC [28]. The new approach described in this paper provides a method for investigating the bioavailability of lutein from vegetables after a single meal, using deuterated spinach or collard greens that have been hydroponically grown with 25 atom-% D_2O [29,30].

2. Material and methods

2.1. Chemicals

All solvents used for the extraction and HPLC, i.e., chloroform, methanol, hexane, ethanol, tetrahydrofurane (THF), and tert-butyl methyl ether (TBME), were HPLC grade and purchased from JT Baker (Phillipsburg, NJ, USA).

2.2. Preparation of the deuterated vegetables

Collard (cultivars Grady Simpson and Bluemax) and spinach (cultivar Melody) were grown hydroponically at the USDA/ARS Children's Nutrition Research Center in Houston, Texas, USA, employing a nutrient solution enriched with 25 atom-% deuterium oxide (D_2O) [29]. Plants were maintained in an environmental growth chamber (Conviron Model PGW36; Winnipeg, Manitoba, Canada) using a 12-hr, 20°C/12-hr, 15°C day/night regime and 70% relative humidity. A combination of incandescent and fluorescent lamps provided light during the day period; intensity of photosynthetically active radiation was 500 μmol of photons m^{-2} s^{-1} at the top of the plants. Plants were started from seeds that were germinated on filter paper; seedlings were planted in polyethylene cups as previously described [30]. Plants were grown hydroponically in a nutrient solution containing the following macronutrients in mM: KNO_3, 10; $NH_4H_2PO_4$, 2; $Ca(NO_3)_2$, 3; $MgSO_4$, 3; and the following micronutrients in μM: $CaCl_2$, 25; H_3BO_3, 25; $MnSO_4$, 2; $ZnSO_4$, 2; $CuSO_4$, 0.5; H_2MoO_4, 0.5; $NiSO_4$, 0.1. Iron was added in chelated form as Fe(III)EDDHA (N,N′-ethylenebis[2-(2-hydroxyphenyl)-glycine]) at 5 μM. MES buffer (adjusted with KOH) was added at 2 mM to maintain nutrient solution pH between 5.4 and 5.8. Nutrient solutions were made by combining stock salt solutions (in H_2O), deionized water (H_2O), and an appropriate amount of D_2O to achieve a final deuterium enrichment of 25 atom-%. All nutrient solutions were constantly aerated with a water vapor-free air supply to provide oxygen to the plant roots. Additional nutrient solution was added to growth containers, as required, in response to plant utilization of water and minerals.

The collard plants were maintained within the growth chamber until harvest at 6 weeks of age. Spinach plants were maintained within an acrylic plastic enclosure (situated inside the growth chamber) until harvest at 4 weeks of age. This enclosure was supplied with supplemental carbon dioxide (CO_2) to maintain a concentration of ~400 ppm CO_2 for the plants (similar to external air). Atmospheric conditions around the plants, therefore, varied between the collard and spinach. Water vapor around the collard leaves was at approximately 0.015 atom-% (i.e., natural abundance), whereas the spinach leaves would have grown within a water vapor environment of approximately 25 atom-%.

At harvest, all leaves were packaged and shipped overnight on ice to the USDA/ARS HNRC at Tufts University in Boston, MA. The vegetables were weighed, chopped, and steamed for five minutes. Afterwards, they were pureed, portioned, and kept at −80°C until being analyzed, or used for the feeding studies.

2.3. Study design

One male and three females were recruited from the general public, with ages ranging from 45 to 65 y and BMI from 21.2 to 29.3 kg/m^2. The subjects (three post menopausal women and one age matched man) were healthy non-smoking adults not having taken vitamin supplements within the last month. They were asked to avoid dark green leafy vegetables for two weeks before the experiment. Informed consent had been obtained from all subjects under the guidelines established by the Human Investigation Review Committee of Tufts University and the Tufts-New England Medical Center. Subjects' characteristics and the kinds and amounts of deuterated vegetables consumed are displayed in Table 1.

The frozen and processed deuterated vegetables were heated in a microwave oven for 2 min before being given to each of the subjects, together with a liquid formulated breakfast that contained 35% of the calories as fat. The liquid breakfast diet (no fiber) was formulated to contain fat (20 g, 35% of total energy), protein (17 g), and carbohydrate (78 g) to provide total energy of 527 kcal. The percentage of energy from saturated, monounsaturated, and polyunsaturated fatty acid were 20, 1.4, and 1.6%, respectively. Five hours after the breakfast, they consumed the same amount of liquid diet as a lunch. Two of the test subjects were given

Table 1
Characteristic data of the volunteers and the amounts of labeled vegetables given to each volunteer.

Subject	Sex	Age	BMI (kg/m^2)	Vegtable (cultivar)	Serving Size	Lutein in the dose (mg)
#1	male	45	29.3	spinach (Melody)	200 g	18.8
#2	female	51	25.1	spinach (Melody)	200 g	18.8
#3	female	58	21.2	collard green (Grady Simpson)	193 g	9.8
#4	female	65	28.7	collard green (Blue Max)	214 g	15.4

spinach while the other two received collard greens. Blood samples were collected before the labeled meal was consumed and at approximately 3 hr (h), 5h, 7h, 9h, 11h, 13h, 1day (d), 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 15 days, 22 days, 29 days, and 34 days after the labeled meal. Due to the volunteers' schedules, some serum samples were collected at alternative time points: following time points were used to collect serum samples: 13 days for subjects #3 & 4 (not 15 days), 16 days for subject #2 (not 15 days) and 20 days for subject # 4 (not 22 days).

The study subjects resided at the HNRC and were provided diets low in carotenoids ($<$ 1 mg carotenoids per day) until 10 d. From 11 d to 34 days, the study subjects were free living.

2.4. Sample preparation

Due to the light sensitivity of carotenoids, the analysis of the serum samples was performed under red light. The serum was separated from whole blood samples (in a Vacutainer™ with no additive) using a serum separator SureSep®II. The samples were centrifuged for 10 min at 4°C and 3000 rpm (Sorvall RT6000 coolable centrifuge, Kendro Laboratory Products, Newtown, CT, USA). Afterwards, the serum was decanted to Cryule vials and kept at -80°C until analyzed.

For the extraction of the carotenoids from the serum samples [31], 4 mL of a mixture of chloroform and methanol (2:1, v/v) and 0.5 mL of a saline solution (0.85%) were added to 500 μL of serum and spiked with 375 μL of the internal standard echinenone (1.25 μM). This mixture was vortexed and then centrifuged for 10 min at 4°C and 3000 rpm. After the removal of the chloroform layer, 5 mL of hexane were added. The mixture was vortexed and centrifuged for 10 min again. The hexane phase was merged with the chloroform extract and dried under nitrogen in a water bath at 40°C. The extracted carotenoids were dissolved in 300 μL of ethanol.

The extraction of the vegetables [32] (spinach and collard greens) was performed by incubating 500 mg of the pureed vegetables together with 10 mL methanol for 1 hr in a shaking incubator at 120 rpm. Afterwards, the mixture was homogenized for 30 s in an ice bath and the probe washed with methanol. The mixture was centrifuged at 3000 rpm for 5 min. The methanol layer was transferred into a 50 mL volumetric flask and the extraction repeated four times with 10 mL of THF, followed by vortexing and centrifugation. The THF layers were combined with the methanol layer and the volume brought up to 50 mL. One mL of the extract was taken, dried under nitrogen, and re-suspended in 1 mL of ethanol. Triplet samples of each vegetable were extracted and the results of the lutein contents of each vegetable were presented in Table 2.

2.5. HPLC analysis

The chromatographic separations were performed on an Agilent 1100 HPLC system (Agilent Technologies, Waldbronn, Germany) system using a UV detector at 450 nm. The separation was done with a highly selective C_{30} RP column (YMC Carotenoid S-3 micron, Waters Inc., Milford, MA, USA) with a particle size of 3 μm [33]. The column dimensions were 150 $\times$ 4.6 mm. The carotenoids were separated with a flow rate of 1 mL/min and by a gradient elution with two mixtures of methanol, tert-butyl methyl ether, and water [mixture A: 83/15/2 (v/v/v), mixture B: 8/90/2 (v/v/v); gradient procedures were: 0 to 1 min 100% A, 1 to 8 min linear gradient to 70% A, 8 to 13 min 70% A, 13 to 22 min linear gradient to 45% A, 22 to 24 min 45% A, 24 to 34 min linear gradient to 5% A, 34 to 38 min 5% A, 38 to 40 min linear gradient to 100% A, and 40 to 50 min 100% A]. The injection volume of a sample was 50 μL.

Table 2
Total area under the labeled lutein response curve (AUC) in serum up to 29 days for each mg of ingested lutein. For subjects #3 & 4, the AUC up to 29d was calculated proportionally between the last two time points.

Subject	Lutein in Vegetable Dose (mg)	AUC of Serum Labeled Lutein [μg-day]		AUC_{29d}: AUC_{15d}	Average Response *[μg-day/mg]
		0 – 15 day	0 – 29 day		
#1	18.8	1689	2734	1.6	145
#2	18.8	1715	2799	1.7	149
#3	9.8	1734	2567	1.5	262
#4	15.4	1279	1965	1.5	128

* This absolute serum lutein response after the vegetable dose is calculated by multiplying the percent enrichment of lutein and the concentration of lutein at each serum sampling time point over 29 days.

2.6. LC/MS analysis

The mass spectrometry was performed with the Bruker Esquire-LC Ion Trap LC/MS$^{(n)}$-System (Bruker Daltonik, Bremen, Germany) with an APCI-Interface and an ion trap. The LC-MS-coupling was done with an Agilent 1100 HPLC system (Agilent Technologies, Palo Alto, CA) equipped with a C30 column (YMC) and software of Bruker Data Analysis Esquire-LC 4.0.

The mass spectra were recorded in a mass region of 540 to 570 m/z. The detection was done using APCI in the positive ion mode. The voltage of the corona needle was optimized, resulting in a current of 4 to 8 μA. The drying and carrier gas was nitrogen at a temperature of 300°C. The temperature of the ionization chamber was set at 300°C.

2.7. Enrichment determination

The use of labeled vegetables, and thus deuterated lutein, provides the means for differentiating between the lutein that has been ingested during the study and the normal serum level (endogenous) by using LC/MS (Fig. 1). For the quantification, the lutein peaks in the EICs of m/z 551 to 554 assigned to endogenous lutein ($\Sigma_{unlabeled}$) and m/z 556 to 562 ($\Sigma_{labeled}$) from the deuterated lutein were individually integrated. The abundance of isotope masses 556 to 562 (abundance measured partially) to m/z 552 to 569 (abundance measured completely) for labeled vegetables (Fig. 1c) was 0.63 for spinach and 0.48 for both collard greens. Based on our analysis on the results, the isotopomer distributions of deuterated lutein are the same in vegetables and serum, these ratios (partial/complete) were used to calculate the whole enrichment from enrichment data measured partially at every time point of serum samples. Subsequently, the enrichment of the supplemented lutein in the serum was calculated by the formula:

$$\text{enrichment (\%)} = \frac{\Sigma\text{labeled} \cdot 100}{\Sigma\text{labeled} + \Sigma\text{unlabeled}}$$

The enrichment determined for each time point at which blood samples were collected was plotted against time to calculate areas under the curve.

2.8. Area under the curve of each mg lutein in the labeled vegetable dose

Whole body serum responses to the lutein in labeled vegetable dose were determined by multiplying the total serum volume (0.0435 L of kilogram body weight) by the concentration of labeled lutein in the circulation. Areas under the serum labeled lutein curves (in μg-day) after the labeled vegetable dose was calculated by using the curve of total serum responses vs. time via Integral-Curve of Kaleidagraph (Synergy Software, Reading, PA). Response in area under the curve of labeled lutein concentration to each mg lutein in the labeled vegetable can be determined. For subjects #3 and #4, the area under the curve (AUC) up to 29 days was calculated proportionally between the last two time points.

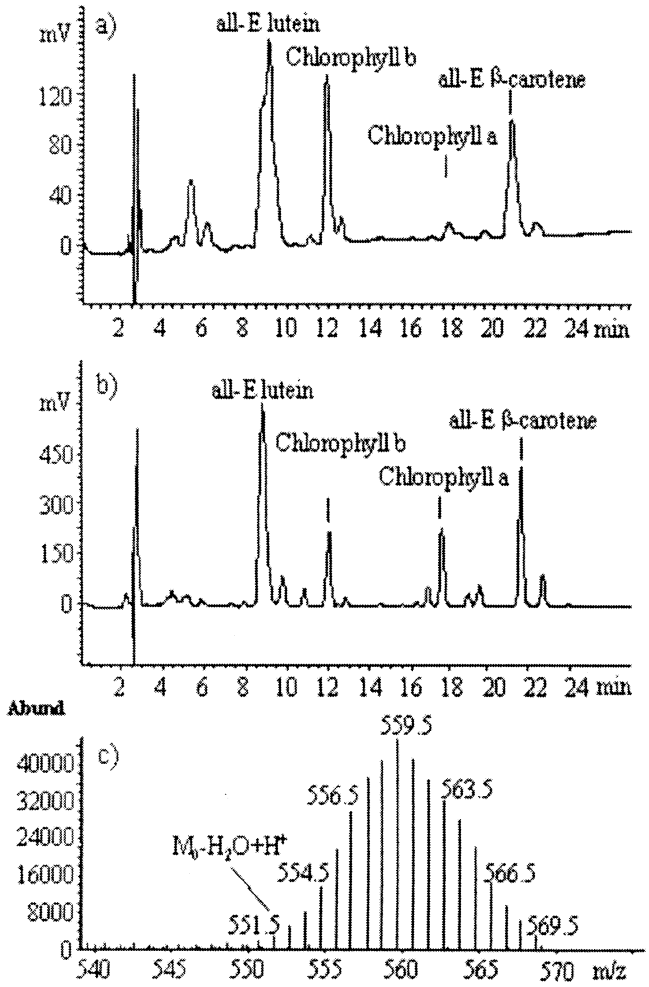

Fig. 1. Separation of the carotenoids from deuterated vegetables [a) spinach and b) collard green] and c) the gaussian isotopomer distribution of the deuterated lutein from labeled spinach.

3. Results

3.1. Quantification of deuterated lutein in labeled vegetables

Initially, it was necessary to determine the amount of deuterated lutein in the spinach and collard greens that had been hydroponically grown in 25 atom-% deuterated water. The carotenoids were separated chromatographically on a highly selective C_{30} RP column using a gradient composed of the eluent methanol, TBME, and water (Fig. 1a is from spinach extraction and Fig. 1b is from collard green extraction). All-trans lutein, all-trans β-carotene and chlorophyll are major phytocomponents detected in the spinach and

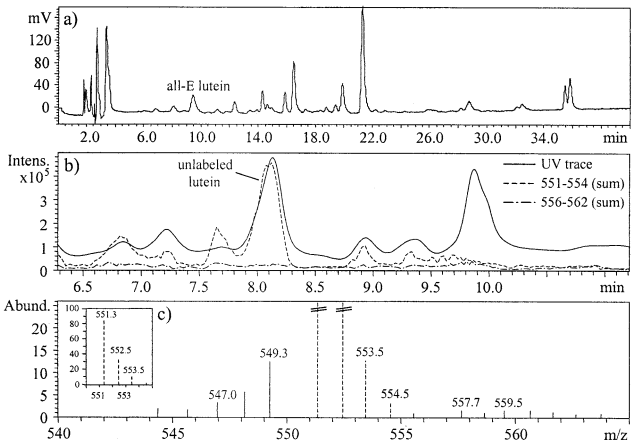

Fig. 2. HPLC-APCI-MS analysis of a serum extract (subject #2) before the supplementation with deuterated spinach [a) HPLC separation of the carotenoids in the serum, b) extracted ion chromatograms (EIC) of unlabeled (m/z 551-554) and labeled (m/z 556-562) lutein, c) mass spectrum at the peak maximum of lutein at 8.2 min].

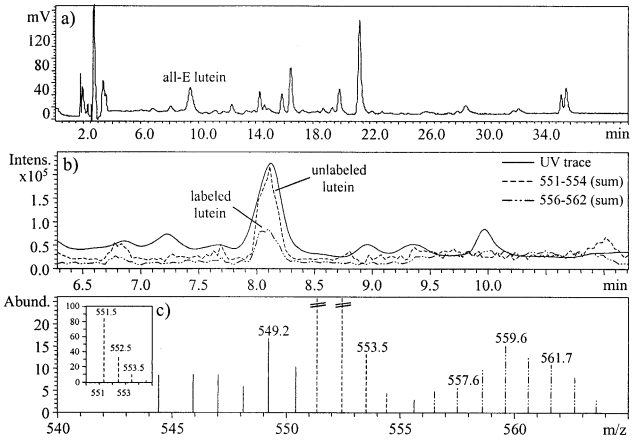

Fig. 3. HPLC-APCI-MS analysis of a serum extract (#2) 2 days after the supplementation with deuterated spinach [a) HPLC separation of the carotenoids in the serum, b) extracted ion chromatograms (EIC) of unlabeled (m/z 551-554) and labeled (m/z 556-562) lutein, c) mass spectrum at the peak maximum of lutein at 8.2 min].

collard greens. The amounts of lutein in 100 g Melody spinach, Grady Simpson collard, and Blue Max collard were 9.4, 5.1, and 7.2 mg, respectfully, as presented in Table 2.

The mass spectrum of the deuterated lutein from the labeled spinach recorded in the positive APCI mode reveals a Gaussian isotopomer distribution that is shown in Fig. 1c. The peak of endogenous lutein is not the molecular ion, but rather the $(\text{lutein} + H - H_2O)^+$ ion at m/z 551. The most abundant isotopomer of labeled lutein is m/z 559 ($^2H_8$-lutein $+ H - H_2O)^+$.

3.2. HPLC-APCI-MS analysis of the serum extracts

The carotenoids were extracted from the serum under red light using chloroform and hexane. Representative chromatograms obtained from the separation of the serum extract at 0h (before) and 1 day after the intake of deuterated spinach (subject #2) performed on a C_{30} RP column are shown in Figs. 2a and 3a, respectively. The main all-E isomer of lutein elutes at 8 to 9 min.

When the chromatographic separation is performed by LC/MS, it is possible to monitor the extracted ion chromatograms (EIC) of the m/z regions 551 to 554 and 556 to 562. The m/z area 551 to 554 is assigned as the predominant natural abundance isotopmers of lutein $(\text{lutein} + H\text{-}H_2O)^+$ while the region of m/z 556 to 562 is the labeled lutein with its different degrees of deuteration.

Prior to the consumption of deuterated vegetables, only the EIC of the endogenous lutein with m/z 551 to 554 was observed from a peak at the HPLC retention time 8.2 min. This is displayed in Fig. 2b and c. The EIC of m/z 556 to 562 assigned as deuterated lutein showed a distinctive peak at 8.2 min in the serum extract collected 1 day after the supplementation with labeled spinach (Fig. 3b). Moreover, the Gaussian isotopomer distribution is clearly visible in the mass spectrum of the peak maximum (Fig. 3c).

3.3. Serum response of lutein after labeled vegetables

Previous studies that sought to determine the bioavailability of carotenoids after the intake of foods or supplements containing carotenoids, simply integrated the corresponding peaks in the HPLC chromatograms and plotted them against time [34]. The concentration of serum lutein was then calculated for each time point by comparing the carotenoid level at this point to the level before the supplementation (baseline lutein concentration). Fig. 4 shows the results of each volunteer from the HPLC analysis on the serum concentration of lutein during the study period. It is possible to observe an increase in the amount of lutein found in the serum during supplementation of the labeled vegetables, but the total serum response determined by the HPLC analysis cannot exclude the contribution of lutein from other sources of lutein in the body or in the diet.

The serum response of labeled lutein (% enrichment) was determined for each time point at which blood samples were drawn and was plotted against time. The absorption-clearance curves of lutein obtained for each volunteer are displayed in Fig. 5 (a and b). The maximum level of enrichment was found between 13 (#2, #3, and #4) and 24 hr (#1) after the supplementation with the deuterated vegetables. The results are also in accordance with the ones from the HPLC quantification. The maximum enrichment was 38 to 42%, except #4, who has a very high enrichment of 67%. The results of the total area under the labeled lutein response curve over 15 days and 29 days are presented in Table 2 and the ratios of $AUC_{29\ days}$ to $AUC_{15\ days}$ in Table 2 showed a constant number of minimal variation from 1.5 to 1.7. Further, these ratios were not affected with the variable changes of lutein concentrations of 1.2, 3.3, 4.0, and 14.6 μg/dL for subjects #4 (from 13 days to 34 days), #1 (from 15 days to 29 days), #2 (from 16 days to 29 days),

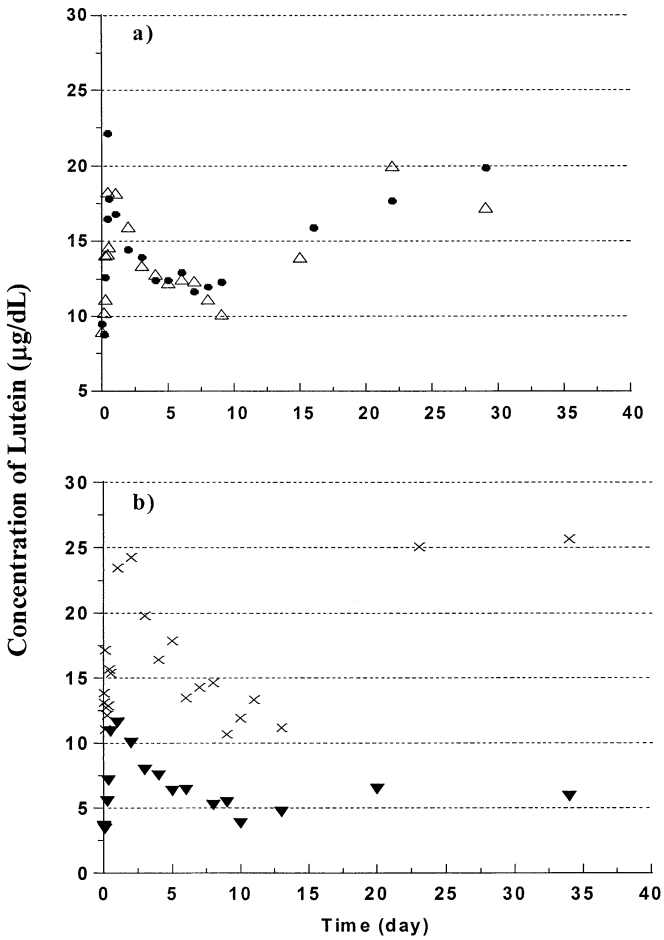

Fig. 4. HPLC quantification of the lutein concentrations in serum after the supplementation of deuterated vegetables. In panel a, open triangle is for subject #1 (spinach, 18.8 mg lutein) and solid circle is for subject #2 (spinach, 18.8 mg lutein). In panel b, cross is for subject #3 (collard green, 9.8 mg lutein) and solid triangle is for subject #4 (collard green, 15.4 mg lutein). Note that subjects were free living after d 10, with lutein intake unregulated through the end of the study.

and #3 (from 13 days to 34 days), respectively, during the free living period as presented in Fig. 4.

The total area under the labeled lutein response curve in serum for each mg of lutein ingested is presented in Table 2. In relative terms, our AUC results (Table 2) show that one mg lutein from spinach may provide about 147 μg-day lutein to healthy adults (integrated over 29 days), when fed as a single dose of 18.8 mg lutein. However, one mg lutein from collard greens may provide from 128 to 262 μg-day lutein (integrated over 29 days), when fed as a single dose of 15.3 or 9.8 mg lutein, respectively.

4. Discussion

Our results show that lutein in spinach and collard greens can be labeled when plants are grown hydroponically with heavy water. In combination with LC/MS, the labeled vegetables can be used to study phytonutrient absorption. We found that 25atom% D_2O in the hydroponic solution can adequately label carotenoids to achieve a peak enrichment of (M + 8) and a range of measurable isotopomers from (M +1) through (M + 18) (Fig. 1). These isotopomers were easily discernable from endogenous unlabeled lutein molecules in the mass spectrometric analysis. Similar isotopomer curves have been reported for deuterated-phylloquinone in heavy water-labeled broccoli [35].

After volunteers took an acute dose of labeled vegetables containing 9.8 to 18.8 mg lutein, we followed the lutein trace in circulation for up to 35 days. Enrichments as low as 8% were obtained for early samples collected during the first few hours after feeding, and also for later samples (time points ≥29 days). We found that the blood samples beyond 35 days cannot be analyzed without compromising accuracy. With the procedures employed in this study, and using a dose as low as 9.8 mg lutein, there was sufficient sensitivity to reliably detect labeled lutein molecules in the serum as late as 34 days post-feeding.

In this study, serum concentration of lutein at later time points during free-living period (after day 10) increased in 3

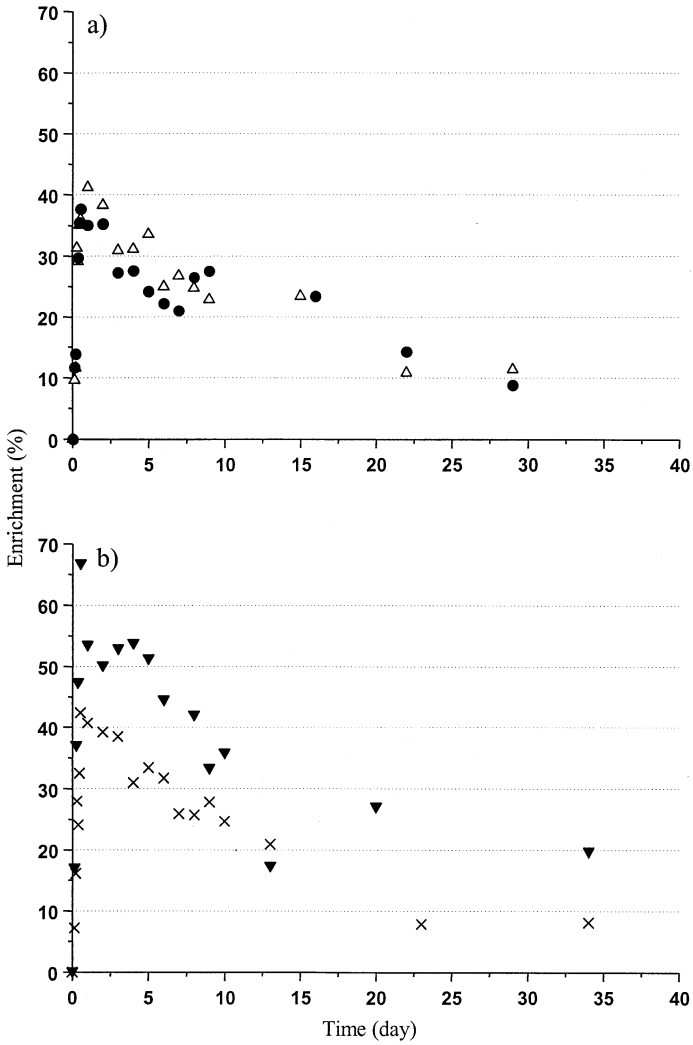

Fig. 5. Serum response of deuterated lutein in percentage enrichment after the ingestion of deuterated vegetables. In panel a, open triangle is for subject #1 (spinach, 18.8 mg lutein) and solid circle is for subject #2 (spinach, 18.8 mg lutein). In panel b, cross is for subject #3 (collard green, 9.8 mg lutein) and solid triangle is for subject #4 (collard green, 15.4 mg lutein).

of 4 subjects. When intake of lutein is higher, serum concentration of lutein would be elevated and the percentage enrichment of lutein may be lower than it would be. However, the labeled lutein from the labeled vegetable dose can still be followed during this period using our current method. The ratios of $AUC_{29\ days}$ to $AUC_{15\ days}$ in Table 2 varied minimally from 1.5 to 1.7 and these ratios were not affected with the variable changes of lutein concentrations of 1.2, 3.3, 4.0, and 14.6 μg/dL for subjects #4 (from 13 days to 34 days), #1 (from 15 days to 29 days), #2 (from 16 days to 29 days), and #3 (from 13 days to 34 days), respectively, during the free living period as presented in Fig. 4. In other words, we did not observe significant effects of the serum concentration of lutein or the changes of serum concentration of lutein on the clearance of serum labeled lutein. This is the advantage of using labeled lutein and LC/MS determination by which dietary changes would not affect the total blood response or apparent bioavailability of the labeled nutrient.

A recent report [36] demonstrated the use of high-precision gas chromatography-combustion interfaced-isotope ratio mass spectrometry (GC-C-IRMS) to quantify plasma appearance of ^{13}C labeled lutein from an algal extract containing 3 mg of perlabeled ^{13}C lutein. The study reported that the highest measured plasma concentration is at 11 (1 subject) and 16 (3 subjects) hours and that there was a three-fold variation among 4 subjects (25 to 38 y) in blood response to a purified lutein in oil dose. Due to limited access to GC-C-IRMS and the availability of high expense of generating ^{13}C- labeled vegetables, we tried to use other readily available resources. The development of the current method will facilitate dietary lutein studies. We observed the highest labeled lutein between 13 and 24 hr after the dose and about two-fold variation among 4 subjects (45 to 65 y) in blood response to a vegetable lutein dose.

Overall, we have shown that it is possible to determine the relative bioavailability of lutein, an important carotenoid

for eye health and function, by administering a single, reasonably sized serving of a deuterated vegetable. It should be acknowledged that our values represent *relative* bioavailabilities, in that we have not captured complete enrichment curves (Fig. 5; curves have not returned to baseline), nor have we been able to account for any lutein that was sequestered/utilized in various biological compartments (e.g., retina, liver, adipose tissues). Nonetheless, this technique should enable comparative investigations of lutein absorption using different lutein-containing foods, different food preparation techniques, or when lutein-containing foods are consumed with varying doses of other carotenoids (i.e., when challenged with potential competitive interactions).

References

[1] Solomons NW. Vitamin A and carotenoids. In: Bowman BA, Russell RM, editors. Present knowledge in nutrition. Washington, DC, USA: International Life Sciences Institute Press, 2001. p. 127–45.

[2] Edge R, McGarvey DJ, Truscott TG. The carotenoids as anti-oxidants – a review. J Photochem Photobiol B Biology 1997;41:189–200.

[3] Rice-Evans CA, Sampson J, Bramley PM, Holloway DE. Why do we expect carotenoids to be antioxidants in vivo? Free Rad Res 1997; 26:381–98.

[4] Young AJ, Lowe GM. Antioxidant and prooxidant properties of carotenoids. Arch Biochem Biophys 2001;385(1):20–7.

[5] Mortensen A, Skibsted LH, Truscott TG. The interaction of dietary carotenoids with radical species. Arch Biochem Biophys 2001;385(1):13–9.

[6] Basu HN, Del Vecchio AJ, Flider F, Orthoefer FT. Nutritional and potential disease prevention properties of carotenoids. J Am Oil Chemists Soc 2001;78(7):665–75.

[7] Hammond BR, Johnson EJ, Russell RM, Krinsky NI, Yeum K-J, Edwards RB, Snodderly DM. Dietary modification of human macular pigment density. Invest Ophthalmol Vis Sci 1997;38:1795–801.

[8] Seddon JM, Ajani UA, Sperduto RD, Hiller R, Blair N, Burton TC, Farber ES, Gragoudas ES, Haller J, Miller DT, Yannuzzi LA, Willett WC. Dietary carotenoids, vitamins A, C and E and advanced age-related macular degeneration – the eye disease case-control study group. JAMA 1994;272(18):1413–20.

[9] Dachtler M, Glaser T, Kohler K, Albert K. Combined HPLC-MS and HPLC-NMR on-line coupling for the separation and determination of lutein and zeaxanthin stereoisomers in spinach and in retina. Anal Chem 2001;73:667–74.

[10] Chaban-Taber L, Willett JM, Seddon JM, Stampfer MJ, Rosner B, Colditz GA, Speizer FE, Hankinson SE. A prospective study of carotenoid and vitamin A intakes and risk of cataract extraction in US women. Am J Clin Nutr 1999;70:509–16.

[11] Brown L, Rimm EB, Seddon JM, Giovannucci EL, Chasan-Taber L, Spiegelman D, Willett WC, Hankinson SE. A prospective study of carotenoid intake and risk of cataract extraction in US men. Am J Clin Nutr 1999;70:517–24.

[12] Street DA, Comstock GW, Salkeld RM, Schueep W, Klag MJ. Serum antioxidants and myocardial infarction. Circulation 1994;90:1154–61.

[13] Ness AR, Powles JW. Fruit and vegetables, and cardiovascular disease: a review. Int J Epidemiol 1997;26:1–13.

[14] Zhang S, Hunter DJ, Forman MR, Rosner BA, Speizer FE, Colditz GA, Manson JE, Hankinson SE, Willett WC. Dietary carotenoids and vitamins A, C and E and risk of breast cancer. J Natl Cancer Inst 1999;91:547–56.

[15] Dorgan JF, Sowell A, Swanson CA, Potischman N, Miller R, Schussler N, Stephenson HE. Relationships of serum carotenoids, retinol, α-tocopherol, and selenium with breast cancer risk: results from a prospective study in Columbia, Missouri (United States). Cancer Causes Control 1998;9:89–97.

[16] Le Marchand L, Hankin JH, Kolonel LN, Beecher GR, Wilkens LR, Zhao LP. Intake of specific carotenoids and lung cancer risk. Cancer Epidemiol Biomarker & Prev 1993;2:183–7.

[17] Slattery ML, Benson J, Curtin K, Ma K-N, Schaeffer D, Potter JD. Carotenoids and colon cancer. Am J Clin Nutr 2000;71:575–82.

[18] Cohen JH, Kristal AR, Stanford JL. Fruit and vegetable intakes and prostate cancer risk. J Natl Cancer Inst 2000;92:61–8.

[19] The Alpha-Tocopherol, Beta-carotene Cancer Prevention Study Group. The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. N Eng J Med 1994;330:1029–35.

[20] Mares-Perlman JA. Too soon for lutein supplements. Am J Clin Nutr 1999;70:431–2.

[21] Food & Nutrition Board. Dietary reference intakes for vitamin C, vitamin E, selenium, and carotenoids. Washington, DC, USA: National Academy Press, 2000.

[22] Prasad KN, Cole W, Hovland P. Cancer prevention studies: past, present, and future directions. Nutr 1998;14:197–210.

[23] Castenmiller JJM, West CE. Bioavailability of carotenoids. Pure & Appl Chem 1997;69(10):2145–50.

[24] Handelman GJ, Nightingale ZD, Lichtenstein AH, Schaefer EJ, Blumberg JB. Lutein and zeaxanthin concentrations in plasma after dietary supplementation with egg yolk. Am J Clin Nutr 1999;70:247–51.

[25] van het Hof KH, West CE, Westrate JA, Hautvast JGAJ. Dietary factors that affect the bioavailability of carotenoids. J Nutr 2000;130:503–6.

[26] van het Hof KH, Brouwer IA, West CE, Haddeman E, Steegers-Theunissen RPM, van Dusseldorp M, Westrate JA, Eskes TKAB, Hautvast JGAJ. Bioavailability of lutein from vegetables is 5 times higher than that of β-carotene. Am J Clin Nutr 1999;70:261–8.

[27] Broekmans WMR, Kloepping-Ketelaars IAA, Schuurman CRWC, Verhagen H, van den Berg H, Kok FJ, van Poppel G. Fruits and vegetables increase plasma carotenoids and vitamins and decrease homocysteine in humans. J Nutr 2000;130:1578–83.

[28] Johnson EJ, Hammond BR, Yeum K-J, Qin J, Wang XD, Castaneda C, Snodderly DM, Russell RM. Relation among serum and tissue concentrations of lutein and zeaxanthin and macular pigment density. Am J Clin Nutr 2000;71:1555–62.

[29] Grusak MA. Intrinsic stable isotope labeling of plants for nutritional investigations in humans. Nutr Biochem 1997;8:164–71.

[30] Grusak MA, Pezeshgi S. Uniformly ^{15}N-labeled soybean seeds produced for use in human and animal nutrition studies: description of a recirculating hydroponic growth system and whole plant nutrient and environmental requirements. J Sci Food Agric 1994;64:223–30.

[31] Tang G, Andrien BA Jr, Dolnikowski G, Russell RM. Determination of the β-carotene-d8 to retinol-d4 conversion in humans using APCI- and ECNCI-MS. Methods Enzym 1997;282:140–54.

[32] Riso P, Porrini M. Determination of carotenoids in vegetable foods and plasma. Internat J Vit Nutr Res 1997;67:47–54.

[33] Yeum K-J, Booth SL, Sadowski JA, Liu C, Tang G, Krinsky NI, Russell RM. Human plasma carotenoid response to the ingestion of controlled diets high in fruits and vegetables. Am J Clin Nutr 1996;64:594–602.

[34] Brown ED, Micozzi MS, Craft NE, Bieri JG, Beecher G, Edwards BK, Rose A, Taylor PR, Smith JC. Plasma carotenoids in normal men after a single ingestion of vegetables or purified β-carotene. Am J Clin Nutr 1989;49:1258–65.

[35] Dolnikowski GG, Sun Z, Grusak MG, Peterson JW, Booth SL. HPLC and GC/MS determination of deuterated vitamin K (phylloquinone) in human serum after ingestion of deterium-labeled broccoli. J Nutr Biochem 2002;13:168–74.

[36] Yao L, Liang Y, Trahanovsky WS, Serfass RE, White WS. Use of a ^{13}C tracer to quantify the plasma appearance of a physiological dose of lutein in humans. Lipids 2000;35:339–48.