Spirulina is an effective dietary source of zeaxanthin to humans

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Abstract
Zeaxanthin is a predominant xanthophyll in human eyes and may reduce the risk of cataracts and age-related macular degeneration. Spirulina is an algal food that contains a high concentration of zeaxanthin. In order to determine the zeaxanthin bioavailability of spirulina for dietary supplementation in humans, spirulina was grown in nutrient solution with 2H2O for carotenoid labelling. Single servings of 2H-labelled spirulina (4·0–5·0 g) containing 2·6–3·7 mg zeaxanthin were consumed by fourteen healthy male volunteers (four Americans and ten Chinese) with 12 g dietary fat. Blood samples were collected over a 45 d period. The serum concentrations of total zeaxanthin were measured using HPLC, and the enrichment of labelled zeaxanthin was determined using LC-atmospheric pressure chemical ionisation-MS (LC-APCI-MS). The results showed that intrinsically labelled spirulina zeaxanthin in the circulation was detected at levels as low as 10 % of the total zeaxanthin for up to 45 d after intake of the algae. A single dose of spirulina can increase mean serum zeaxanthin concentration in humans from 0·06 to 0·15 nmol/l, as shown in our study involving American and Chinese volunteers. The average 15 d area under the serum zeaxanthin response curve to the single dose of spirulina was 293 nmol £ d/mmol (range 254–335) in American subjects, and 197 nmol £ d/mmol (range 154–285) in Chinese subjects. It is concluded that the relative bioavailability of spirulina zeaxanthin can be studied with high sensitivity and specificity using 2H labelling and LC-APCI-MS methodology. Spirulina can serve as a rich source of dietary zeaxanthin in humans.

Key words: Zeaxanthin: Spirulina: Bioavailability: 2H labelling

Zeaxanthin and its structural isomer lutein are the most prevalent xanthophylls found in the human body. They are highly concentrated in the retina of human eyes. Zeaxanthin is the major component in the central macula, while lutein is predominant in the peripheral area(1,2). It is believed that zeaxanthin and lutein function as blue-light filters and reactive oxygen species scavengers in the retina to protect the cells from oxidative damage(3,4).

Numerous studies have suggested that zeaxanthin and lutein are crucial for visual health. In animal models (in monkeys), zeaxanthin and lutein have been found to be important for the development and maintenance of normal distribution of the retinal pigment epithelium(5,6); retinal zeaxanthin has also been shown to protect the photoreceptors from light-induced damage in quail eyes(7,8). In human subjects, some epidemiological and clinical researchers have demonstrated that high levels of zeaxanthin and lutein intake are associated with significantly decreased risks of cataract and age-related macular degeneration(9–11).

Because humans cannot synthesise xanthophylls, dietary intake of xanthophyll-containing foods is critical to provide these phytonutrients to our bodies. In the US population, the average intake of lutein and zeaxanthin together is about 2 mg/d(12), which is three times less than the dose of lutein and zeaxanthin that has been linked with a decreased risk of age-related macular degeneration and cataracts.
(6 mg/d)\(^{(11)}\). Therefore, lutein and zeaxanthin supplements have been suggested for visual health in people with low dietary intake, especially for older adults.

However, although lutein is abundant in many green leafy vegetables such as kale and spinach, only a few kinds of food contain high concentrations of zeaxanthin\(^{(13,14)}\). In cooked egg-yolk, maize and orange pepper, zeaxanthin concentrations are about 587, 202 and 1665 µg/100 g fresh weight, respectively\(^{(15)}\). Spirulina is an alga that is rich in carotenoids, including zeaxanthin\(^{(15–18)}\). In the strain of spirulina studied in the present investigation, the concentration of zeaxanthin as a dried powder can reach 74 000 µg/100 g\(^{(19)}\). Previous studies in animal models as well as in human subjects have demonstrated that β-carotene from spirulina has a high bioavailability and a high bioconversion to vitamin A factor\(^{(19)}\); however, no studies on spirulina zeaxanthin have been reported.

In the present report, the enrichment of spirulina zeaxanthin in serum and its bioavailability in humans have been determined. A single serving of intrinsically \(^2\)H-labelled spirulina was consumed by American and Chinese healthy males. Their plasma samples were collected up to 45 d post-spirulina supplementation. The concentration and isotopic enrichment of spirulina zeaxanthin were analysed using \(C_{30}\) column-based HPLC and LC-atmospheric pressure chemical ionisation-MS (LC-APCI-MS). The results were used to calculate a relative bioavailability (the change in plasma concentration in response to a test meal)\(^{(20)}\), which demonstrated that spirulina is a rich source of dietary zeaxanthin in both American and Chinese volunteers.

Materials and methods

Subjects and study design

The present study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Institutional Review Board of Tufts Medical Center and the Institute of Nutrition and Food Hygiene Ethical Review Committee of the Chinese Academy of Preventative Medicine. Informed consent was obtained from all subjects under the guidelines established.

The human studies were conducted in China and the USA in 2006 and 2008, respectively. For this study, four American males (two African Americans and two Caucasians) and ten Chinese males were recruited separately. The volunteers were healthy, non-smoking adults and were not taking vitamin supplements at the time of the study. All subjects were instructed to eat a low-carotenoid diet for 2 weeks before taking the study dose. On day 1, each subject was given a spirulina supplement of 4 g (for American volunteers) or 5 g (for Chinese volunteers) with a carotene-free liquid formulated breakfast. The ingredients of this liquid breakfast contained banana, coconut milk, whey protein, glucose polymer, white sugar and water, which provided 12 g fat (26% of total energy), 4 g protein (4% of total energy) and 74 g carbohydrate (70% of total energy), with a total energy content of 1668 kJ (399 kcal).

Fasting blood samples (10 ml) were collected from all subjects before supplementation as the baseline. Blood samples (10 ml) were collected from all subjects at 0 (fasting blood, just before breakfast), and 6, 8, 11 and 13 h after intake of the supplement on day 1. Blood was drawn at 0, 5 and 11 h on day 2, and at 0 and 11 h on day 3. Fasting blood samples (10 ml) were obtained on days 4, 5, 6, 7, 11, 13, 15, 19, 23, 27, 32, 39 and 45. Due to the schedules of the volunteers, time points varied slightly (±1 d) for some subjects and two subjects had a shorter period of intervention (a minimum of 23 d).

Chemicals and materials

Most of the solvents used in this study including chloroform, methanol (MeOH), hexane, ethanol, tert-butyl methyl diethyl ether (MTBE), tetrahydrofuran were HPLC-grade and purchased from Sigma (St Louis, MO, USA). Diethyl ether was from Fisher Scientific (Fair Lawn, NJ, USA). \(NH_2\) cartridge columns were from Phenomenex (Torrance, CA, USA).

Preparation of \(^2\)H labelled spirulina

\(^2\)H labelled spirulina (\(Spirulina platensis\)) was grown in liquid culture at the US Department of Agriculture Agricultural Research Service, Children’s Nutrition Research Center in Houston, TX, USA as previously described\(^{(19)}\). Briefly, a liquid stock culture of spirulina cells was obtained from the University of Texas Culture Collection of Algae (UTEX no. LB2340; University of Texas, Austin, TX, USA), inoculated into 20-litre glass carboys, and grown in a sterilised artificial seawater medium enriched with 23 atom % \(^2\)H2O. After 10–12 d of growth, the spirulina cultures were harvested, freeze-dried and shipped to the Carotenoids and Health Laboratory of the US Department of Agriculture Human Nutrition Research Center on Aging at Tufts University (Boston, MA, USA), where the freeze-dried spirulina was powdered, packed into capsules and stored at \(-80^\circ C\) immediately.

Spirulina capsules for the China study were shipped by air, on dry ice, to the National Institute for Nutrition and Food Safety, Beijing, China. All spirulina capsules were stored at \(-80^\circ C\) until being analysed or consumed by the study subjects.

Sample preparation

Whole blood samples were protected from light using aluminium foil, incubated at \(37^\circ C\) for 30 min, and centrifuged at \(4^\circ C\), 800 g for 15 min to separate the serum. Serum samples collected from Chinese subjects were stored at \(-20^\circ C\) first (less than 2 months) and then shipped by air, on dry ice, to the Carotenoids and Health Laboratory of HNRCA in Boston, MA, USA, where the samples were stored at \(-80^\circ C\) until analysis. Due to the light sensitivity of carotenoids, the extractions and analyses of carotenoids were performed under red light.
To extract carotenoids from spirulina, 1·0 mg of dried spirulina powder was mixed with 10 ml MeOH for 1 h in a shaking incubator at 120 rpm. The mixture was centrifuged at 3000 rpm for 5 min, and the MeOH extract was transferred into a 50 ml volumetric flask. The residue was further extracted with 10 ml of tetrahydrofuran, vortexed and centrifuged at 3000 rpm for 5 min. Extraction of tetrahydrofuran was repeated three or four times, and all tetrahydrofuran extracts were combined with the MeOH extract in the same flask. The final volume of the spirulina extract was adjusted to exactly 50 ml in the volumetric flask, and 1 ml of the extract was transferred to a 10 ml glass tube, dried under N₂ and resuspended with 1 ml of ethanol for HPLC and LC-APCI-MS analyses.

To extract carotenoids from human serum, 500 μl serum samples, 500 μl of 0·85 % saline, 60 μl internal standard (eichinenone in ethanol with absorbance of 0·1–0·2 measured at 460 nm), and 5 ml CHCl₃–MeOH (2:1, v/v) were added into a 5 ml glass tube, and also transferred to the column. Under a gentle vacuum, the column containing the serum extracts was washed with 3 ml of hexane once, and eluted with 10 ml CHCl₃. The CHCl₃ solution was added to the 5 ml glass tube, dried under N₂ and resuspended with 1 ml of ethanol for HPLC analysis.

For LC-APCI-MS analysis, serum was extracted as done previously, but the final dried residue was resuspended with 100 μl of CHCl₃. The CHCl₃ solution was added to the centre of an aminopropyl column (500 mg/3 ml) in the Vac Elut apparatus, which was conditioned with 2 ml of hexane. An additional 100 μl of CHCl₃ was used to wash the glass tube, and also transferred to the column. Under a gentle vacuum, the column containing the serum extracts was washed with 3 ml of hexane once, and eluted with 3 ml of diethyl ether with 3 % acetic acid. The elution was collected in a 5 ml glass tube, dried under N₂ and resuspended with 120 μl of ethanol to make ready for HPLC analysis.

For HPLC analysis

The concentrations of xanthophylls in spirulina and human serum were analysed using a Waters HPLC coupled with a Waters 994 Programmable Photodiode Array Detector (Waters Corporation, Milford, MA, USA) and a semi-micro bore C₃₀ column (3 μm, 150 mm × 3·0 mm; YMC, Wilmington, NC, USA). The HPLC mobile phase A contained MeOH, MTBE and 1·5 % ammonium acetate in water (83:15:2, by vol.); and phase B contained MeOH, MTBE and 1·0 % ammonium acetate in water (8:90:2, by vol.). Gradient procedures (flow rate 1 ml/min) were changed as follows: 0 % B isocratic (0–2 min), 0 % B – 5 % B linear gradient (2–10 min), 5 % B – 55 % B linear gradient (10–18 min), 55 % B – 95 % B linear gradient (18–20 min), 95 % B – 0 % B linear gradient (20–21 min), 0 % B isocratic (21–40 min). The total run time was 40 min and the injection volume was 20 μl. The UV–Vis spectra of all carotenoids were recorded in the range of 250–600 nm, and peak identification was based on comparisons with the retention times and absorption spectra of known carotenoid standards in our laboratory. Data acquisition and processing were performed using Empower Pro software (version 2·0; Waters Corporation).

LC-atmospheric pressure chemical ionisation-MS analysis

Analysis of zeaxanthin enrichment was performed with an Agilent LC-MS (Agilent Technologies, Santa Clara, CA, USA) equipped with an Agilent HPLC, autosampler, UV detector, HP1100 MSD with APCI interface and a C₃₀ column (3 μm, 150 mm × 4·6 mm, Bischoff Chromatography, Leonberg, Germany). The HPLC mobile phase A contained MeOH, MTBE and 1·5 % ammonium acetate in water (88:12:0, by vol.); and phase B contained MeOH, MTBE and 1·0 % ammonium acetate in water (8:90:2, by vol.)). Gradient procedures (flow rate 1 ml/min) were changed as follows: 0 % B isocratic (0–2 min), 0 % B – 5 % B linear gradient (2–10 min), 5 % B – 55 % B linear gradient (10–18 min), 55 % B – 95 % B linear gradient (18–20 min), 95 % B – 0 % B linear gradient (20–21 min), 0 % B isocratic (21–40 min). The total run time was 40 min and the injection volume was 60 μl. The UV–Vis spectra of peaks were recorded in the range of 250–600 nm and the mass spectra were recorded using the following parameters: polarity, positive; mass range, m/z 550–590; fragmentor, 90; gain, 20·00; threshold, 150; and stepsize, 0·1. Data acquisition and process were performed using ChemStations software (Agilent Technologies).

Data process and statistical analysis

The enrichment of labelled zeaxanthin was calculated by integrating the areas of labelled and unlabelled zeaxanthin in extracted ion chromatographs and by using the following equations (1–3):

\[
\sum \text{Unlabelled zeaxanthin} = \sum \text{areas of } m/z 569 - 571. \quad (1)
\]

\[
\sum \text{Labelled zeaxanthin} = \sum \text{areas of } m/z 573 - 583. \quad (2)
\]

\[
\frac{\left( \sum \text{labelled zeaxanthin} \times 100 \right)}{\left( \sum \text{labelled zeaxanthin} + \sum \text{unlabelled zeaxanthin} \right)} \quad (3)
\]

The serum response of labelled zeaxanthin was calculated using equation 4, in which 0·0435 is the serum volume in litres/kg of body weight:

\[
\text{Serum response of labelled zeaxanthin (nmol)} = \frac{\text{enrichment of labelled zeaxanthin in total zeaxanthin} \times \text{total serum zeaxanthin concentration} \times \text{body weight (in kg)} \times 0·0435}{\text{litres/kg of body weight}}. \quad (4)
\]
Average serum response (nmol £ up to 15 d (AUC0–15d) was calculated directly or calculated
software, Reading, PA, USA). For all subjects, the AUC
Integral-curve of Kaleidagraph software (version 3.5; Synergy
Chinese subjects.
baseline concentrations of xanthophylls and serum responses
significance of differences of age, body weight, BMI values,
San Diego, CA, USA). An unpaired
Prism for Windows software (version 4.0; GraphPad Software,
(6) 9-cis-carotene.
Baseline concentration of serum lutein (mol/l)* 0·12–0·18 0·16 0·03 0·27–0·52 0·39 0·09
* Baseline concentration was measured 4 d before the supplementation of spirulina.
† AUC was measured over 15 d for all subjects.
‡ Average serum response is the 15 d area under the labelled zeaxanthin response curve per μmol of consumed spirulina zeaxanthin.

The area under the curve (AUC) of labelled zeaxanthin was calculated by using the curve of serum response of labelled zeaxanthin (nmol, as y axis) v. time (d, as x axis) via Integral-curve of Kaleidagraph software (version 3.5; Synergy Software, Reading, PA, USA). For all subjects, the AUC up to 15 d (AUC0–15d) was calculated directly or calculated proportionally between the two nearest time points.
The average response to labelled spirulina zeaxanthin in the circulation was calculated using equation 5:
Average serum response to labelled spirulina zeaxanthin
(nmol × d/μmol) = AUC0–15 d of labelled
spirulina zeaxanthin/consumed zeaxanthin dose (μmol).

All statistical analyses were performed using Graphpad Prism for Windows software (version 4.0; GraphPad Software, San Diego, CA, USA). An unpaired t test was used to assess the significance of differences of age, body weight, BMI values, baseline concentrations of xanthophylls and serum responses to labelled spirulina zeaxanthin between American and Chinese subjects.

Results

Characteristics of American and Chinese subjects
As previously mentioned, two groups of middle-aged, healthy, non-smoking males were recruited in the USA (n 4) and China (n 10), respectively. The age range of American subjects is 46–52 (49·3 (SD 2·8)) years; weight range is 68·9–83·5 (76·3 (SD 6·1)) kg; and BMI range is 23·1–26·5 (25·5 (SD 1·6)) kg/m2. The age range of Chinese subjects is 41–57 (48·1 (SD 7·7)) years; weight range is 52·2–78·4 (66·7 (SD 8·1)) kg; and BMI range is 17·9–27·4 (23·4 (SD 3·1)) kg/m2. Although the mean values of age, body weight and BMI of American subjects were higher than those of Chinese subjects, there were no statistically significant differences between them for these characteristics.
The carotenoid concentrations of all subjects in the two groups were in the reported ranges21 (data not shown). The range of lutein concentrations was 0·12–0·18 μmol/l in the American group and 0·27–0·52 μmol/l in the Chinese group, while the range of zeaxanthin concentrations was 0·04–0·07 and 0·05–0·09 μmol/l in the American and Chinese groups, respectively.

Quantification of 2H-labelled zeaxanthin in spirulina
HPLC analyses showed that in the spirulina produced for this study, trans-zeaxanthin and trans-β-carotene were the two major carotenoids and cryptoxanthin, 13-cis-β-carotene and 9-cis-β-carotene were also present in low concentrations (Fig. 1). There was no lutein contained in the spirulina that we studied, and echinenone was too small to affect the experiment (Fig. 1).

Due to the different times at which the human studies were conducted in the USA (2008) and China (2006), the 2H-labelled spirulina was harvested in different batches. The concentration of spirulina zeaxanthin was 0·66 mg/g in the batch for American volunteers and 0·74 mg/g for Chinese volunteers; therefore, the dose of labelled spirulina zeaxanthin was 2·63 mg (4·61 μmol) for American volunteers and 3·68 mg (6·46 μmol) for Chinese volunteers (Table 1).

Using LC-APCI-MS, the most abundant protonated molecules of unlabelled zeaxanthin were identified at m/z 569 (zeaxanthin + H+), and ions of anhydrozeaxanthin at m/z 551 (zeaxanthin + H+ – H2O) with an abundance of 12·6 % (Fig. 2a). About 99 % of zeaxanthin in the labelled spirulina was enriched with 2H, and Gaussian distribution of isotopomers of zeaxanthin had the most abundant

Fig. 1. Separation of carotenoids from 2H-labelled spirulina in this study by HPLC analysis. Peaks were identified as: (1) trans-zeaxanthin; (2) chlorophyll; (3) cryptoxanthin; (4) 13-cis-β-carotene; (5) all-trans-β-carotene; (6) 9-cis-β-carotene.

| Subjects... | American | | | Chinese | | |
|------------|----------|--------|--------|----------|--------|
| Batch of spirulina (year) | | | | Range | Mean |
| 2008 | 2·63 |
| 2006 | 3·68 |
| Dosage of zeaxanthin (mg) | | | | Range | Mean |
| 0·12–0·18 | 0·16 |
| 0·04–0·07 | 0·06 |
| 0·12–0·20 | 0·15 |
| Concentration of zeaxanthin 1 d post-supplementation (μmol/l) | | | | Range | Mean |
| 59–65 | 62 |
| 1167–1542 | 1346 |
| 998–1851 | 1279 |
| Average serum response (nmol × d/μmol) | | | | Range | Mean |
| 254–335 | 293 |
| 154–285 | 197 |
| † Average serum response is the 15 d area under the labelled zeaxanthin response curve per μmol of consumed spirulina zeaxanthin.

Table 1. Baseline concentrations of serum lutein and zeaxanthin, and serum response to labelled spirulina zeaxanthin in American and Chinese subjects (Ranges, mean values and standard deviations)
enrichment of $^{2}$$^{2}$H at $m/z$ 577 (zeaxanthin + $^{2}$H$_{8}$ + H$^{+}$; Fig. 2(b)). Similar isotopomer distribution of anhydrozeaxanthin ions (zeaxanthin + $^{2}$H$_{8}$ + H$^{+}$ – H$_{2}$O, zeaxanthin + $^{2}$H$_{8}$ + H$^{+}$ – 2H$_{2}$O) at $m/z$ 559 and $m/z$ 541 were also observed, with the abundance of 15-1 and 7-5%, respectively (Fig. 2(b)).

**Enrichment of labelled spirulina zeaxanthin in circulation in human subjects**

The representative HPLC chromatographs and mass spectra of human serum zeaxanthin (from American subject 2) before and 48 h after supplementation with spirulina are shown in Fig. 3. The HPLC chromatographs demonstrate that serum extracts before and after supplementation shared similar carotenoid profiles (Fig. 3). All trans-lutein eluted at 6-9 min, and all trans-zeaxanthin eluted at 8-5 min. The amount of lutein decreased slightly 48 h post-supplementation (Fig. 3(a), before supplementation), but the amount of zeaxanthin greatly increased 48 h post-supplementation (Fig. 3(a), after supplementation).

According to the distribution of zeaxanthin mass (Fig. 2), the peaks of $m/z$ 569–571 were assigned as endogenous unlabelled zeaxanthin, while those of $m/z$ 573–583 were assigned as the $^{2}$H-labelled zeaxanthin from spirulina. Extracted ion chromatographs of unlabelled and labelled zeaxanthin showed that, before the supplementation, no labelled zeaxanthin was detectable at 8-5 min (Fig. 3(b), before supplementation); however, 48 h after supplementation, both labelled and unlabelled zeaxanthin were present (Fig. 3(b), after supplementation). The Gaussian isotoptamer distribution of $^{2}$H-labelled zeaxanthin was clearly visible after supplementation (Fig. 3(c), after supplementation), but not before supplementation (Fig. 3(c), before supplementation), in the mass spectrum of the zeaxanthin peak maximum.

**Serum responses to labelled spirulina zeaxanthin in American and Chinese volunteers**

Although we followed the labelled zeaxanthin trace in the circulation up to 45 d, only data over 21 d are presented in the graphs. The concentrations of serum zeaxanthin increased dramatically about 1–2 d post-supplementation in all subjects (data not shown). The mean value of serum zeaxanthin concentration 1 d after supplementation increased from baseline of 0.06 to 0.11 μmol/l in American subjects and from baseline of 0.07 to 0.15 μmol/l in Chinese subjects (Table 1). Over the 21 d of this study, the serum concentrations of total zeaxanthin fluctuated in some subjects (e.g. American subject 1 and Chinese subject 7), but were relatively stable in others (e.g. American subject 2 and Chinese subject 10; Fig. 4).

In all subjects, the enrichment of labelled zeaxanthin in the circulation reached a maximum between 1 and 3 d post-supplementation, and then decreased with little variation (Fig. 5 and data not shown). The amount of serum labelled zeaxanthin followed a similar pattern (Fig. 5). The mean highest value of labelled zeaxanthin as percentage of total zeaxanthin in American subjects was 62% (range 59–65%), and was 49% in Chinese subjects (range 43–56%; Table 1).

**Area under the curve of labelled spirulina zeaxanthin responses in American and Chinese subjects**

The AUC of labelled zeaxanthin was integrated using amounts of labelled zeaxanthin $v.$ time over 15 d due to the detectability of the zeaxanthin. American subjects had a mean value of 1346 nmol × d and Chinese subjects had a mean value of 1279 nmol × d (Table 1). The mean value of the average serum response, which is the 15 d area under the serum labelled zeaxanthin response curve (in nmol × d) divided by the consumed zeaxanthin dose (in μmol), was 293 nmol × d/μmol in American subjects (range 254–335), and was 197 nmol × d/μmol in Chinese subjects (range 154–285).

**Discussion**

In the present study, we utilised $^{2}$H labelling and LC-APCI-MS to determine the bioavailability of spirulina zeaxanthin in human subjects. The peaks of enrichment of (M + $^{2}$H$_{8}$) and measurable isotoptamers from (M + $^{2}$H$_{14}$) of labelled zeaxanthin were easily discernible from unlabelled zeaxanthin in human serum extracts (Fig. 3). After the study, subjects took a single dose of spirulina, and the labelled zeaxanthin was detected as early as 1 d post-supplementation.
Fig. 3. LC-atmospheric pressure chemical ionisation-MS analysis of serum extracts (American subject 2) before and after supplementation with $^2$H-labelled spirulina. (a) HPLC separation of the carotenoids in the serum. (b) Extracted ion chromatograms (EIC) of unlabelled ($m/z$ 569–571, —) and labelled ($m/z$ 573–583, —) zeaxanthin. —, UV450. (c) Mass spectra at the peak maximum of zeaxanthin before and 48 h after supplementation with $^2$H-labelled spirulina.
and enrichments could be measured in circulation up to 45 d post-feeding (data not shown). Therefore, the serum response to labelled zeaxanthin from spirulina was sensitively detected with minimal influences by their daily dietary intake of zeaxanthin.

Our present study showed that spirulina is a good dietary source of zeaxanthin which can effectively increase serum zeaxanthin concentration in humans. There are several studies reporting the influence of dietary zeaxanthin on subject plasma zeaxanthin concentrations (22–26). In one study with older adults, egg-yolk supplementation of a beef tallow diet or a maize oil diet increased plasma zeaxanthin concentrations by 142 or 114%, respectively (26). A similar study of a 12-week egg intervention also showed that egg intake significantly increased serum zeaxanthin concentrations in women (22). In a recently reported study, consumption of four egg-yolks/d for 5 weeks in older adults increased serum zeaxanthin concentrations from 0·03 to 0·06 µmol/l (23).

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**Fig. 4.** Serum concentrations of total zeaxanthin (in µmol/l) after supplementation with spirulina over 21 d in representative subjects (American subjects 1, 2 and Chinese subjects 7, 10).

**Fig. 5.** Serum responses to labelled zeaxanthin after supplementation with spirulina in representative subjects (American subjects 1, 2 and Chinese subjects 7, 10). $^2$H-labelled spirulina zeaxanthin as percentage of total zeaxanthin (--- and •, left axis) and amount of labelled zeaxanthin (in nmol, --- and ○, right axis) in the circulation over 21 d are presented.
In our study, a single dose of 4.0 or 5.0 g spirulina significantly increased the zeaxanthin concentrations in the circulation of both American and Chinese subjects. One day after supplementation, the mean concentration of total serum zeaxanthin increased from 0.06 to 0.11 μmol/l in American subjects (2.63 mg zeaxanthin dose) and from 0.07 to 0.15 μmol/l in Chinese subjects (3.68 mg zeaxanthin dose; Table 1). Because egg-yolks contain a substantial amount of cholesterol, spirulina could be a good, alternative source of zeaxanthin for those individuals seeking to limit their dietary cholesterol intake. Consumption of vegetables such as spinach and maize at dietary levels have been reported to result in no increases in plasma zeaxanthin concentrations.

There are several factors that might be contributing to the high bioavailability of spirulina zeaxanthin in humans. First, dried spirulina powder contains an extremely high concentration of zeaxanthin, about 50–300 times higher than that in cooked egg-yolk, maize or pepper. In the present study, a single dose of 2.6 or 3.7 mg zeaxanthin was administered to subjects, which might be difficult to achieve if other diets are chosen. Second, the spirulina cell wall is composed of proteins and peptidoglycans that can be digested easily, which helps zeaxanthin to be released from the food matrix. In addition, spirulina contains high concentrations of NEFA that can reach 9–14 % of the dry weight of spirulina. Since fat can increase the bioavailability of carotenoid absorption, the lipid matrix contained in spirulina may facilitate the absorption of zeaxanthin. We observed a significant difference of average serum response to a single dose of spirulina zeaxanthin in middle-aged American and Chinese males (Table 1). However, it should be noted that the difference we observed was from two small groups (four American subjects, ten Chinese subjects), and no conclusion on racial differences of zeaxanthin absorption could be made at this point. Since it is known that both age and sex may affect the serum response to carotenoids, the substantial response to spirulina zeaxanthin should be further investigated in other populations. With the present study, our data strongly suggested that spirulina is a rich dietary zeaxanthin source, which might benefit the general population for visual health.

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