Spirulina is an effective dietary source of zeaxanthin to humans

Bolan Yu[1,2], Jie Wang[3], Paolo M. Suter[4], Robert M. Russell[2], Michael A. Grusak[5], Yin Wang[6], Zhixu Wang[7], Shian Yin[3] and Guangwen Tang[2]*

[1]*Third Affiliated Hospital of Guangzhou Medical University, Guangzhou, People's Republic of China*
[2]*Jean Mayer US Department of Agriculture Human Nutrition Research Center on Aging at Tufts University, 711 Washington Street, Boston, MA, USA*
[3]*National Institute for Nutrition and Food Safety, Beijing, People's Republic of China*
[4]*University Hospital, Zurich and Nestlé Foundation, Lausanne, Switzerland*
[5]*US Department of Agriculture/Agricultural Research Service, Children's Nutrition Research Center, Baylor College of Medicine, Houston, TX, USA*
[6]*Zhejiang Academy of Medical Sciences, Hangzhou, People's Republic of China*
[7]*Nanjing Medical University, Nanjing, People's Republic of China*

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 ^2H_2O 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 μmol/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/μmol (range 254–335) in American subjects, and 197 nmol × d/μmol (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

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Abbreviations: AUC, area under the curve; MeOH, methanol; MTBE, methyl *tert*-butyl diethyl ether; LC-APCI-MS, LC-atmospheric pressure chemical ionisation-MS.

* **Corresponding author:** Dr G. Tang, fax +1 617 556 3344, email guangwen.tang@tufts.edu

(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[13]. 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), tetrahydrofurane 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 % ^2H_2O. 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°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°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°C for 30 min, and centrifuged at 4°C, 800 **g** for 15 min to separate the serum. Serum samples collected from Chinese subjects were stored at −20°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°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 tetrahydrofurane, vortexed and centrifuged at 3000 rpm for 5 min. Extraction of tetrahydrofurane was repeated three or four times, and all tetrahydrofurane 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 N_2 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 (echinenone in ethanol with absorbance of 0·1–0·2 measured at 460 nm), and 5 ml $CHCl_3$–MeOH (2:1, v/v) were added into 15 ml disposable glass tubes. The mixture was vortexed, centrifuged at 3000 rpm for 5 min, and the lower layer was removed to another 15 ml tube. After removal of the chloroform layer, 3 ml hexane and 1 ml ethanol were added to the remaining aqueous phase, vortexed and centrifuged again. The upper layer was combined with the $CHCl_3$–MeOH extract. All samples were extracted in duplicates, and their chloroform and hexane layers were combined together, dried under N_2 and finally resuspended in 120 µl ethanol to make ready 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 $CHCl_3$. The $CHCl_3$ 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 $CHCl_3$ 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 N_2 and resuspended with 120 µl of ethanol to make ready for LC-APCI-MS analysis.

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 C_{30} 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 (85:12:3, by vol.); and phase B contained methanol, MTBE and 1·0 % ammonium acetate in water (8:90:2, by vol.). The gradient procedure, at a flow rate of 0·4 ml/min, was as follows: 0 % B isocratic (0–3 min), 0 % B – 20 % B linear gradient (3–10 min), 20 % B – 55 % B linear gradient (10–18 min), 55 % B – 95 % B linear gradient (18–33 min), 95 % B – 0 % B linear gradient (33–37 min), 0 % B isocratic (37–40 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, HP1100MSD with APCI interface and a C_{30} 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 (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 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 Chem-Stations 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):

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

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

Enrichment of labelled zeaxanthin from spirulina (%)

$$= \left(\sum \text{labelled zeaxanthin} \times 100 \right) \Big/ \left(\sum \text{labelled zeaxanthin} \right. \\ \left. + \sum \text{unlabelled zeaxanthin} \right). \quad (3)$$

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:

Serum response of labelled zeaxanthin (nmol)
$$= \text{enrichment of labelled zeaxanthin in total zeaxanthin} \\ \times \text{total serum zeaxanthin concentration} \\ \times \text{body weight (in kg)} \times 0.0435. \quad (4)$$
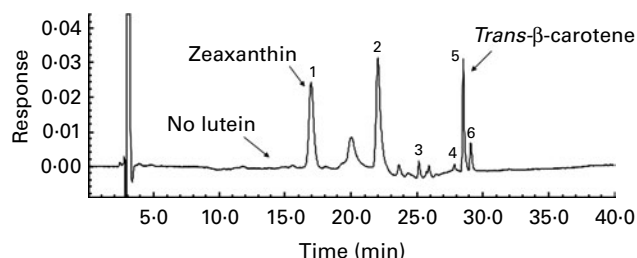
Fig. 1. Separation of carotenoids from ^{2}H-labelled spirulina in this study by HPLC analysis. Peaks were identified as: (1) *trans*-zeaxanthin; (2) chlorophyll; (3) cryptoxanthin; (4) 13-*cis*-β-carotene; (5) all-*trans*-β-carotene; (6) 9-*cis*-β-carotene.

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 (day, 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 ($AUC_{0-15 d}$) 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

$$(nmol \times d/\mu mol) = AUC_{0-15 d} \text{ of labelled} \qquad (5)$$

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/m^2. The age range of Chinese subjects is 41–57 (48·1 (SD 5·7)) 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/m^2. 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 ranges[21] (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 ^{2}H-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 ^{2}H-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$^+$ − H$_2$O) with an abundance of 12·6% (Fig. 2(a)). About 99% of zeaxanthin in the labelled spirulina was enriched with ^{2}H, and Gaussian distribution of isotopomers of zeaxanthin had the most abundant

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)

Subjects…	American			Chinese		
	Range	Mean	SD	Range	Mean	SD
Batch of spirulina (year)		2008			2006	
Dosage of zeaxanthin (mg)		2·63			3·68	
Baseline concentration of serum lutein (µmol/l)*	0·12–0·18	0·16	0·03	0·27–0·52	0·39	0·09
Baseline concentration of serum zeaxanthin (µmol/l)*	0·04–0·07	0·06	0·01	0·05–0·09	0·07	0·01
Concentration of serum zeaxanthin 1 d post-supplementation (µmol/l)	0·09–0·14	0·11	0·02	0·11–0·24	0·15	0·04
Highest value of labelled zeaxanthin as percentage of total zeaxanthin (%)	59–65	62	NS	43–56	49	NS
AUC of labelled zeaxanthin (nmol × d)†	1167–1542	1346	153	998–1851	1279	293
Average serum response (nmol × d/µmol)‡	254–335	293	33	154–285	197	45

AUC, area under the curve.
* 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.
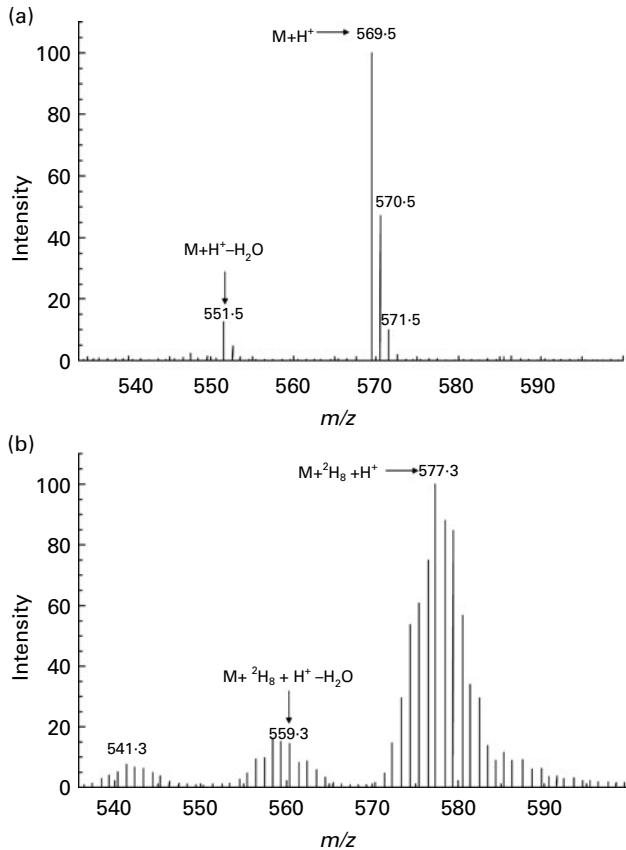
Fig. 2. Mass spectra of unlabelled and labelled zeaxanthin analysed by LC-atmospheric pressure chemical ionisation-MS. (a) Molecular mass of unlabelled zeaxanthin standard; (b) isotopomer distribution of zeaxanthin fraction from ^{2}H-labelled spirulina.

enrichment of 8 ^{2}H at m/z 577 (zeaxanthin $+ {}^2H_8 + H^+$; Fig. 2(b)). Similar isotopomer distribution of anhydrozeaxanthin ions (zeaxanthin $+ {}^2H_8 + H^+ - H_2O$, zeaxanthin $+ {}^2H_8 + H^+ - 2H_2O$) 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 isotopomer 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 $(M + {}^2H_8)$ and measurable isotopomers from $(M + {}^2H_1)$ through $(M + {}^2H_{14})$ 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

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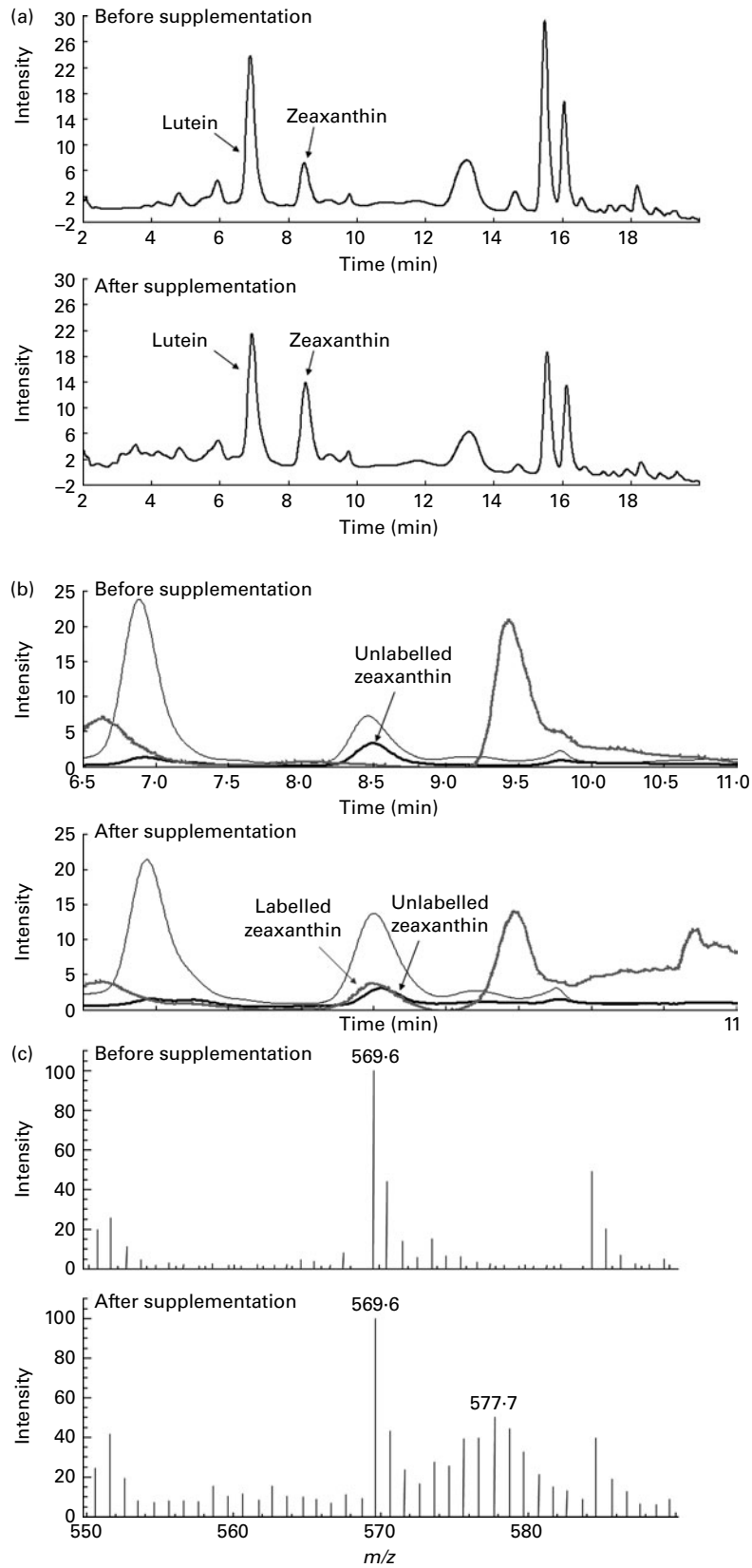
Fig. 3. LC-atmospheric pressure chemical ionisation-MS analysis of serum extracts (American subject 2) before and after supplementation with ^{2}H-labelled spirulina. (a) HPLC separation of the carotenoids in the serum. (b) Extracted ion chromatograms (EIC) of unlabelled (m/z 569–571, ▬▬) and labelled (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.
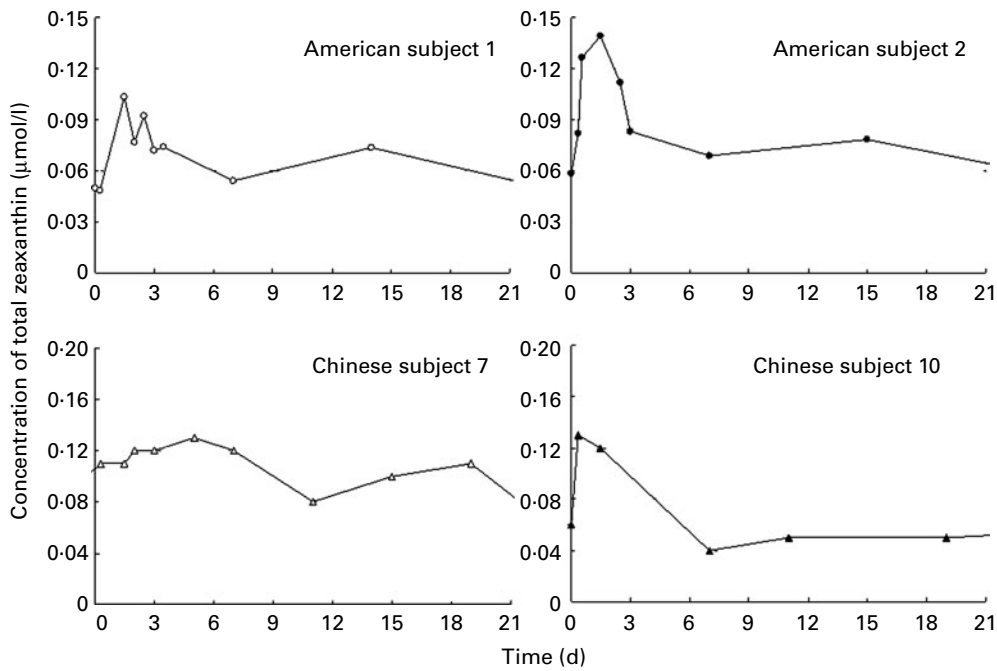
Fig. 4. Serum concentrations of total zeaxanthin (in μmol/l) after supplementation with spirulina over 21 d in representative subjects (American subjects 1, 2 and Chinese subjects 7, 10).

(Fig. 5), 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].
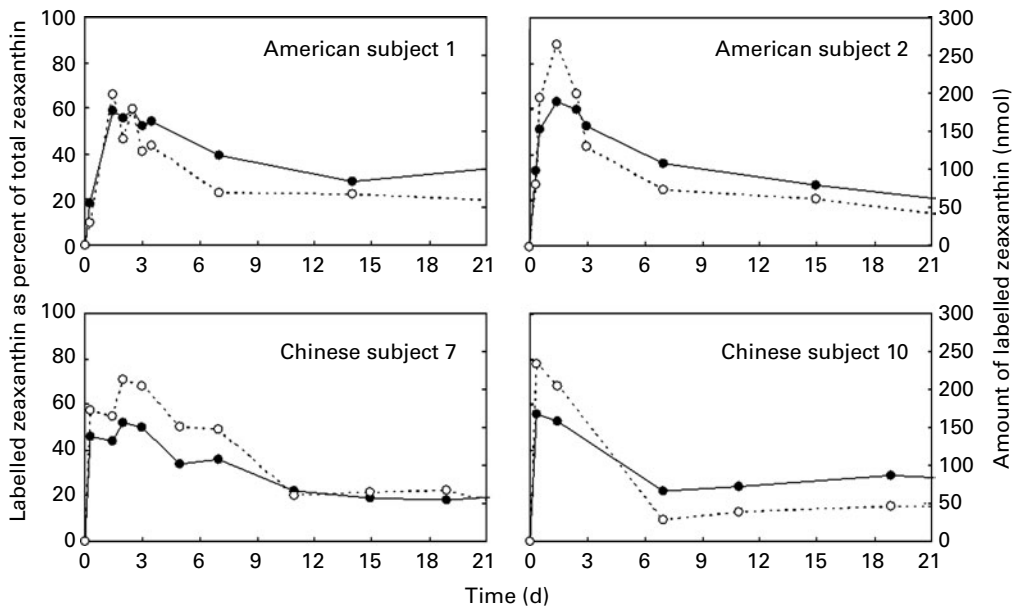
Fig. 5. Serum responses to labelled zeaxanthin after supplementation with spirulina in representative subjects (American subjects 1, 2 and Chinese subjects 7, 10). [2]H-labelled spirulina zeaxanthin as percentage of total zeaxanthin (—— and ●, left axis) and amount of labelled zeaxanthin (in nmol, ······ and ○, right axis) in the circulation over 21 d are presented.

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