

β -Carotene–vitamin A equivalence in Chinese adults assessed by an isotope dilution technique

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The present study was carried out to determine the conversion factor of synthetic ²H-labelled β -carotene to vitamin A in Chinese adults by using a stable-isotope dilution technique. Fifteen healthy volunteers aged 50–60 years were recruited for a 55 d experiment. The volunteers (nine males and six females) were each given a physiological dose of [²H₈] β -carotene (6 mg) in oil on the first day of the experiment, and a reference dose of [²H₈]retinyl acetate (3 mg) in oil was given on the fourth day. Serum samples were collected at 0, 3, 5, 7, 9, 11, and 13 h on the first and the fourth days of the study, daily for 10 d, and then weekly from days 14 to 56. β -Carotene and retinol were extracted from serum and isolated by HPLC, and their enrichments were respectively determined by using GC–electron capture negative chemical ionisation-MS and LC–atmospheric pressure chemical ionisation interface-MS. Four of the subjects exhibited β -carotene to vitamin A conversion factors of >29.0:1 on a molar basis and were termed ‘poor converters’. In the eleven normal converters (seven males and four females), the calculated conversion factors of β -carotene to retinol ranged from 2.0:1 to 12.2:1 with an average of 4.8 (SD 2.8):1 on a molar basis, and from 3.8:1 to 22.8:1 with an average of 9.1 (SD 5.3):1 on a weight basis. The 52 d post-intestinal absorption conversion was estimated to be about 30% of the total converted retinol.

β -Carotene: Vitamin A: Biological conversion efficiency: Isotope dilution technique

Vitamin A is needed for vision, growth, reproduction, cellular differentiation and proliferation, and for the integrity of the immune system. Vitamin A deficiency can have serious health consequences such as blindness (Solomons, 2001), and increased mortality from infection (World Health Organization, 1995). Conversely, excessive intakes of vitamin A can result in teratogenicity (Rothman *et al.* 1995), liver damage and increased bone loss (Solomons, 2001). Dietary β -carotene is the main provitamin A carotene in food and does not result in vitamin A toxicity even when ingested at high doses. Although β -carotene has been considered as a safe vitamin A source (Food and Agriculture Organization/World Health Organization, 1967; Olson, 1986), its conversion efficiency to vitamin A in man is uncertain.

The retinol equivalence of dietary β -carotene has been defined classically as 6 μ g β -carotene being equal to 1 μ g retinol (Food and Agriculture Organization/World Health Organization, 1967; National Research Council, 1989; Chinese Nutrition Society, 2000). In China, this 6:1 factor is still used (Chinese Nutrition Society, 2000),

although a factor of 12:1 has recently been promulgated in the USA and Canada (US Institute of Medicine, 2001). The conversion efficiency of β -carotene to vitamin A is influenced by many factors and varies widely with dose and different physiological situations (Ribaya-Mercado *et al.* 2000; Tang *et al.* 2000). The 6:1 factor of β -carotene to vitamin A was based on depletion–repletion studies (Goodman *et al.* 1966; Blomstrand & Werner, 1967; Sauberlich *et al.* 1974), and reflects the conversion factor of β -carotene to vitamin A in vitamin A-depleted subjects. This factor would therefore probably not be applicable to a relatively well-nourished population.

The conversion of β -carotene to vitamin A cannot be quantified in well-nourished human subjects by administering unlabelled β -carotene due to the inability to distinguish newly formed retinol from the body reserves, though the triacylglycerol-rich lipoprotein (TRL) model allows quantification with non-physiological doses. The conversion of β -carotene to vitamin A was therefore investigated in well-nourished subjects by administering isotopically labelled β -carotene ([²H₈] β -carotene) and [²H₈]retinyl

Abbreviations: APCI, atmospheric pressure chemical ionisation; AUC, area under the curve; ECNCI, electron capture negative chemical ionisation; HNRCA, Human Nutrition Research Center on Aging at Tufts University; RAc, retinyl acetate.

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acetate (RAc) as the vitamin A reference (Tang *et al.* 1997, 1998, 2000, 2003). By this method, the newly administered labelled β -carotene and its metabolite, retinol, together with the labelled reference dose of retinol can be traced clearly at physiological dose levels.

Materials, subjects and methods

Labelled compounds

[$^2\text{H}_8$] β -carotene (11, 11', 19, 19, 19, 19', 19', 19'- $^2\text{H}_8$ -labelled β -carotene, 82.0% in the all-*trans* form, 8.0% in the 13-*cis* form, 4.2% in the 9-*cis* form and 3.4% in the 15-*cis* form; BASF Products, Ludwigshafen, Germany), dissolved in maize oil and contained in gelatin capsules, was provided to the National Institute for Nutrition and Food Safety (Beijing, China) by the Jean Mayer US Department of Agriculture Human Nutrition Research Center on Aging at Tufts University (HNRCA), Boston, MA, USA. The crystalline [$^2\text{H}_8$] β -carotene dissolved in ethanol was checked by HPLC before it was dissolved in oil for experimental use, and the chemical purity spectroscopically of the labelled β -carotene was 90.6%. It also contained [$^2\text{H}_7$] β -carotene (15.7%), [$^2\text{H}_6$] β -carotene (0.3%), and [^1H] β -carotene (2.9%) as measured by atmospheric pressure chemical ionisation (APCI)-MS (Tang *et al.* 2003). The oral doses of [$^2\text{H}_8$] β -carotene were corrected by a purity factor of 90.6% in data processing.

[$^2\text{H}_8$]RAc (10, 14, 19, 19, 19, 20, 20, 20- $^2\text{H}_8$ -labelled RAc) (Cambridge Isotope Laboratory product, Andover, MA, USA) was also dissolved in maize oil and, contained in gelatin capsules, provided to the National Institute for Nutrition and Food Safety by the HNRCA. The purity of [$^2\text{H}_8$]RAc was > 98%, and that of the ^2H -labelled fraction was 99.95% (0.05% was unlabelled RAc) with 67.2% as [$^2\text{H}_8$]RAc, 14.1% as [$^2\text{H}_7$]RAc and 2.3% as [$^2\text{H}_6$]RAc. There was about 15% natural ^{13}C in the [$^2\text{H}_8$]RAc. No correction was made for the [$^2\text{H}_8$]RAc doses in the data analysis.

Subjects

Subjects were recruited from three rural villages of China's eastern Shandong Province, where the villagers had available plenty of cereal and other vegetable foods in their diets, but only limited animal foods. With the help of village doctors, experimental information and recruitment messages were advertised among the villagers. Forty villagers aged 50–60 years expressed their willingness to be volunteers and after informed consent accepted an extensive physical examination with blood screening. Fifteen volunteers (nine males and six females) were selected upon physical examination and enrolled to participate in the study. Informed written consent for all procedures was obtained from all volunteers under the guidelines established both by the ethical review committee of the Institute of Nutrition and Food Hygiene, Chinese Academy of Preventive Medicine and the institutional review board of Tufts University, New England Medical Center.

All subjects had the following characteristics: 50–60 years old; non-smokers and not having taken vitamin A

or β -carotene supplements or other nutrient supplements within the last month; having body weights within 20% of their standard weight for height ($\text{BMI} \leq 30 \text{ kg/m}^2$); absence of symptomatic cardiac disease or uncontrolled hypertension on physical examination; negative tests for *Helicobacter pylori* and Hepatitis B surface antigen; no parasitism or fat malabsorption (i.e. no parasite eggs on faecal examination and no oil globules found on microscopic stool inspection using Sudan III); no history of bleeding disorders; no history of gastric, intestinal, liver, pancreatic or renal disease; no portion of the stomach or the intestine surgically removed; no history of intestinal obstruction or malabsorption; no chronic alcoholism; no convulsive disorder; no abnormality in screening blood or urine samples. All subjects had a serum retinol concentration in the lower level of the normal range (0.7–1.4 $\mu\text{mol/l}$), with seven subjects having serum retinol concentrations lower than 1.0 $\mu\text{mol/l}$.

Study design

A metabolic research unit was established in the local town (Ji-ning) hospital. A dietitian gave dietary instructions to the subjects to avoid large amounts of carrots, dark green leafy vegetables and liver products as well as alcohol and smoking during 2 weeks of family (free-living) life before entering the experiment. After the 2-week preparation period, participants were housed in the metabolic research unit for a 10 d residential stay. After this they were once again free-living from the 11th to the 56th day of the study and followed the same dietary instructions. The dietitian followed up with all participants on a weekly basis to ensure dietary compliance.

On the morning of day 1, a fasting serum sample (10 ml) was drawn from each subject. Then at 0 h each subject ingested ten gelatin capsules containing a total of 6 mg [$^2\text{H}_8$] β -carotene (11011 nmol) in 6 g maize oil together with a semi-liquid, retinol- and β -carotene-free breakfast (soft noodles with soup, with 25% energy from fat). At 5 h after the breakfast, the subjects consumed the same semi-liquid diet as a lunch. In the evening (10 h after the breakfast) the volunteers received a supper of low β -carotene (about 0.1 mg) and vitamin A (about 0.05 mg) content with 30% energy as fat (steamed wheat-flour bread, fish and white Chinese cabbage cooked with peanut oil). On the fourth day of the study, the volunteers consumed a capsule containing 3.0 mg [$^2\text{H}_8$]RAc (8915 nmol) in 170 mg maize oil with the same semi-liquid diets as used before. At 5 h after the breakfast they consumed the same retinol- and β -carotene-free meal as a lunch, and 10 h after the breakfast they received the same supper of low β -carotene and vitamin A content as was used on day 1. For the first 9 d of the study, the volunteers were provided all meals of a low β -carotene and vitamin A content consisting of wheat flour, soyabean tofu, white fish, white chicken meat, and light-coloured vegetables. Serum samples of 10 ml were collected by venepuncture at 0, 3, 5, 7, 9, 11, and 13 h of the 1st and the 4th days of the experiment, and fasting (overnight) serum samples were also collected on the mornings of the 2nd, 3rd, 5th, 6th, 7th, 8th, 9th, 10th, 14th, 21st, 28th, 35th, 42nd, 49th, and 56th day.

The experimental scheme of dosing, meals and the timing of blood collections are shown in Fig. 1.

The blood samples were transferred to no-additive tubes covered with Al foil to prevent light damage and set at room temperature (25°C) for 0.5 h. The samples were then centrifuged at 800 g for 15 min at room temperature. Serum was separated, transferred to 2 ml vials and stored at -20°C until the end of the experiment and then transported on dry ice to Beijing and kept at -70°C there, and then to Boston (within 5 months; Craft *et al.* 1988). The samples were kept at -70°C in Boston until they were analysed (3 months). The serum samples were always kept either in the dark or under red light conditions during processing, transport, and analysis. The serum samples were analysed by the Carotenoids and Health Laboratory of the Jean Mayer US Department of Agriculture HNRCA (Boston, MA, USA).

Methods

Instrumentation. The HPLC system consisted of two Waters 510 pumps, a Waters 717 plus autosampler, a YMC C₃₀ column from Waters Inc. (Milford, MA, USA), or a C₁₈ column from Perkin-Elmer Inc. (Norwalk, CT, USA), a model 7950 HPLC column temperature controller (Johnes Chromatography Ltd, Hengoed Mid Glam, Wales, UK), a Waters 994 Programmable Photodiode Array Detector, and a Gilson FC 203 fraction collector (Middletown, WI, USA), supported by a Waters networking *Millennium* computer system. The detector was set at 340 nm for retinoids and at 450 nm for carotenoids (Tang *et al.* 1993; Yeum *et al.* 1996).

GC-electron capture negative chemical ionisation (ECNCI)-MS was carried out using an Agilent 5973N (Agilent, Folsom, CA, USA), and the GC column was a DB-1 (Agilent, Folsom, CA, USA). The temperature of the column oven and on-column injector was programmed from 50°C to 285°C at 15°C/min. The GC-MS interface temperature was set at 285°C. He gas was used as the carrier gas for GC. The analytes were detected by a quadrupole mass spectrometer, using 2.1×10^{-4} torr of methane

as the moderator gas for ECNCI. The ion source temperature was 150°C.

LC-APCI-MS was carried out using an Agilent 1100 LC (Andover, MA, USA), equipped with a C₁₈ Prizm column (Keystone Scientific, PA, USA), and a Bruker Esquire LC mass spectrometer (Billerica, MA, USA).

High-performance liquid chromatography analysis of serum samples. Saline (50 μ l; 0.85% NaCl), 100 μ l RAc-echinenon in ethanol (as internal standard substances), and 3 ml chloroform-methanol (2:1, v/v) were added to 100 μ l of serum sample. The mixture was vortexed and centrifuged for 10 min at 4°C and at 800 g. The chloroform layer was collected. Hexane (2 ml) was then added to the aqueous layer to re-extract fat-soluble nutrients. The hexane layer was combined with the chloroform layer and evaporated under N₂ on an N-EVAP (Organomation Associates, Inc., South Berlin, MA, USA). The residue was dissolved in 100 μ l ethanol using a 40 s vortex and 20 s sonication, and 50 μ l was injected onto an HPLC equipped with a YMC C₃₀ column to measure carotenoids and retinol concentrations (Tang *et al.* 1993; Yeum *et al.* 1996; Johnson *et al.* 2000).

The HPLC mobile phase was methanol-methyl-*tert*-butyl ether-water (83:15:2, by vol. (solvent A) or 8:90:2, by vol. (solvent B), with 1.5% ammonium acetate in the water), as described by Yeum *et al.* (1996). The concentrations of β -carotene and retinol were quantified, and, together with the percentage isotopic enrichment of retinol and β -carotene (determined by GC-MS and LC-MS), were used to calculate the molar enrichments of [²H₄]retinol, [²H₈]retinol, and [²H₈] β -carotene in the processed sera samples.

Gas chromatography-electron capture negative chemical ionisation-mass spectrometry analysis. First, a 200 μ l serum sample was extracted following the procedure mentioned earlier. The extract residue was re-suspended in 70 μ l acetonitrile-tetrahydrofuran (2:1, v/v), and 50 μ l of it was injected onto the HPLC equipped with a Perkin-Elmer C₁₈ column to separate the retinol fraction. The mobile phase was acetonitrile-tetrahydrofuran-water with 1.5% ammonium acetate in water (50:20:30, by vol. (solvent A) or 50:44:6, by vol. (solvent

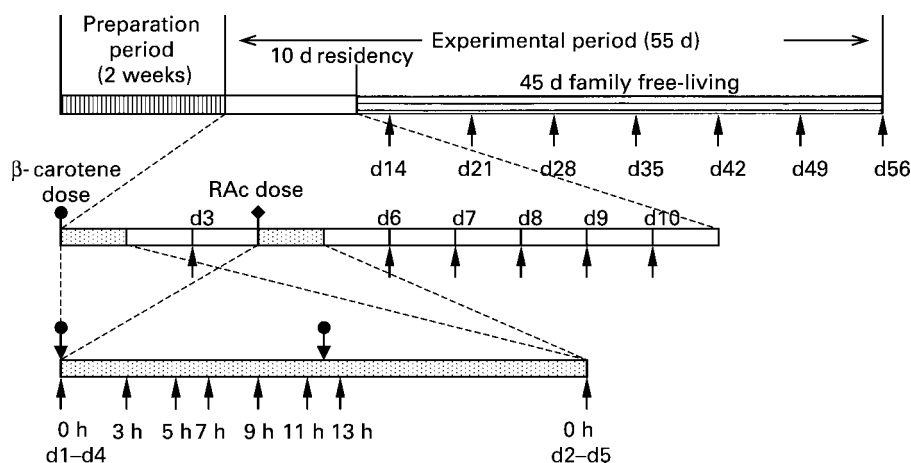


Fig. 1. The experimental scheme of dosing, meals and the timing of blood-sample collection. The experiment covered 55 d, beginning on day 1 (d1) and ending on day 56 (d56). (\uparrow), Blood-sample collection; (\downarrow), semi-liquid diet; (\bullet), β -carotene dose; (\blacklozenge), retinyl acetate (RAc) dose.

B)) as described by Tang *et al.* (2003). In this system, the retinol eluted at 10 min. The elution was collected from 9.0 to 11.0 min by a fraction collector, and dried under N₂. N, O-bis(trimethylsilyl) trifluoroacetamide (40 µl) with 10% trimethylchlorosilane (Pierce, Rockford, IL, USA) was added to the residue in the test tube. The tube was capped with a ground glass stopper and was heated at 70°C for 30 min. The reaction mixture of retinyl trimethylsilyl ether and the unreacted N, O-bis(trimethylsilyl) trifluoroacetamide and trimethylchlorosilane was transferred by a glass pipette to a brown vial with a conical-shaped glass insert and was analysed by GC–ECNCSI-MS to determine the enrichment of [²H₄]retinol and [²H₈]retinol (Tang *et al.* 1998, 2003).

The derivatisation reaction mixture (1 µl) was injected into the GC–ECNCSI-MS. The mass spectrometer was set to scan repetitively between 260 and 280 Da. The percentage enrichment of [²H₄]retinol derived from [²H₈]β-carotene was calculated by integrating the peak areas under the re-constructed mass chromatogram of the negative ions at m/z 271 (²H₃), 272 (²H₄ + ¹³C-²H₃) and 273 (¹³C-²H₄) divided by the total area response of the labelled and unlabelled retinol ions. The percentage enrichment of [²H₈]retinol derived from [²H₈]RAC was calculated by integrating the peak area under the re-constructed mass chromatogram of the negative ions at m/z 274 (²H₆), 275 (²H₇), 276 (²H₈), 277 (¹³C-²H₈), and 278 (¹³C₂-²H₈) divided by the total area response of the labelled and unlabelled retinol fragment ions. The percentage enrichment measured by GC–MS and the concentration of retinol in the serum was used to calculate the concentration of labelled retinol in the circulation (Tang *et al.* 2003).

Liquid chromatography–atmospheric pressure chemical ionisation–mass spectrometry analysis. A 2 ml serum sample was extracted with chloroform–methanol (2:1, v/v) as described earlier (Tang *et al.* 2003). The extract was purified through NH₂ cartridge columns using hexane as the eluent. The β-carotene–hexane eluent was evaporated under N₂. The residue was re-suspended in 70 µl methyl-*tert*-butyl ether–methanol (2:1, v/v), and 50 µl of it was injected onto an HPLC system equipped with a C₃₀ column. The HPLC mobile phase was methanol–methyl-*tert*-butyl ether–water, and a C₃₀ column was used (Tang *et al.* 2003).

The β-carotene fraction from the HPLC separation was collected and dried under N₂. The purified β-carotene fraction was evaporated under N₂, re-suspended in ethanol by vortexing and sonication, and injected into an LC–APCI-MS. The LC mobile phase was 95% methanol and 5% ethanol–methanol–tetrahydrofuron (75:20:5, by vol.). The mass spectrometer was set to scan the range of 535 to 550 Da in 0.1-Da steps with a scan speed of 5.25 scans/s. The actual enrichment of labelled β-carotene in serum was determined at m/z (M + H)⁺ of 537 (¹H), 538 (¹³C-¹H), 539 (¹³C-¹³C-¹H), 544 (²H₇), 545 (²H₈ + ¹³C-²H₇), 546 (¹³C-²H₈, ¹³C-¹³C-²H₇), and 547 (¹³C-¹³C-²H₈) with a Bruker Data Analysis Esquire-LC MS Processing, version 1.6m (Billerica, MA, USA).

Calculations of areas under the curves of labelled retinol or β-carotene in the serum. Total serum responses to the [²H₈]β-carotene dose and the [²H₈]RAC dose were

defined by multiplying the total serum volume (0.0435 of body weight; Diem, 1962) and the concentrations of [²H₄]retinol, [²H₈]retinol, and [²H₈]β-carotene in the circulation. The areas under the curves (AUC) in nmol × d were used to represent total serum responses to the [²H₈]β-carotene and the [²H₈]RAC doses, and calculated by using the curves of total serum responses *v.* time (nmol × d) (Tang *et al.* 2003). The integral calculus of the AUC was carried out using the Integral-Curve of Kaleidagraph (v3.51; Synergy Software, Reading, PA, USA).

Calculations of vitamin A equivalence. It was assumed that [²H₄]retinol formed from [²H₈]β-carotene followed the same serum kinetics as [²H₈]retinol formed from the quantitatively known [²H₈]RAC. [²H₄]retinol formed from the β-carotene dose was calculated by comparing the AUC of the serum [²H₄]retinol response and the AUC of the vitamin A reference dose (8915 nmol [²H₈]RAC). This calculated value yields the retinol equivalence of the β-carotene dose. The calculating formula is as follows (Tang *et al.* 2003):

$$\begin{aligned} & \text{[}^2\text{H}_4\text{]retinol formed from the } \beta\text{-carotene dose (nmol)} \\ &= \frac{\text{AUC of [}^2\text{H}_4\text{]retinol}}{\text{AUC of [}^2\text{H}_8\text{]retinol}} \times 8915. \end{aligned}$$

Calculations of conversion factor. The amount of a given oral dose of β-carotene was compared with the amount of vitamin A derived from the β-carotene dose in order to define the β-carotene to vitamin A conversion factor by using the following formula (Tang *et al.* 2003):

$$\begin{aligned} & \text{Molar conversion factor of } \beta\text{-carotene to retinol} \\ &= \frac{\text{[}^2\text{H}_8\text{]}\beta\text{-carotene dose (nmol)}}{\text{[}^2\text{H}_4\text{]retinol formed from the [}^2\text{H}_8\text{]}\beta\text{-carotene dose (nmol)}}. \end{aligned}$$

Because the ingested labelled β-carotene dose was of 90.6% chemical purity, the amount was corrected from 6 mg to 5.436 mg in calculating the conversion factor of β-carotene to vitamin A. Therefore, β-carotene dose (nmol) = 6 mg × 90.6%/544.9 (mg/mmol) × 10⁶ = 9976 nmol. There were *cis* isomers in the labelled β-carotene doses but they were not a major component of the dose. The contribution of the *cis* isomers of β-carotene to the conversion factor was not evaluated separately.

Calculations of the calculated area under the curve of [²H₄]retinol and post absorption conversion of β-carotene to retinol. The [²H₄]retinol formed from the [²H₈]β-carotene dose was assumed to follow the same serum kinetics as the [²H₈]retinol formed from the (quantitatively known) [²H₈]RAC dose on the assumption that all [²H₄]retinol from the [²H₈]β-carotene was produced in the intestine only. The period of 24 h was chosen as a cut-off time for the intestinal conversion of β-carotene to retinol. The [²H₄]retinol:[²H₈]retinol formed on day 1 value should be constant throughout the whole experiment if no further conversion occurs. Thus, a presumed AUC value over time can be calculated for [²H₄]retinol based on the AUC of

$[^2\text{H}_8]$ retinol as follows (Tang *et al.* 2003):

$$\begin{aligned} & \text{Calculated (presumed) AUC of } [^2\text{H}_4]\text{retinol at } n \text{ d} \\ &= (n \text{ d AUC of } [^2\text{H}_8]\text{retinol}/1 \text{ d AUC of } [^2\text{H}_8]\text{retinol}) \\ & \quad \times (1 \text{ d AUC of } [^2\text{H}_4]\text{retinol}), \end{aligned}$$

where n is 6, 13, 20, 27, 34, 41, and 52.

The difference of the actual measured AUC of $[^2\text{H}_4]$ -retinol and the calculated (presumed) AUC of $[^2\text{H}_4]$ retinol was deemed to come from the contribution of post-absorption (i.e. extra-intestinal) conversion of $[^2\text{H}_8]$ β -carotene to retinol. The following formula was used to calculate the percentage post-absorption conversion of β -carotene to retinol at different time points:

$$\begin{aligned} & \text{Percentage retinol formed due to post-absorption} \\ & \text{conversion} = 100 \times (n \text{ d AUC of } [^2\text{H}_4]\text{retinol} \\ & \quad - n \text{ d calculated AUC of } [^2\text{H}_4]\text{retinol})/n \\ & \quad \text{d AUC of } [^2\text{H}_4]\text{retinol}, \end{aligned}$$

where n is 6, 13, 20, 27, 34, 41 and 52.

Calculations of total liver vitamin A store. The total liver vitamin A store can be estimated when the oral administered dose of ^2H -labelled vitamin A becomes equilibrated with the body pool. The equilibration time is reported to be about 19 d (Furr *et al.* 1989). The modified Bausch–Rietz equation (Olson, 1986; Furr *et al.* 1989; Haskell *et al.* 1998; Tang *et al.* 2002) was used to estimate the body store of vitamin A using 18, 21, and 25 d serum $[^2\text{H}_4]$ retinol enrichment data.

Data processing and statistical analysis. The unpaired t test was used to compare differences in age, BMI, retinol, and carotenoids (β -carotene, α -carotene, lutein + zeaxanthin, cryptoxanthin, lycopene), as well as differences in the AUC of $[^2\text{H}_4]$ retinol, $[^2\text{H}_8]$ retinol, and $[^2\text{H}_8]$ β -carotene. It was used to compare equivalents and conversion factors between sexes and between normal converters and poor converters. In addition, ANOVA was used to determine differences among the AUC of $[^2\text{H}_4]$ retinol, $[^2\text{H}_8]$ retinol and $[^2\text{H}_8]$ β -carotene and the calculated AUC of $[^2\text{H}_4]$ -retinol, as well as conversion factors at different days. Systat v9.0 (SAS Institute Inc., Cary, NC, USA) was

used for statistical calculations. A difference was considered to be significant at $P \leq 0.05$.

Results

The characteristics of the subjects and their baseline serum concentrations of retinol and carotenoids are presented in Table 1. There were no significant differences between the sexes for age, BMI, serum retinol, β -carotene, α -carotene, lutein + zeaxanthin, cryptoxanthin, or lycopene.

The serum response and conversion factor at 52 d after the dose

Individual data of sex, age, BMI, and AUC of $[^2\text{H}_8]$ β -carotene, AUC of $[^2\text{H}_4]$ retinol from the $[^2\text{H}_8]$ β -carotene dose, AUC of $[^2\text{H}_8]$ retinol from the $[^2\text{H}_8]$ RAC dose, vitamin A equivalent of the $[^2\text{H}_8]$ β -carotene dose as well as the calculated conversion factors of β -carotene to retinol on a molar basis and by weight at 52 d for each subject are presented in Table 2.

All fifteen subjects responded well to the $[^2\text{H}_8]$ RAC reference dose. AUC of $[^2\text{H}_8]$ retinol formed from the $[^2\text{H}_8]$ RAC dose in all subjects ranged from 1994 to 7304 nmol \times d, as shown in Table 2. However, the responses to the $[^2\text{H}_8]$ β -carotene dose differed greatly with AUC of $[^2\text{H}_4]$ retinol ranging from 39 to 2545 nmol \times d. The responses of subjects no. 4, 11, 14 and 15 to the $[^2\text{H}_8]$ β -carotene dose were very low, as presented in Table 2. The poor responses of the four subjects to the $[^2\text{H}_8]$ β -carotene dose were first speculated to be due to interference at m/z 272 of the chromatograph in the GC–MS analysis. However, repeated GC–MS analysis on their serum samples showed a clear and weak $[^2\text{H}_4]$ retinol peak, suggesting that these four subjects responded much less robustly to an oral dose of $[^2\text{H}_8]$ β -carotene. Here, the four poor converters were termed as poor converters (see later) and separated from the other subjects in the data analysis. The statistical results for all subjects, the eleven normal converters, and the four poor converters are each presented in Table 2. The average conversion factors after a 6 mg β -carotene dose for all subjects (nine males and six females) were 15.6 (SD 19.8):1 (range of 2.0:1 to 58.1:1) on a molar basis and 29.3 (SD 37.2):1

Table 1. Subject characteristics and fasting serum concentrations of retinol and carotenoids at the beginning of the study (Mean values and standard deviations)

	Male (n 9)		Female (n 6)		Total (n 15)	
	Mean	SD	Mean	SD	Mean	SD
Age (years)	56.4	3.8	53.2	2.4	55.1	3.6
BMI (kg/m^2)	23.8	2.9	27.0	4.1	25.1	3.7
Retinol ($\mu\text{mol}/\text{l}$)	1.3	0.5	1.0	0.2	1.2	0.4
β -Carotene ($\mu\text{mol}/\text{l}$)	0.30	0.15	0.38	0.16	0.33	0.15
α -Carotene ($\mu\text{mol}/\text{l}$)	0.02	0.01	0.03	0.01	0.02	0.01
Lutein and zeaxanthin ($\mu\text{mol}/\text{l}$)	0.6	0.2	0.6	0.2	0.6	0.2
Cryptoxanthin (nmol/l)	68.5	37.0	120.1	63.4	89.1	53.9
Lycopene (nmol/l)	1.9	2.0	3.1	3.1	2.4	2.5

Table 2. Serum response areas under the curves (AUC) of [²H₈]β-carotene, [²H₄]retinol (from the [²H₈]β-carotene dose) and [²H₈]retinol (from the [²H₈]retinyl acetate (RAc) dose), and calculated retinol equivalents and conversion factors of [²H₈]β-carotene to vitamin A of fifteen subjects at 52 d after the doses

Subject no.	Sex	Age	BMI	AUC of [² H ₈]β-carotene at 52 d (nmol × d)	AUC of [² H ₄]retinol from [² H ₈]β-carotene dose at 52 d (nmol × d)	AUC of [² H ₈]retinol from [² H ₈]RAc dose at 52 d (nmol × d)	Vitamin A equivalents	Conversion factor	
								By mol	By weight
Normal converters (eleven subjects; seven males and four females)									
1	Male	50	24.0	538	653	4044	1441	6.9	13.0
2	Male	60	20.8	986	283	3080	819	12.2	22.9
3	Male	50	23.8	1301	1888	5119	3288	3.0	5.7
5	Male	60	24.9	2024	2545	4562	4974	2.0	3.8
6	Male	56	23.2	1233	806	3750	1917	5.2	9.8
7	Male	60	20.3	1498	714	3748	1698	5.9	11.0
8	Male	60	21.6	1186	621	2338	2369	4.2	7.9
9	Female	57	28.6	2877	1376	3282	3737	2.7	5.0
10	Female	53	28.8	1324	913	3626	2244	4.5	8.3
12	Female	51	23.7	1642	868	2772	2793	3.6	6.7
13	Female	55	29.9	2255	1460	3926	3315	3.0	5.7
Mean		55.6	24.7	1533	1102	3659	2599	4.8	9.1
SD		4.1	3.7	646	658	787	1180	2.8	5.3
Poor converters (four subjects; two males and two females)†									
4	Male	54	24.2	682	129	4208	273	36.6	68.7
11	Female	51	27.9	1174	77	2022	342	29.2	54.8
14	Female	52	20.6	526	39	1994	175	56.9	106.9
15	Male	53	30.0	259	141	7304	172	58.1	109.1
Mean		52.5	25.6	660*	97*	3882	240**	45.2**	84.9**
SD		1.3	4.2	384	47	2506	82	14.5	27.3
All subjects (fifteen subjects; nine males and six females)									
Mean		54.8	25.0	1300	834	3718	1970	15.6	29.3
SD		3.8	3.7	670	723	1341	1470	19.8	37.2

Mean value is significantly different to that for normal converters: * $P < 0.05$, ** $P < 0.01$ (independent samples *t* test).

† The subjects were termed as poor converters who exhibited conversion factors of β-carotene to retinol as $> 29.0:1$ by mol.

(range of 3.8:1 to 109.1:1) on a weight basis. However, as noted earlier, since the conversion factors did not fall into a normal distribution, subjects who exhibited conversion factors $> 29.0:1$ on a molar basis were designated as poor converters, and special attention was paid to them. In the four poor converters (two males and two females), the homologous conversion factors were 45.2 (SD 14.5):1 (range of 29.2: to 58.1:1) on a molar basis, and 84.9 (SD 27.3):1 (range of 54.8: to 109.1:1) on a weight basis. In the eleven normal converters (seven males and four females), the average conversion factors of β-carotene to retinol was 4.8 (SD 2.8):1 (range of 2.0:1 to 12.2:1) on a molar basis and 9.1 (SD 5.3):1 (range of 3.8:1 to 22.9:1) on a weight basis. The inclusion of the data from the four subjects who were poor converters made the standard deviation much greater. The serum response curves after the [²H₈]β-carotene dose and the [²H₈]RAc reference dose of one representative 'normal converter' subject are presented in Fig. 2 and one representative 'poor converter' subject in Fig. 3.

The mean serum responses of the 52 d AUC of [²H₈]β-carotene and the AUC of [²H₄]retinol formed from the [²H₈]β-carotene dose, and the AUC of [²H₈]retinol formed from the [²H₈]RAc dose showed no significant difference between males and females, either among all subjects or the eleven normal converters. Further, no significant correlation was found between the conversion factors and BMI, age, or baseline levels of retinol and carotenoids.

Comparison between the normal converters and the poor converters

From the comparison between the four poor converters and the eleven normal converters (Table 2), no significant difference was found in age, BMI, baseline retinol, baseline carotenoids, and none had intestinal parasites or fat malabsorption. Also, no significant differences were found in the baseline concentrations of serum retinol and carotenoids between the poor converters and the normal converters. The four poor converters exhibited a similar average serum response AUC of [²H₈]retinol from the [²H₈]RAc dose at 52 d (3882 (SD 2506) nmol × d) as compared with the eleven normal converters (3659 (SD 787) nmol × d). However, the average 52 d AUC of [²H₄]retinol from the [²H₈]β-carotene dose (97 (SD 47) nmol × d) and the average AUC of [²H₈]β-carotene (660 (SD 384) nmol × d) in the poor converters was significantly lower than that in the normal converters (1102 (SD 658) and 1533 (SD 646) nmol × d, respectively) (both $P < 0.05$). Therefore, the average conversion factor of β-carotene to retinol in the normal converters (4.8 (SD 2.8):1 on a molar basis) was as about ten times more efficient than in the poor converters (45.2 (SD 14.5):1 on a molar basis) ($P < 0.01$).

β-Carotene post-absorption conversion

The AUC of total [²H₈]retinol from the [²H₈]RAc dose and [²H₄]retinol from the [²H₈]β-carotene dose at 1, 6, 13, 20,

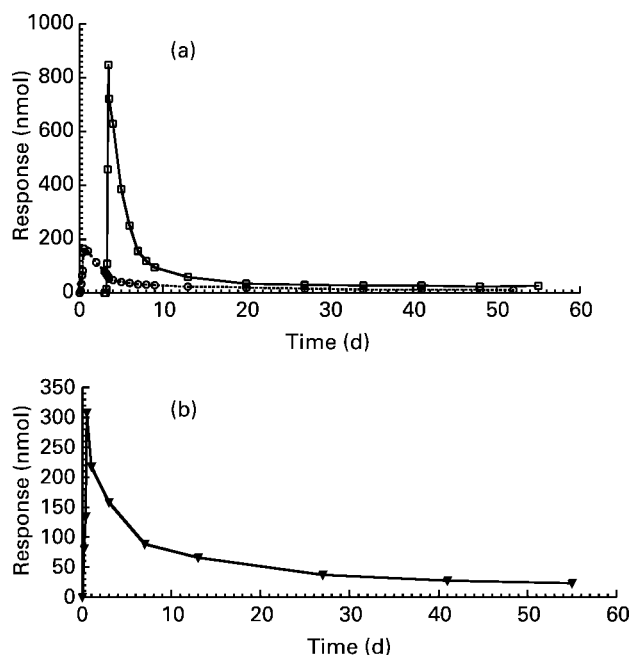


Fig. 2. Serum response curves of (a) [$^2\text{H}_4$]retinol ($-\circ-$), [$^2\text{H}_8$]retinol ($-\square-$), and (b) [$^2\text{H}_8$] β -carotene ($-\blacktriangledown-$), after dosing with 6 mg [$^2\text{H}_8$] β -carotene (day 0) and 3 mg [$^2\text{H}_8$]retinyl acetate (day 3) in one representative normal converter subject (subject no. 9).

27, 34, 41, and 52 d after the corresponding doses are presented in Table 3. All the AUC increased over time. But the increase of the AUC of [$^2\text{H}_4$]retinol over time was more than that of the AUC of [$^2\text{H}_8$]retinol over time. This result indicates that there was an additional post-intestinal retinol formation from the [$^2\text{H}_8$] β -carotene over time. This additional [$^2\text{H}_4$]retinol formed after intestinal conversion can be estimated by using the difference of

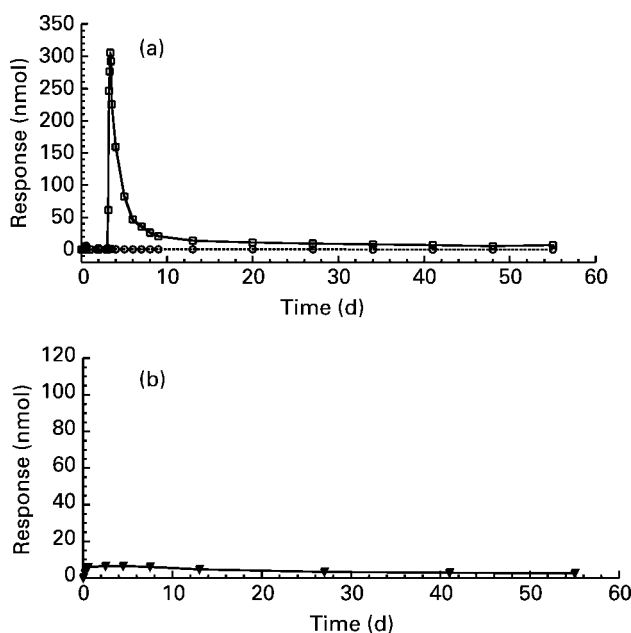


Fig. 3. Serum response curves of (a) [$^2\text{H}_4$]retinol ($-\circ-$), [$^2\text{H}_8$]retinol ($-\square-$), and (b) [$^2\text{H}_8$] β -carotene ($-\blacktriangledown-$), after dosing with 6 mg [$^2\text{H}_8$] β -carotene (day 0) and 3 mg [$^2\text{H}_8$]retinyl acetate (day 3) in one representative poor converter subject (subject no. 14).

the actual measured AUC of [$^2\text{H}_4$]retinol and the calculated AUC of [$^2\text{H}_8$]retinol. It is reasonable to use 1 d as a cut-off time to define the intestinal conversion of β -carotene (Tang *et al.* 2003). This additional [$^2\text{H}_4$]retinol accounted for 19.7, 22.7, 26.3, 27.8, 28.6, 29.5 and 30.1% of the total converted retinol at 6, 13, 20, 27, 34, 41, and 53 d after the β -carotene dose, respectively ($P < 0.05$).

The average total conversion factors at 1, 6, 13, 20, 27, 34, 41 and 53 d were 7.0 (SD 3.3), 5.6 (SD 2.7), 5.3 (SD 2.9), 5.1 (SD 2.8), 5.0 (SD 3.0), 4.9 (SD 2.9), 4.9 (SD 2.9) and 4.8 (SD 2.8):1 on a molar basis, respectively. Although these factors were not statistically different ($P > 0.05$) due to relatively large standard deviations, it is obvious that the trend toward better conversion with time was due to additional post-intestinal β -carotene conversion.

The total liver vitamin A store

The average values of the calculated total liver vitamin A stores (μmol) based on 18, 21, and 25 d [$^2\text{H}_8$]retinol enrichment data are: 212 (SD 92), 227 (SD 90), and 250 (SD 91), respectively for the eleven normal converters; 275 (SD 183), 296 (SD 195), and 326 (SD 211), respectively for the four poor converters; 229 (SD 119), 245 (SD 122), and 270 (SD 129), respectively for all the subjects. The differences among values of total liver vitamin A stores based on 18, 21, and 25 d [$^2\text{H}_8$]retinol enrichment data did not reach statistical significance for the normal converters *v.* the poor converters *v.* all subjects (one-way ANOVA, both $P > 0.05$). No difference was found between the total liver vitamin A stores in the normal converters and the poor converters at different time points (unpaired *t* test, both $P > 0.05$).

Discussion

Fifteen healthy volunteers aged 50–60 years were selected from rural villages of eastern China, and given each a high physiological dose (6 mg in oil) of [$^2\text{H}_8$] β -carotene and [$^2\text{H}_8$]RAc (3 mg in oil) as a vitamin A reference dose to evaluate the conversion efficiency of 6 mg β -carotene to vitamin A. The average intake of dietary carotenoids by the Chinese population is 2.9 mg/d based on the 1992 National Nutrition Survey (Institute of Nutrition and Food Hygiene, Chinese Academy of Preventive Medicine, 1996). The labelled β -carotene or vitamin A in oil capsules was ingested together with a designed diet consisting of local foods with 25% energy from fat. These farmer volunteers had plenty of cereal and other vegetable foods available, but very limited animal foods in their usual diets. They had a range of baseline serum retinol concentrations between 0.70 and 1.40 $\mu\text{mol/l}$ (200–400 $\mu\text{g/l}$) (seven of them had concentrations $< 1.0 \mu\text{mol/l}$, indicating that they were in marginal to low normal vitamin A status; Chinese Nutrition Society, 2000).

In the present experiment, the mean 52 d conversion factor of β -carotene to vitamin A at a dose level of 6 mg was 29.3 (SD 37.2):1 by weight. Because of the very poor conversion in four volunteers, the inclusion of these poor converters made the β -carotene conversion data asymmetrically distributed and the mean value was difficult to

Table 3. Serum response areas under the curves (AUC) of [²H₄]retinol and [²H₈]retinol at 1, 6, 13, 20, 27, 34, 41 and 52 d after the [²H₈]β-carotene and [²H₈]retinyl acetate (RAc) doses, and the percentage of post-absorption conversion and the conversion factors of β-carotene to retinol at different days for eleven normal converters (seven males and four females)

(Mean values and standard deviations)

Study day	AUC of [² H ₈]retinol from [² H ₈] RAc*** (nmol × day)		AUC of [² H ₄]retinol from [² H ₈] β-carotene *** (nmol × d)		Calculated AUC of [² H ₄]retinol****† (nmol × d)		Retinol derived from post-absorption conversion (%)***‡		β-carotene conversion factor ratio (on a molar basis)*	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	556	87	110	66	–	–	0		7.1	3.4
6	1850	332	483	327	378	248	19.7	17.8	5.6	2.7
13	2404	495	653	415	496	337	22.7	20.5	5.4	2.9
20	2730	579	777	484	564	391	26.3	21.2	5.1	2.8
27	2979	621	871	534	615	424	27.8	22.7	5.0	3.0
34	3202	664	945	572	661	457	28.6	22.7	5.0	2.9
41	3382	724	1012	613	700	491	29.5	22.8	4.9	2.9
52	3659	787	1102	658	758	533	30.1	22.1	4.8	2.8

Significance of differences between days by ANOVA: *NS, **P<0.05, ***P<0.001.

† Calculated AUC of [²H₄]retinol at n d = (n d AUC of [²H₈]retinol/1 d AUC of [²H₈]retinol) × (1 d AUC of [²H₄]retinol), where n is 6, 13, 20, 27, 34, 41 and 52.

‡ Percentage retinol of post-absorption conversion = (n d AUC of [²H₄]retinol – n d calculated AUC of [²H₄]retinol)/n d AUC of [²H₄]retinol, where n is 6, 13, 20, 27, 34, 41 and 52.

interpret. Thus, the four poor converters were separated from the other normal converters in the data analysis. The mean 52 d conversion factor of 6 mg β-carotene to vitamin A in the eleven normal converters was 9.1 (SD 5.3):1 by weight, with the highest conversion efficiency factor being 3.8:1 and the lowest conversion efficiency factor being 22.8:1 by weight, whereas the four poor converters had a mean 52 d conversion factor of 84.9 (SD 27.3):1 by weight. The results from the eleven normal converters are similar to those previously reported by Tang *et al.* (2003) in American adults using the same methodology. These authors reported a 53 d conversion factor after a 6 mg β-carotene dose in oil of 9.1:1 (by weight) with a variation from 2.4:1 to 20:1 in twenty-two volunteers aged 40–70 years (twelve females and ten age-matched males). However, there are some interesting differences between the American subjects studied by Tang *et al.* (2003) *v.* the Chinese subjects from the present experiment.

First, while the normal converters in the present study exhibited a similar efficiency of β-carotene conversion to vitamin A, as indicated by the average conversion factor of 9.1 as in the normal converter Chinese subjects, the

mean serum response AUC of [²H₄]retinol after the [²H₈]β-carotene dose in the Chinese normal converters (1102 (SD 658) nmol × d for 52 d) was almost double that seen in the American subjects (569 (SD 385) nmol × d for 53 d). Similarly, the mean serum response AUC of [²H₈]retinol after the [²H₈]RAc dose in the normal converter Chinese subjects was almost double that seen in the American subjects (3659 (SD 787) nmol × d for 52 d in the Chinese *v.* 1798 (SD 1139) nmol × d for 53 d in the Americans), as presented in Table 4. Even the poor converters had much greater AUC of [²H₈]retinol. The average serum ²H₈ response curves both for the Chinese and the American subjects, as illustrated in Fig. 4, show that the [²H₈]retinol enrichment of the Chinese subjects reached a peak at as high as 800 nmol, whereas that of the American subjects only reached a peak of one half that, about 400 nmol. Compared with the data from the American subjects, the doubled AUC of [²H₄]retinol and [²H₈]retinol in the Chinese normal converters produced a comparative conversion factor but slightly less vitamin A equivalents (2599 (SD 1180) *v.* 3414 (SD 2298) nmol, NS), as seen in Table 4. The reason for the doubled AUC of [²H₄]retinol and the doubled AUC of [²H₈]retinol in the Chinese subjects as compared

Table 4. Comparison of areas under the curves (AUC) of [²H₄]retinol from the [²H₈]β-carotene dose and [²H₈]retinol from the [²H₈]retinyl acetate (RAc) dose, as well as vitamin A equivalents and conversion factor of β-carotene to vitamin A between the eleven normal converters in the present study and the American volunteers in the Tang *et al.* (2003) study

(Mean values, standard deviations and ranges)

Data sources ...	Tang <i>et al.</i> (2003) (n 22, time = 53 d)			Present paper (n 15, time = 52 d)		
	Mean	SD	Range	Mean	SD	Range
AUC of [² H ₄]retinol from [² H ₈] β-carotene dose (nmol × d)*	569	385	144–1535	1102	658	283–2545
AUC of [² H ₈]retinol from [² H ₈] RAc dose (nmol × d)*	1798	1139	618–5123	3659	787	2338–5119
Vitamin A equivalents (nmol)	3414	2298	1021–8478	2599	1180	819–4974
Conversion factor:						
By weight	9.1	5.7	2.4–20.2	9.1	5.3	3.8–22.9
By mol	4.8	3.1	1.3–10.8	4.8	2.8	2.0–12.2

* Mean value is significantly different between the two populations (P<0.05).

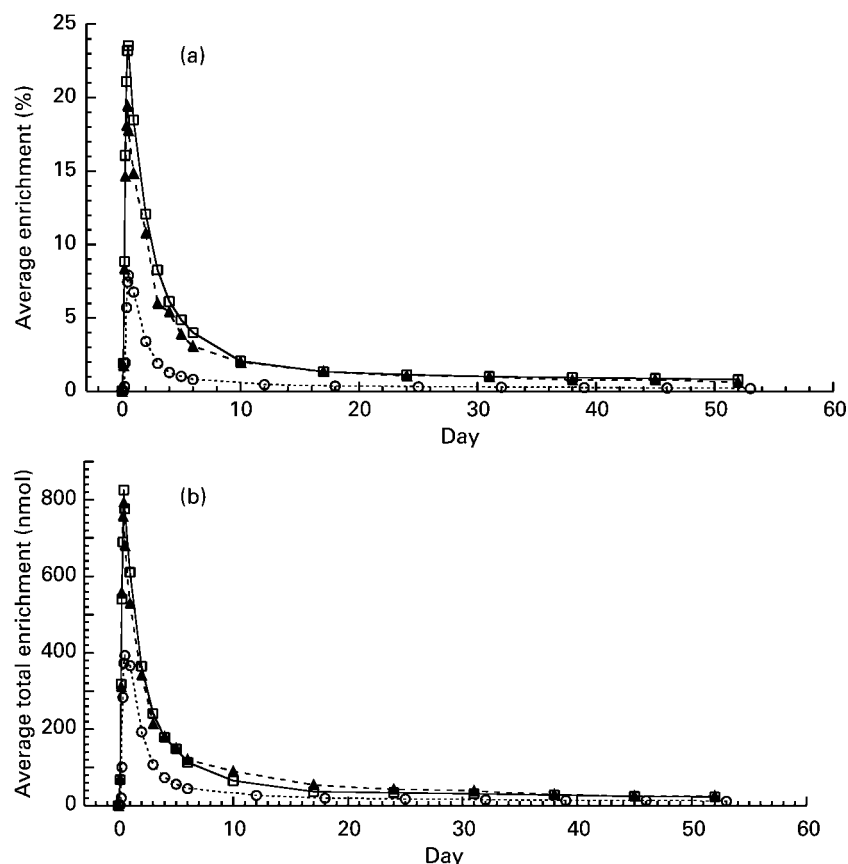


Fig. 4. Comparison of average serum $[^2\text{H}_8]$ retinol enrichment between the American volunteers (twenty-two subjects, twelve females and ten males; \circ -) from the Tang *et al.* (2003) study and the Chinese volunteers of the present study. Fifteen Chinese farmer volunteers (nine males and six females) were studied after a 6 mg dose of $[^2\text{H}_8]$ β -carotene and a $[^2\text{H}_8]$ retinyl acetate reference dose using the same design as in the Tang *et al.* (2003) experiment. Among the fifteen subjects, eleven (seven males and four females) responded well to the $[^2\text{H}_8]$ β -carotene dose (termed as normal converters; \square -) and four (two males and two females) responded poorly to the $[^2\text{H}_8]$ β -carotene dose (termed as poor converters; \blacktriangle -).

with the American subjects after the same $[^2\text{H}_8]$ β -carotene dose and $[^2\text{H}_8]$ RAc reference dose is uncertain, but it can be speculated that as the Chinese subjects had smaller vitamin A stores in the liver, the retinol converted from the $[^2\text{H}_8]$ β -carotene dose or absorbed from the RAc dose was less diluted in the body pool and stayed in the circulation longer than in the American subjects. Similar observations in experiments on rats with high *v.* marginal vitamin A status were reported by Green *et al.* (1987). That is, the curves of plasma retinol kinetics in rats of marginal vitamin A status showed a higher AUC than that in rats of high vitamin A status. Thus, it is possible that the higher serum AUC responses are mainly due to the difference of body stores of vitamin A between the two populations.

Although it is believed, at present, that 50–70% of an oral dose of vitamin A will be absorbed, variability in the absorption of an oral vitamin A dose is of concern when the conversion efficiency of β -carotene to vitamin A is being evaluated using an isotope dilution technique with retinol as the reference dose. This is because the derived conversion factors are relative to the ingested reference vitamin A dose response. As mentioned earlier, the higher AUC response of the Chinese subjects are mainly due to the lower body stores of vitamin A and, thus, might not imply the better absorption of vitamin A

by the Chinese population. However, it is not clear if the absorption of vitamin A is different in the Chinese population from that in the US population.

The results of conversion factors in the present study are different from the conversion factors previously determined using a depletion–repletion study design to determine the β -carotene equivalence (conversion factor) to vitamin A. The reported β -carotene equivalence at the daily dose of 1200–1500 μg β -carotene from the depletion–repletion method usually ranges from 2.0:1 to 3.8:1 on a weight basis (Hume & Krebs, 1949; Sauberlich *et al.* 1974). The major differences between the earlier studies and the present study are (1) the vitamin A status of the subjects being studied and (2) the amount of β -carotene dose. The higher conversion efficiencies of β -carotene to vitamin A in earlier studies were obtained from experiments on vitamin A-depleted subjects *v.* the lower conversion efficiency seen in the better-nourished subjects in the present study. It was reported that the activity of intestinal β -carotene cleavage enzyme in vitamin A-sufficient rats is 50% of that in vitamin A-deficient rats (Villard & Bates, 1986). Further, the doses of β -carotene used in these early studies (1.2–1.5 mg/d) were lower than the present study dose (6 mg) and the conversion of β -carotene to vitamin A might be more efficient using a 1 mg dose than a 6 mg

dose of β -carotene. However, based on the present experiment and that of Tang *et al.* (2003), the conversion factor of 6 mg β -carotene to vitamin A appears very similar in both Chinese and American study populations.

The intestine is the most important organ for the conversion of β -carotene to vitamin A, but other organs and tissues such as liver, fat, lung, and kidney are also capable of converting β -carotene to retinoids (Wang *et al.* 1991). In the present paper 24 h was used as the cut-off time to distinguish the intestinal conversion of β -carotene to retinol from the post-intestinal conversion of β -carotene to retinol (Tang *et al.* 2003). It was found that the post-intestinal conversion of vitamin A accounted for 19.7 to 30.1 % of the total converted retinol over the period of 6 to 52 d, respectively. The present results show that almost one-third of the total vitamin A formed from 6 mg oral β -carotene was due to extra-intestinal (post-absorptive) cleavage in the Chinese adults. The present results differ from those from the study of Tang *et al.* (2003), in which it was reported that the average of 19 % of total vitamin A formed from a β -carotene dose was from extra-intestinal cleavage in a well-nourished American population. It is possible that the percentage of post-intestinal conversion of β -carotene to retinol may also be related to a subject's vitamin A status.

The four poor converters in the present study showed strikingly poor responses to the labelled β -carotene dose. This indicates that the absorption and conversion of β -carotene is impaired in some human subjects, in whom dietary retinol can be absorbed normally. Lin *et al.* (2000), from the University of California, Davis, reported five poor responders in their experiment on eleven American women aged 19–39 years, in which they used crystalline β -carotene ($> 15 \mu\text{mol}$) and vitamin A in gelatin capsules (not mixed in oil). However their poor responders also had much low absorption of vitamin A doses. The subjects in the present experiment deserve further study to explore the possible reasons (for example, genetic) for their low response to the labelled β -carotene and poor conversion of β -carotene to vitamin A.

In summary, the conversion of β -carotene to vitamin A in Chinese adults has been quantitatively determined by using a stable-isotope reference method. An average conversion factor of 9.1 μg β -carotene equivalent to 1 μg retinol was found in eleven Chinese adults aged 50–60 years who were judged to be normal (or good) converters. The 52 d post-intestinal absorption conversion was estimated to account for 30 % of the total converted retinol. Four volunteers were found to be poor converters, who responded very poorly to the labelled β -carotene dose but quite well to the labelled vitamin A dose and exhibited β -carotene conversion efficiency to retinol as $> 29:1$ on a molar basis. Further investigations on possible genetic characteristics on β -carotene conversion to vitamin A in this population may provide a better understanding on the vitamin A value of β -carotene in man.

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