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Low and high responders to pharmacological doses of β-carotene: proportion in the population, mechanisms involved and consequences on β-carotene metabolism

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Abstract The aim of this study was to assess the interindividual variability of chylomicron β-carotene response to a pharmacological load of β-carotene in the population, to identify the mechanisms responsible for this variability, and to evaluate its consequences on β-carotene status and metabolism. The variability, as estimated by the 3-h chylomicron β-carotene response to 120 mg β-carotene in 79 healthy male volunteers, was high (CV = 61%), but it was unimodal and all the subjects had detectable chylomicron β-carotene. In 16 subjects randomly selected among the 79, the interindividual variability of the triglyceride-adjusted chylomicron (β-carotene + retinyl palmitate) response (0–12.5 h area under the curve) was high (CV = 54%), suggesting that there is a high interindividual variability in the efficiency of intestinal absorption of β-carotene. The chylomicron β-carotene response was correlated (r = 0.50, P < 0.05) with the chylomicron triglyceride response. The β-carotene status, as assessed by β-carotene concentration in buccal mucosal cells, was correlated (r = 0.73, P < 0.05) with the triglyceride-adjusted chylomicron β-carotene response, i.e., with the ability to respond to β-carotene. The triglyceride-adjusted chylomicron retinyl-palmitate response was correlated (r = 0.55, P < 0.05) with the triglyceride-adjusted chylomicron β-carotene response. Plasma all-*trans* retinoic acid slightly, but significantly, increased (+40%) 3 h after the β-carotene load, but this increase was not related to the triglyceride-adjusted β-carotene response. In conclusion, the ability to respond to β-carotene is highly variable, but there is probably a very small proportion of true non-responders to pharmacological doses of β-carotene in the healthy population. This variability is apparently mainly due to interindividual differences in the efficiency of intestinal absorption of β-carotene and in chylomicron metabolism. The ability to respond to β-carotene can affect the β-carotene status and the provitamin A activity of β-carotene, but it has apparently no effect on the amount of retinoic acid appearing in the plasma after the ingestion of a pharmacological dose of β-carotene.—Borel, P., P. Grolier, N. Mekki, Y. Boirie, Y. Rochette, B. Le Roy, M. C. Alexandre-Gouabau, D. Lairon, and V. Azais-Braesco. **Low and high responders to pharmacological doses of β-carotene: proportion in the population, mechanisms involved, and consequences on β-carotene metabolism. *J. Lipid Res.* 1998.** 39: **2250–2260.**

Supplementary key words provitamin A activity • retinoic acid • chylomicron • retinyl-palmitate • β-carotene status • erythropoeitic protoporphyria

β-Carotene (β-C) is one of the most abundant carotenoids found in the human diet. Interest in its metabolism originates because it accounts for a significant part of human vitamin A intake through its provitamin A activity. This interest has recently been reinforced for two reasons. First, a better knowledge of β-C metabolism is required to explain the contradictory results about the protective effects of β-C towards lung cancer and cardiovascular diseases, obtained in epidemiological studies (1–3) and in supplementation trials (4–6). Second, data on the nutritional and physiological factors that affect its provitamin A activity can help to explain the discrepancy between recent results which suggest that dark-green leafy vegetables are not efficient in improving vitamin A status (7), and earlier results which suggest that plant sources (8) or purified (9) β-C improve vitamin A status.

Studies in animal models and in humans have greatly improved our knowledge of how β-C is absorbed, metabolized, and transported to tissues (10–14). In summary, β-C is absorbed by the enterocyte via a passive diffusion mechanism (15). In the enterocyte, a fraction of β-C is converted into retinyl esters (16). Uncleaved β-C and retinyl esters are then incorporated in the chylomicrons and transported to the liver. β-C is then either stored in the liver or transported to extrahepatic tissues by very low density lipoproteins (VLDL).

Abbreviations: AUC, area under the curve; β-C, β-carotene; CM, chylomicron; RP, retinyl palmitate; TG, triacylglycerols.

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Although the general process of the intestinal absorption and plasma transport of β-C in humans is relatively well understood, several points remain unclear. Among these, one appears very important for human health. It concerns the high interindividual variability of the plasma β-C response to a single oral dose of β-C (17–22) and the fact that some studies have identified subjects who were apparently nonresponders to β-C (23, 24). Depending on the study design, a nonresponder to β-C is defined as either a subject who has no significant increase of β-C concentration in the blood after the ingestion of a single high dose of β-C, or a subject who has no significant increase in blood β-C concentration after ingestion of a β-C-rich diet for several weeks. This low, or lack of, response may originate from an impaired uptake of β-C from the intestinal mucosa, from an extensive conversion of β-C into vitamin A, from an inefficient incorporation of β-C into chylomicrons, or from variability in lipoprotein metabolism. At present, there are insufficient data to allow the relative contributions of these factors to this phenomenon to be estimated.

Consistent low or high β-C response could have important consequences for human health. Indeed, it can be speculated that nonresponders would not benefit from the suspected antioxidant properties of β-C and, thus, could be more susceptible than responders to diseases in which free radicals are involved. Conversely, hyperresponders may cleave β-C inefficiently and thus may use β-C poorly as a source of vitamin A. Finally, if the reason for the lack of response is a very efficient cleavage of β-C into vitamin A, it can be hypothesized that these subjects might have an abnormally high increase in plasma retinoic acid concentration after the ingestion of pharmacological doses of β-C.

The aim of this study was to evaluate the interindividual variability of β-C response to a β-C load in the healthy population, to identify the mechanism(s) involved, and to estimate the consequences of a high or a low response on β-C status and on β-C provitamin A activity. The study was divided into two parts. In the first experiment, we measured the chylomicron β-C, retinyl palmitate (RP), and triacylglycerol (TG) responses 3 h after the intake of a β-C-rich fatty meal, in a large group of subjects (79 healthy male volunteers) to evaluate the interindividual variability of β-C response in the healthy population. In the second experiment, we measured, in 16 subjects randomly selected among the 79, the whole postprandial chylomicron β-C, RP, and TG response to a β-C-rich fatty meal, in order to identify the mechanisms responsible for the interindividual variability of chylomicron β-C response and to evaluate the physiological implications for a high or low responder to β-C in terms of β-C status and of β-C metabolism.

SUBJECTS AND METHODS

Subjects

Seventy-nine young male volunteers were recruited for this study. They were healthy, as was verified by clinical examination and disease history, and had no symptoms of fat malabsorption. Informed written consent was obtained from all the volunteers and the study was approved by the local medical ethical committee of the regional university hospital complex of Clermont-Ferrand (France). Forty-six subjects did not smoke and the other 33 smoked fewer than 20 cigarettes/d (mean: 4.5 cigarettes/d). The subjects had not taken vitamin supplements for at least 3 months before the experiments. Their lean and fat masses were estimated by bioelectric impedance measurements by means of a BIA 101A instrument (RJL systems, Mt. Clemens, MI). Their physical characteristics are listed in **Table 1**.

Usual diets of the subjects

The usual diets of the subjects were monitored, between the 1 June and 25 July, through a 3-day food recall which was completed before the experiment. This diary was analyzed for nutrient composition by using a diet analyzer software (GENI, Micro 6, Nancy, France). The database of the software, derived from the Regal table (25), was complemented for carotenoids with a carotenoid food-composition database (26). This database was kindly provided by Gary R. Beecher from the Beltsville Agricultural Research Center (Beltsville, MD). Key data from the diet analysis are given in Table 1.

β-Carotene status of the subjects

The β-C status was evaluated by measuring β-C in plasma and in buccal mucosal cells. Plasma β-C was measured in fasting subjects who had been asked not to modify their dietary habits during the week preceding the experiment.

Buccal mucosal cells were collected by buccal mucosal scraping (27, 28) as follows. After having rinsed their mouths with drinking water, the subjects brushed the inside of their cheeks with a wet soft toothbrush, 10 times on each side. Then, they rinsed their cheeks twice with 25 mL 0.9% NaCl. Mucosal cells were spun down at 1400 *g* for 10 min. The supernatant was discarded and the cell pellet was washed with 15 mL cold phosphate-buffered saline (PBS). The tubes were then flushed with nitrogen and stored at −80°C until analysis (29). For the analysis, the cells were resuspended in 1.2 mL cold PBS. The cell suspensions were sonicated for two periods of 1.5 min each. Two hundred microliters of the mixture was used for protein measurement (30) and 1 mL for β-C measurement (as described below). Results were expressed as nmol β-C/g protein.

TABLE 1. Physical characteristics and dietary habits of the 79 subjects

Variable	Mean	CV (%)
Age (y)	23.6 ± 0.5	16.3
Body mass index (kg/m^2)	22.3 ± 0.3	10.8
Lean (kg)	60.9 ± 0.8	9.6
Fat (kg)	10.8 ± 0.7	48.4
Dietary habits		
Energy (kJ/d)	11952 ± 344	23.9
Protein (g/d)	112 ± 4	32.8
Fat (g/d)	116 ± 3	24.3
Carbohydrates (g/d)	323 ± 11	29.2
β-carotene (mg/d)	2.16 ± 0.41	157.3

Values are means ± SEM. Lean and fat masses of the subjects were estimated by bioelectric impedance measurements. Dietary habits were estimated with a 3-day food recall which was completed in the month before the experiment. The diary was analyzed for nutrient composition by using a diet analyzer software. The original database of the software (25) was complemented for carotenoids with a carotenoid food-composition database (26).

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Experimental design

The study was divided into two parts. In the first experiment, called "assessment of the variability of chylomicron β-C response in the population", we chose to study about 80 subjects so that we could detect, at a risk $\alpha = 5\%$, 30% of nonresponders with a confidence interval of 10%. The 79 subjects ingested a β-C-rich meal and the concentration of β-C, RP, and TG was measured in the chylomicrons (CM) collected 3 h after meal intake. This time-point was chosen because previous studies (31–33) have shown that the CM concentration reaches its maximum about 3 h after intake of meals containing similar amounts of fat. In the second experiment, called "mechanisms involved and consequences on β-C metabolism," 16 subjects were randomly selected among the 79. We did not find any significant difference between this subgroup and the initial group regarding the physical parameters measured as well as the dietary and smoking habits. The 16 subjects ingested two successive meals. The first meal contained β-C, while the second meal, given 6 h later, did not. We provided a second meal containing long-chain TGs, but no β-carotene, 6 h after the first meal intake, in order to permit the secretion into the lymph of β-carotene that could have been absorbed in the enterocyte but not secreted into the lymph during the first postprandial period. This was done because we have recently found that, after a meal containing a pharmacological dose of β-carotene, a significant fraction of β-carotene is recovered in the CM secreted during the postprandial period that follows the next meal (34). Blood samples were collected after a 12-h fasting period and every hour for 6 h after each meal intake. Retinoic acid was measured in blood samples collected after a 12-h fasting period and 3 h after the first meal intake. TG, β-C, and RP were measured in CM prepared from all blood samples collected.

The meals were composed of: bread (40 g), wheat semolina (60 g cooked and hydrated with 120 mL water), cooked egg whites (35 g), an oil-in-water emulsion (about 100 mL), a nonfat yogurt (125 g), and 300 mL water. The meals contained less than 1 μg vitamin A and carotenoids as estimated by the diet analyzer software. The emulsion, prepared by Inocosm (Chatenay-Malabry, France), contained 40 g sunflower oil, 1 g emulsifiers (egg lecithins and plant ceramides), and 60 mL water. In the case of the β-C-rich meals, it also contained 120 mg (23) all-*trans* β-C in the form of a β-C 30% oil suspension (Hoffmann-La Roche, Basel, Switzerland) which had been mixed with the sunflower oil before preparing the emulsions. The total amount of the β-C and TG required for all the subjects was blended at the same time so that the mixture given to all the volunteers had exactly the same β-C content. The homogeneity of the β-C dispersion in the oil was verified by HPLC measurements of β-C concentration of several samples of the emulsion. The coefficient of variation for 8 measurements was less than 4%.

The meals were ingested within 20 min. The time 0 of each experiment was set at the middle of the first meal.

Chylomicron preparation

Blood was collected in EDTA Vacutainers and plasma was prepared immediately by centrifugation (4°C, 10 min, 910 *g*).

The $S_f > 1000$ fraction containing CM plus large CM-remnants was isolated on the day of experiments from 2 mL plasma layered under 3 mL 0.9% NaCl, by ultracentrifugation at 10°C (24,000 *g* for 1 h) in a Beckman (Palo Alto, CA) 40.3 rotor (31–35). CM were stored at −80°C until analysis (29).

Analytical determinations

Triacylglycerols (TG), cholesterol, and phospholipids were determined by enzymatic procedures (36–38) with commercial kits (Boehringer, Mannheim, Germany). Apolipoprotein A-I and apolipoprotein B were assayed by immunoturbidimetry (Behring Werke A. G., Marburg, Germany). LpA-I, Lp[a], and apolipoprotein C-III were assayed by electroimmunodiffusion (Sebia, Issy-les-Moulineaux, France).

Plasma and buccal mucosal cell β-C was extracted twice with ethanol and hexane and quantified by reverse-phase HPLC on a Kontron (Zurich, Switzerland) apparatus with detection by light absorbance at 450 nm. The column was a Zorbax ODS (250 × 40 mm, 5 μm) purchased from Interchim (Montluçon, France) and the mobile phase was a mixture of acetonitrile–dichloromethane–methanol 70:20:10 (v/v/v). Echinenone and all-*trans* β-C (Hoffmann-La Roche, Basel, Switzerland) were used as internal and external standards, respectively. Quantifications were carried out by use of Kontron MT2 software. Overall recovery yields ranged from 65 to 100%. The limit of detection was around 1 ng.

Retinyl palmitate (RP) was extracted as described for β-C. It was quantified by reverse-phase HPLC with detection at 325 nm. The column was a C18-nucleosil (250 × 4.6 mm, 5 μm) purchased from Touzart et Matignon (Paris, France), and the mobile phase was 100% methanol. RP (Hoffmann-La Roche) was used as external standard. Retinyl laurate, which had been synthesized according to Azaïs-Braesco et al. (39), was used as internal standard. Quantifications were carried out by use of Kontron MT2 software. Overall recovery yields ranged from 75 to 100%. The limit of detection was around 5 ng.

Retinoic acid was analyzed as follows. Two mL of plasma was adsorbed on a C18-packed column (Sep-pak plus, Waters, St Quentin-en-Yvelines, France). The very polar compounds adsorbed on the column were eliminated first by 3 mL 2% (w/v) ammonium acetate. Then, retinoic acid was removed from the column by using 3 mL methanol. This methanol solution was evaporated to dryness under a stream of nitrogen and the retinoic acid was subsequently re-dissolved in a known small volume of methanol. This retinoic acid-methanol solution was injected onto a 600-717-996 Waters HPLC system, run by the Millenium™ software. The reverse-phase column was a C18 Puresil (Waters, 150 × 4.6 mm, 5 μm) and the compounds were eluted by a mixture of methanol–ammonium acetate 80:20 (v/v). (13-*cis*)-Retinoic acid and all-*trans* retinoic acid were eluted at 6.3 min and 7.9 min, respectively. Identification of compounds was based both on the retention time and online UV absorption spectra, compared to those of authentic standards (Sigma Chimie, La Verpillière, France). Quantification was carried out by external calibrations using these standards, then corrected for the extraction yield as determined by the recovery of [11,12-$^3H_2$]all-*trans* retinoic acid (NEN, Paris, France, specific activity 51 Ci/mmol) used as an internal standard. Quantifications were carried out by use of Millenium™ software. Overall recovery yields ranged from 60 to 86%. The limit of detection was around 5 ng. All mobile phase solvents were from Carlo Erba Reactifs (Nanterre, France).

Calculations and statistical analysis

Results are expressed as means ± SEM. Areas under the curves (AUC) were calculated by the trapezoidal rule. Correlation coefficients and their probability levels were obtained from linear regression analyses. When a variable was related to more than one factor, a multiple regression model was elaborated to estimate the respective contribution of these factors. The statistical significance ($P < 0.05$) of the differences found between the postprandial values and the fasting values was assessed by one-way analysis of variance (ANOVA) for paired values with time as the factor (31–33, 40). When a significant (<0.05) *P* value was obtained, differences between means were assessed by the Fisher's PLSD (protected least significant difference) test. The statistical significance ($P < 0.05$) of the differences found between the fasting plasma retinoic acid values and the corresponding postprandial values was assessed by the Student's *t* test for paired values.

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These statistical comparisons were performed with the StatView SE+Graphics software version 1.03 (Abacus, Berkeley, CA).

RESULTS

Physical characteristics and dietary habits of the 79 subjects

As shown in Table 1, the subjects had a typical Western diet with a moderate energy consumption. Protein, carbohydrate, fat, and alcohol accounted for 15.8%, 45.2%, 36.8%, and 2.2% of energy, respectively. The amount of carotenoids ingested per day was about 5 mg, and β-C was the main carotenoid ingested (2.2 mg), followed by lycopene (1.6 mg), xanthophylls (lutein + zeaxanthin) (0.8 mg), and α-carotene (0.4 mg). The interindividual variability of β-C intake was very high with a CV of 157% (**Table 2**).

β-Carotene status of the 79 subjects

Figure 1 and Table 2 show that regardless of the marker of β-C status used, i.e., fasting plasma β-C (Fig. 1A) or buccal mucosal cell β-C (Fig. 1B), the β-C status was highly variable, with CV of 76% and 257% for β-C of plasma and buccal mucosal cells, respectively. Note that the distribution of β-C in the plasma was unimodal whilst it was more heterogeneous in the buccal mucosa cells. There was no significant relationship between plasma and buccal mucosa cell β-C. There was no significant relationship between smoking habits and plasma or mucosal cell β-C.

First experiment: Assessment of the variability of chylomicrons β-carotene response in the population

The frequency distribution of the CM β-C concentration measured 3 h after the β-C load in the 79 subjects is shown in **Fig. 2A**. The concentration of β-C recovered in the CM fraction was highly variable in the population. More precisely, the difference between the subject who had the lowest concentration (17.7 nmol/L) and the subject who had the highest concentration (302.7 nmol/L) was about 17-fold and the CV was 61% (Table 2). However, note that β-C was detected in the CM fraction of all subjects. Figure 2B and Table 2 show that the distribution of the concentration of TG in the CM fraction had a variability similar to that of β-C, with a ratio of about 14 between the subject who had the lowest concentration (102 μmol/L) and the subject who had the highest (1412 μmol/L), and a CV of 68%. Adjustment of CM β-C concentrations by CM TG (Fig. 2C) did not attenuate the variability of CM β-C concentrations because the CV of the TG-adjusted CM β-C concentration was similar to that for CM β-C (Table 2). Finally, there was no significant relationship between the smoking habits of the subjects and the CM β-C response or the TG-adjusted CM β-C response.

Figure 3A shows the frequency distribution of CM RP concentrations measured 3 h after the β-C load. As observed for CM β-C and CM TG, the interindividual variability was very high, with a CV of 94%. Moreover, 10 subjects had no detectable RP in their CM fraction. Adjustment of CM RP concentrations by CM TG (Fig. 3B) increased this variability with a CV of 164%. Finally, the CM RP/β-C ratio (Fig. 3C) had the highest variability of all the parameters measured, with a CV of 221%. Again there was no significant effect of the smoking habits on this response.

Second experiment: Mechanisms involved and consequences on β-carotene metabolism

Plasma lipid-metabolism parameters. Several parameters of lipid metabolism were measured in the fasting plasma of the 16 subjects who took part in the second experiment. The results of these measurements are shown in **Table 3**.

Chylomicron β-carotene response. **Figure 4** shows the CM β-C and TG responses measured in the 16 subjects who ingested two successive meals in the second experiment. The curves in bold print represent the mean response of the 16 subjects, and the other curves represent the minimal and maximal responses, as assessed by 0–12.5 h AUC, obtained among the 16 subjects.

As shown in Fig. 4A, there was a significant increase in CM β-C concentration in the postprandial period. This concentration reached a first peak at 3 h after β-C intake and then decreased slightly. After the intake of the second meal, which did not provide β-C, the CM β-C concentration increased, peaked at 7.5 h, and then decreased until 12.5 h. Two main observations can be made. First, the CM

TABLE 2. Interindividual variabilities

	Mean	SEM	95 % CI	CV (%)	Centiles 10th	50th	90th
β-Carotene intake (mg/d)	2.2	0.4	(1.4–3.0)	157	0.03	0.77	6.70
Fasting plasma β-carotene (nmol/L)	233.9	20.1	(193.6–274.2)	76	78.0	192.8	403.3
Buccal mucosal cell β-carotene (nmol/g protein)	2.8	0.8	(1.1–4.5)	257	0	1.3	4.5
Chylomicrons[a]							
Triglyceride (μmol/L)	502.4	38.4	(426–578.8)	68	173.9	395.3	1086.5
β-Carotene (nmol/L)	98.0	6.8	(84.5–111.5)	61	40.0	83.8	196.7
Retinyl palmitate (nmol/L)	76.9	8.3	(60.4–93.4)	94	0	65.8	192.2
β-Carotene/triglyceride (nmol/μmol)	0.23	0.02	(0.20–0.26)	61	0.11	0.20	0.42
Retinyl palmitate/triglyceride (nmol/μmol)	0.19	0.04	(0.12–0.26)	164	0	0.13	0.40
Retinyl palmitate/β-carotene	1.0	0.26	(0.48–1.52)	221	0	0.6	1.9

Number of subjects, 79; CI, confidence interval; CV, coefficient of variation.

[a] Chylomicrons prepared from a blood sample collected 3 h after the intake of a meal which contained 40 g triglycerides and 120 mg β-carotene as the only source of vitamin A.

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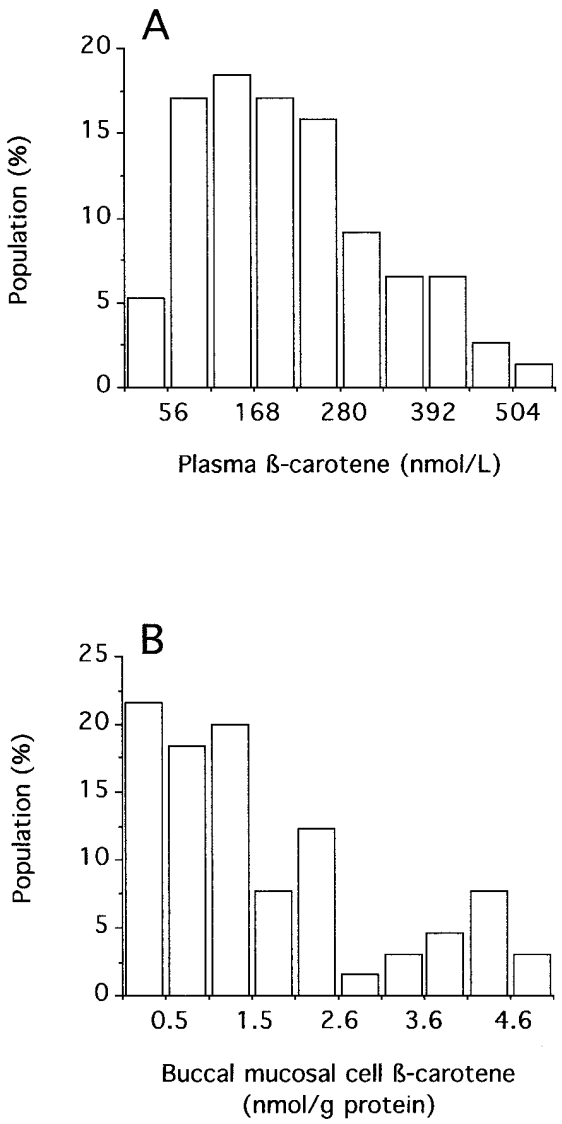

Fig. 1. Frequency distribution graphs of the β-carotene concentration in plasma (A) and buccal mucosal cells (B) in 79 subjects.

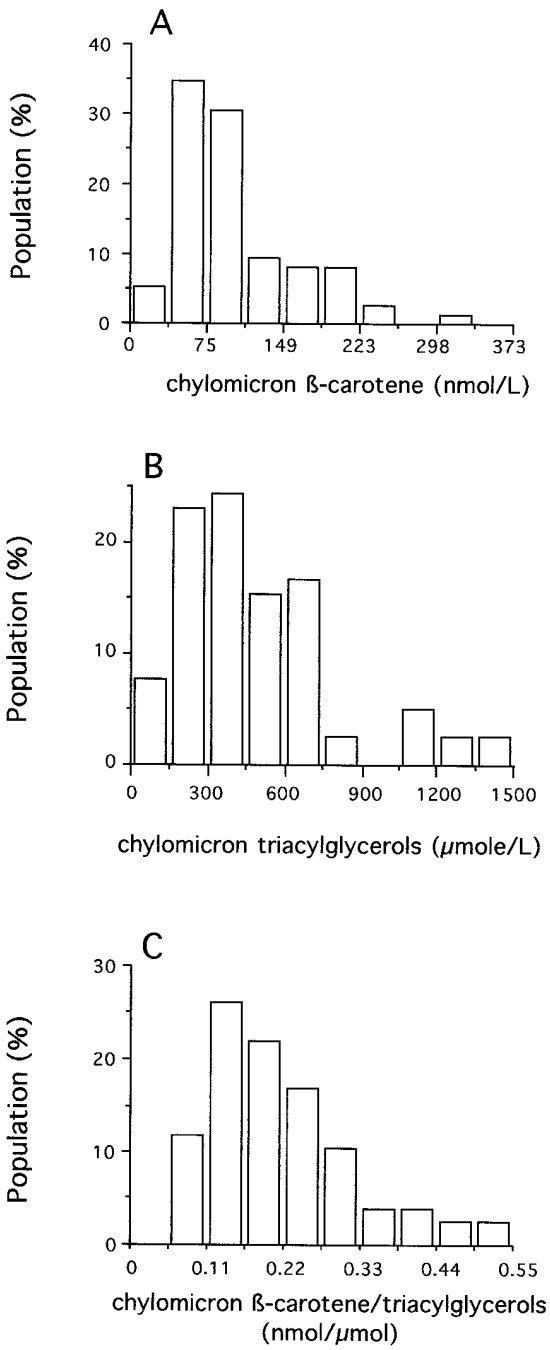

Fig. 2. Frequency distribution graphs of the chylomicron β-carotene (A), chylomicron triacylglycerol (B) and chylomicron β-carotene/triacylglycerol (C) responses obtained 3 h after the intake of a β-carotene-rich meal (120 mg β-carotene) by 79 subjects.

β-C response to the second meal was equivalent to that obtained after the first meal. Second, the interindividual variability of CM β-C response (0–12.5 h AUC) was very high with a ratio of about 9 between the highest response (0–12.5 h AUC = 3495 nmol/L·h) and the lowest response (AUC = 372 nmol/L·h), and a CV of 70%. Note that, after TG-adjustment, the CM β-C response had a lower interindividual variability, but remained very high, i.e., a CV of 60%. In agreement with the results obtained in the first experiment, there was no effect of smoking on the CM β-C response.

Chylomicron triacylglycerol response. The CM TG response to the two successive meals is shown in Fig. 4B. The CM TG responses obtained after intake of each meal (0–6 h AUC and 6–12.5 h AUC) were very similar. The interindividual variability of CM TG response (0–12.5 h AUC) was similar to that observed for CM TG concentrations measured at 3 h in the 79 subjects with CVs of 64 and 68%, respectively.

Chylomicron retinyl-palmitate response. As shown in **Fig. 5A**, the CM RP concentration increased after the intake of the first meal, which contained β-C as the only source of vitamin A. It also increased after the intake of the second meal, which did not provide vitamin A. The response obtained was very similar to that observed for CM β-C with two peaks at 3 and 7.5 h. The interindividual variability of the CM RP response was high with a ratio of about 7 between the lowest response (139 nmol/L·h) and the highest (977 nmol/L·h) and a CV of 57%. As observed for β-C, TG adjustment of CM RP response only slightly attenuated the in-

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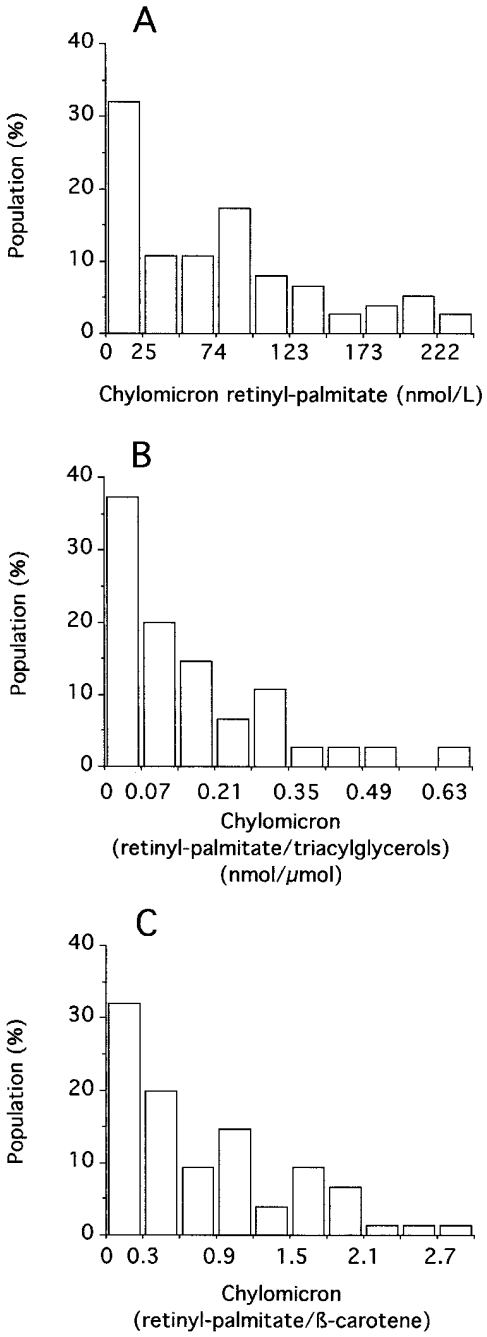

Fig. 3. Frequency distribution graphs of the chylomicron retinyl-palmitate (A), chylomicron retinyl-palmitate/triacylglycerol (B), and chylomicron retinyl-palmitate/β-carotene (C) responses obtained 3 h after the intake of a β-carotene-rich meal by 79 subjects. Note that the only source of vitamin A in this meal was β-carotene.

terindividual variability, i.e., a CV of 52%. Note again that no significant relationship was observed between the dietary habits of the subjects and the CM RP response.

Chylomicron retinyl-palmitate/β-carotene ratio. The CM RP/β-C ratio was relatively constant during the postprandial period (Fig. 5B), but there was a great interindividual variability between the subjects (CV = 60%). There was no relationship between this ratio and the smoking habits of the subjects.

TABLE 3. Fasting plasma lipid-metabolism parameters of the 16 subjects

	Concentration
Triacylglycerol (mmol/L)	1.20 ± 0.09
Phospholipid (mmol/L)	2.89 ± 0.13
Cholesterol (mmol/L)	4.82 ± 0.24
HDL cholesterol (mmol/L)	1.13 ± 0.09
LDL cholesterol (mmol/L)	2.54 ± 0.16
VLDL cholesterol (mmol/L)	1.15 ± 0.08
Apolipoprotein A-I (g/L)	1.16 ± 0.06
Apolipoprotein B (g/L)	0.86 ± 0.06
Apolipoprotein C-III (g/L)	0.11 ± 0.01
Lp[a] (g/L)	0.31 ± 0.07
LpAI (g/L)	0.49 ± 0.04
LpAI/AII	0.67 ± 0.04

Values are means ± SEM. The parameters were measured on blood samples collected after 12 h fasting.

Plasma retinoic acid response. **Table 4** shows the mean plasma retinoic acid concentrations measured after fasting and 3 h after the β-C load in the 16 subjects. There was a significant increase in the plasma all-*trans* retinoic acid concentration after the meal, but there was no significant variation in the plasma concentration of 13-*cis* retinoic acid. Note that there was no significant relationship between the dietary habits of the subjects and the fasting or postprandial retinoic acid concentrations.

DISCUSSION

In humans, most β-C taken up in the enterocyte is secreted as unchanged β-C and RP in the chylomicron fraction. Thus, the appearance of β-C and RP in this fraction, after the intake of a β-C load, is a reliable indicator of β-C absorption and gives valuable information on the efficiency of β-C conversion into vitamin A (41). For several reasons, we used 120 mg β-C, which is much higher than the estimated intake of about 5 mg per day from typical diets. The first reason was so that nonresponders to β-C could be indentified unequivocally. Indeed, for reasons of sensitivity, there is a risk that the use of a dietary dose of β-C and the classical HPLC method could lead to subjects who were in fact low responders being wrongly identified as nonresponders. The second reason was that pharmacological doses of β-C, i.e., from 9 times to 60 times the dietary intake estimates, have been used in recent studies on β-C metabolism in humans (17, 21–24, 42–47) making the comparison between previous results and the present results easier. The third reason was that pharmaceutical doses of β-C are commonly used in the treatment of erythropoeitic protoporphyria (48) and were also used in recent intervention studies performed to assess the effect of β-C on cancer incidence (5, 6). As the results of these studies are controversial, and because it has been suggested that the unexpected results obtained could have been due, in part, to the interindividual variability of β-C metabolism, it is particularly important to have a better understanding of the interindividual variability of β-C response to such doses in humans.

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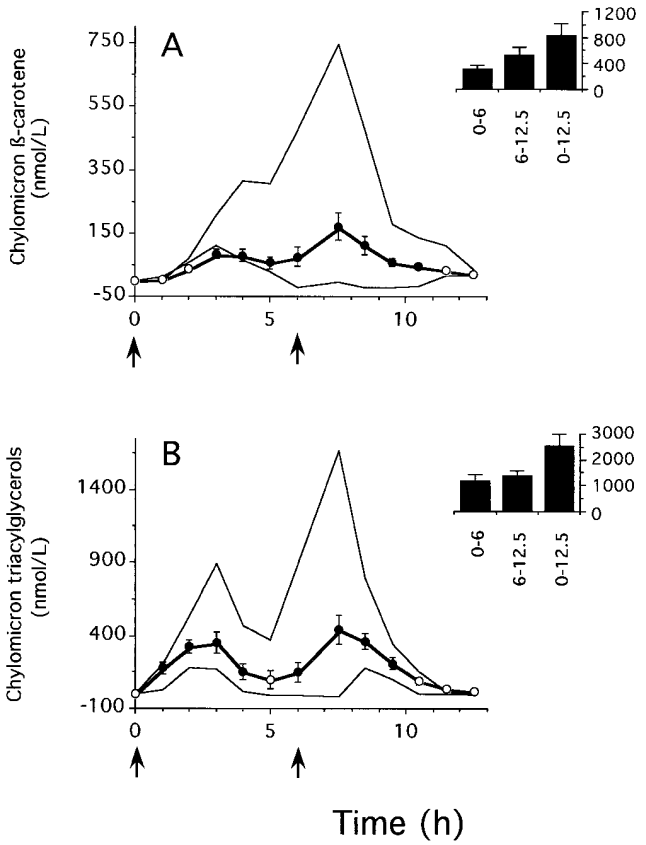

Fig. 4. Chylomicron β-carotene (A) and triacylglycerol (B) responses obtained after the intake of two successive meals by 16 subjects. The first meal provided 120 mg β-carotene and 40 g triacylglycerol while the second provided 40 g triacylglycerol without β-carotene. The arrow shows the time at which the meals were ingested. Inserts: area under the curve (AUC) of the responses obtained after the first meal (0–6 h), after the second meal (6–12.5 h), or after both meals (0–12.5 h). The thick curves show the mean response ± SEM of the 16 subjects while the thin curves show the responses of the subjects who had the lowest and the highest responses (as estimated by their 0–12.5 h AUC). A filled symbol at a given time indicates that there is a significant difference between the mean concentration at this time and the corresponding fasting (0 h) concentration (ANOVA for paired values, $P < 0.05$).

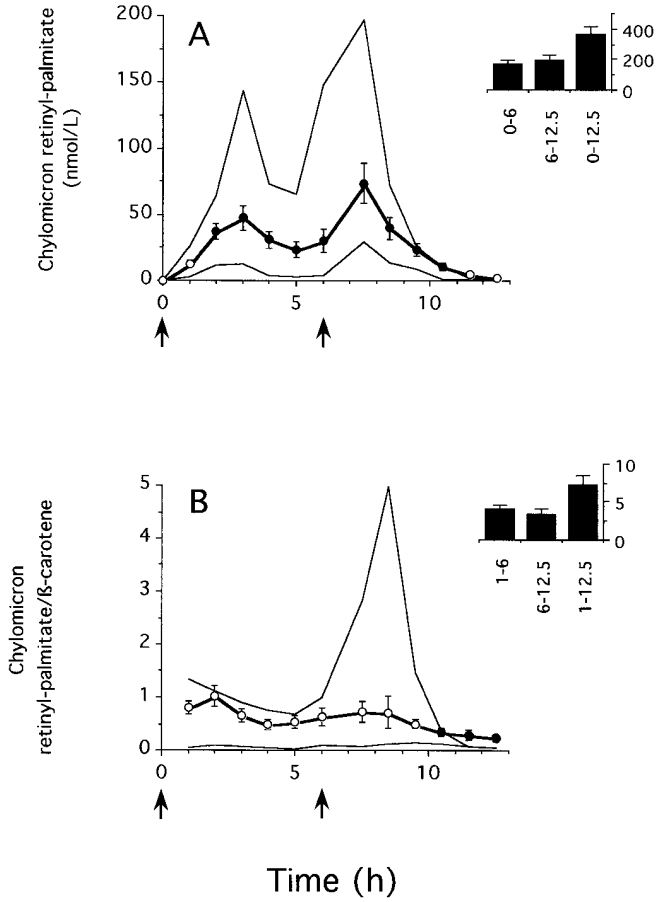

Fig. 5. Chylomicron retinyl-palmitate (A) and retinyl-palmitate / β-carotene (B) responses obtained after the intake of the two successive meals by the 16 subjects. For other explanations see Fig. 4 legend.

Because it is possible that β-C can be incorporated not only into the CM secreted in the first postprandial period after a β-C load, but also in the CM secreted after subsequent meals, we gave a second meal to the subjects to avoid underestimating the β-C response. Nevertheless, the magnitude of the CM β-C and RP responses observed after the second meal was unexpected because, in a previous study (41), only 1 subject out of 10 had appreciable β-C and RP responses after the second meal. This apparent discrepancy is probably due to the different doses of β-C used because we gave 120 mg whereas Van Vliet, Schreurs, and Van Den Berg (41) gave 15 mg β-C. The most likely hypothesis to explain this finding is that a large fraction of the absorbed β-C was not incorporated in the CM secreted after the first meal, because of the limited solubility of β-C in TG (49), and was temporarily stored in the enterocyte until the TGs provided by the next meal enabled the β-C to be incorporated into the CM.

Additional experiments are needed to verify this hypothesis. Nevertheless, these data indicate that an accurate measurement of the chylomicron β-C response to a high oral dose of β-C should take into account the additional response following a susequent meal in order to avoid large underestimations.

Variability of chylomicron β-carotene response after a single oral dose of β-carotene in the healthy population

Four conclusions can be drawn concerning the interindividual variability of CM β-C responses in the healthy population.

First, as described by several authors (17–19), this variability is very high. More precisely, we found a CV of 61% for the interindividual variability of CM β-C concentration at 3 h in the 79 subjects, and a CV of 68% for the interindividual variability of CM β-C response (0–12.5 h AUC) in the 16 subjects. Nevertheless this variability is not, as generally believed, exceptionally high because it is similar to the variability observed for CM TG response, i.e., CVs of 68 and 72% in the 79 and the 16 subjects, respectively. Note that the high interindividual variability of the CM TG response does not mean that the subjects had a highly

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TABLE 4. Plasma retinoic acid response to an all-*trans* β-carotene load in 16 subjects

	Fasting Plasma Concentration	Postprandial Plasma Concentration	Postprandial versus Fasting[a]
All-*trans* retinoic acid (nmol/L)	4.96 ± 0.43	6.96 ± 0.70	$P = 0.0017$
13-*Cis* retinoic acid (nmol/L)	4.95 ± 0.63	5.13 ± 0.77	$P = 0.8564$

Fasting plasma retinoic acid concentrations were measured after 12 h fasting and postprandial concentrations were measured 3 h after the intake of a test meal that provided 120 mg all-*trans* β-carotene. Values are means ± SEM.

[a] The statistical comparisons between the postprandial and the fasting plasma retinoic acid concentrations were made by the Student's *t*-test for paired values.

variable absorption efficiency for TG, which is assumed to be higher than 95% in healthy subjects for this amount of TG ingested, but rather means that the subjects had very different rates of fat absorption and/or CM clearance.

Second, in agreement with a previous study (21) the distribution of CM β-C concentration is unimodal. Indeed, there was no clear separation between low responders and high responders.

Third, there is apparently no, or only very low, incidence of true nonresponders to pharmacological doses of β-C in the healthy population. Evidence for this is that *i*) in the first experiment, all the 79 subjects had β-C in the CM fraction collected 3 h after the β-C load, and *ii*) in the second experiment, all the 16 subjects had a CM β-C response. This observation was noteworthy because, before this experiment, there was doubt about whether there is a high proportion of true nonresponders in the healthy population. Indeed, three studies (18–19, 41) found no true nonresponders among large samples of the population, whilst two other studies (23, 24), using doses of β-C similar to that used in our study, report a high proportion of nonresponders, i.e., 7 out of 11 in the Johnson and Russell study (23) and 3 out of 7 in the Stahl et al. study (24). In fact, Johnson and Russell (23) defined as nonresponders the subjects who had a peak plasma β-C response (AUC) below the level of the mean response plus two SDs for a control group (who did not take β-C). Nevertheless, these subjects had a significant increase in β-C concentration at 9 h post-dose in the plasma and at 3 h post-dose in the chylomicrons, suggesting that they were in fact low responders rather than non-responders. The results of Stahl et al. (24) are more difficult to explain, but the fact that they used a mixture of β-C isomers (with 9-*cis* β-C as the main *cis* isomer) instead of the pure all-*trans* isomer can explain the difference observed.

Finally, the ability to respond to β-C seems to be a constant characteristic of the subjects because we found a significant correlation between the TG-adjusted CM β-C concentrations measured in the 16 subjects at 3 h in the two separate experiments ($r = 0.52$, $P < 0.05$).

Mechanisms involved

In agreement with previous studies (19–20, 23) we did not find any relationship between the β-C response to a single β-C load and any of the physical characteristics measured, the dietary habits, or the smoking habits of the subjects (Table 1). Also, we did not find any relationship between the β-C response and the baseline plasma β-C levels. Thus, these factors apparently do not affect the β-C response. Given the current knowledge about β-C metabolism (10–14), three likely mechanisms can be proposed to explain the interindividual variability in CM β-C response.

i) The efficiency of intestinal absorption of β-C may be variable in the population. Although we did not directly measure the interindividual variability of β-C absorption, we estimated it by measuring the interindividual variability of the CM (β-C + RP)/TG ratio. In the first experiment, i.e., in the 79 subjects, this ratio was measured at one postprandial point, i.e., at 3 h, whilst in the second experiment, i.e., in the 16 subjects, it was measured at each postprandial point, so that a complete postprandial ratio could be calculated. The difference between the subject who had the lowest ratio and the subject who had the highest was about 11-fold in the first experiment and about 7-fold in the second experiment, with CVs of 55% and 54%, respectively. Moreover, there was a positive correlation ($r = 0.60$, $P < 0.05$) between the ratios measured at 3 h in the two experiments. Thus, the efficiency of intestinal absorption of β-C seems highly variable in healthy humans but is apparently a constant characteristic of the individuals. The reason for this high variability is unknown but it explains, at least in part, the high variability of CM β-C response to dietary β-C.

ii) The efficiency of intestinal conversion of β-C into vitamin A may be variable in the population. This hypothesis can be assessed by evaluating the relationship between the CM β-C/TG response (0–12.5 h AUC) and the CM RP/TG response. As we found a positive relationship ($r = 0.55$, $P < 0.05$) between these two responses, and not the negative relationship that would be expected in that case, we point out that the higher the amount of β-C absorbed, the higher the amount of RP that is secreted in the CMs. Thus, the conversion of β-C into vitamin A apparently has no major effect on CM β-C response.

iii) The metabolism of CM, i.e., the secretion rate and the clearance rate of CM, may be variable in the population and may affect the CM β-C response. Because, in humans, newly absorbed β-C is almost exclusively secreted in the CM, the positive relationship ($r = 0.50$, $P < 0.05$) found between the CM β-C response and the CM TG response suggests that CM β-C response depends on CM metabolism, i.e., on the rate of CM secretion and clearance.

Thus, the interindividual variability of CM β-C response to a β-C load, i.e., the ability to respond to β-C, appears to be due mainly to differences between individuals in the efficiency of intestinal absorption of β-C and in CM metabolism.

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Ability to respond to β-C and the ability to convert β-C into vitamin A apparently affect the β-C status

It is logical to suspect that the high interindividual variability of β-C status is partly explained by the dramatic variation of β-C intake in the population. This was confirmed by the significant relationship ($r = 0.30$, $P < 0.05$, n = 79) found between β-C intake and β-C status (as estimated by plasma β-C). However, it is reasonable to consider that both the ability to respond to β-C and the ability to convert β-C into vitamin A can also affect this status.

The effect of the ability to respond to β-C on the β-C status of the subjects was evaluated by measuring the relationships between this ability, evaluated by the 0–12.5 h CM β-C/TG response, and the β-C status of the subjects, evaluated by the concentration of β-C in the fasting plasma or in the buccal mucosal cells. The results obtained depended on the marker of β-C status used, i.e., there was a strong positive correlation ($r = 0.61$, $P = 0.048$, n = 16) between the CM β-C/TG response and the concentration of β-C in the buccal mucosal cells, whereas there was no significant relationship between the CM β-C/TG response and the fasting plasma β-C concentrations. As the very high variability of dietary β-C intake might have distorted these relationships, we adjusted the β-C status for β-C intake. After this adjustment, the relationship between the CM β-C/TG response and the concentration of β-C in the buccal mucosal cells was strengthened ($r = 0.73$, $P = 0.016$), but the lack of relationship between the β-C/TG response and the plasma β-C concentrations persisted.

The effect of the ability of the subjects to convert β-C on their β-C status was evaluated by measuring the relationship between the CM RP/β-C response and the β-C status because the CM RP/β-C ratio has been proposed to reflect the efficiency of β-C conversion into vitamin A (41). The results obtained showed that the concentration of β-C in the buccal mucosal cells, when adjusted for β-C intake, was negatively correlated with the CM RP/β-C response ($r = -0.63$, $P = 0.036$), while the concentration of β-C in the fasting plasma was not correlated with this response.

The finding that the ability to respond to β-C and the ability to convert β-C into vitamin A were related to the β-C status when it was estimated by buccal mucosal cell β-C, but were not when the β-C status was estimated by the plasma β-C, can reasonably be explained by the fact that plasma β-C concentrations are probably affected more by recent β-C intake than are tissue β-C concentrations.

To summarize, as the ability to respond to β-C and the ability to convert β-C into vitamin A were found to be related to the β-C status, we suggest that these capabilities probably affect the β-C status. A multiple regression model makes it possible to estimate the respective contribution of these two factors: β-C concentration in the buccal mucosal cells (μg β-C (adjusted for β-C intake)/mg proteins) = 0.00237 β-C/TG response (nmol/μmol) − 0.38997 RP/β-C response + 0.20643. This model explains 54% ($P < 0.05$) of the variation of β-C status (adjusted for β-C intake).

Ability to respond to β-C probably affects the apparent provitamin A activity of β-C

β-C is the main provitamin A carotenoid in the human diet, thus it is particularly important to evaluate the interindividual variability of provitamin A activity of β-C, and to check whether the ability to respond to β-C can affect β-C provitamin A activity. Because a significant proportion of β-C is converted into vitamin A in the intestine, and because the resulting retinyl esters are mostly incorporated in the CM (10–14), measuring the CM RP response to a β-C load in different subjects provides an acceptable means of estimating the interindividual variability of the provitamin A activity of β-C.

Observing of the CM RP responses makes it possible to draw several conclusions about the apparent provitamin A activity of β-C in humans. *i*) Most healthy subjects are able to convert β-C into vitamin A, at least at the intestinal level. Although in the first experiment we found that about 13% of the 79 subjects had no detectable RP in the CM fraction collected 3 h after the β-C load, when the RP was measured in the whole postprandial period, i.e., in the second experiment, all the subjects, even those who had no detectable RP in the CM fraction collected at 3 h in the first experiment, had a positive CM RP response (0–12.5 h AUC). It is likely that variations in the kinetics of appearance of RP in the CM fraction may explain why some subjects can have undetectable RP at a given time in the postprandial period.

ii) The interindividual variability of the apparent provitamin A activity of β-C is high in healthy humans. This conclusion was drawn from the fact that the CV of the CM RP response (0–12.5 h AUC) was 65% (n = 16), and that there was a ratio of 11 between the subjects with the lowest/highest responses.

iii) The efficiency of β-C conversion into vitamin A appears to be highly variable in the population as shown by the 60% CV of the β-C/RP response. This probably affects the provitamin A activity of β-C in different subjects.

iv) CM metabolism affects the apparent provitamin A activity of β-C, as shown by a positive relationship ($r = 0.75$, $P < 0.001$) between CM RP response (0–12.5 h AUC) and CM TG response (0–12.5 h AUC).

v) The apparent provitamin A activity of β-C is related to the ability to respond to β-C; the TG-adjusted CM RP response (0–12.5 h AUC) was positively related ($r = 0.55$, $P < 0.05$) with the TG-adjusted CM β-C response (0–12.5 h AUC).

To summarize, the apparent provitamin A activity of β-C, i.e., the RP response to a β-C load, is highly variable in healthy humans. This high variability suggests that the dietary equivalence between β-C and retinol, which is assumed to be 6 μg β-C for 1 μg retinol, varies greatly among different subjects. This variability is likely to be due to interindividual differences in the intestinal conversion of β-C into vitamin A, but the ability of the subjects to respond to β-C and the CM metabolism of the subjects are also contributing factors. It is reasonable to suggest that the pharmacological amount of β-C used has not exceeded the capability of intestinal β-C dioxygenase to

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cleave β-C in all the subjects because we did not find the similar amount of RP in the CM of the different subjects that would be expected in that case. Thus the interindividual differences in the intestinal conversion of β-C into vitamin A may be due to interindividual differences in the intestinal level of β-C dioxygenase activity.

There is a slight increase in plasma all-*trans* retinoic acid concentration after an all-*trans* β-C load but the ability to respond to β-C is not related to this increase

The consumption of large doses of preformed vitamin A supplements by pregnant women is associated with birth defects (50). Numerous animal studies have confirmed that preformed vitamin A and some of its metabolites, particularly all-*trans*-retinoic acid, are teratogenic (51). Several studies in vitro have shown that retinoic acid can also be produced from β-C (13). However, the effect of dietary β-C on plasma retinoic acid concentration has, to our knowledge, never been described in humans. Our study shows for the first time that plasma all-*trans*-retinoic acid concentration can increase (+40% above the fasting value) after the ingestion of a pharmacological dose of all-*trans*-β-C. Although this result is based only on one time point, i.e., 3 h post-meal, the significant ($P = 0.0017$) difference between the fasting and the postprandial value suggests that this observation is of physiological significance. However, two findings suggest that this increase seems to cause no danger to embryogenesis. First, only the all-*trans* isomer increased, whereas 13-*cis*-retinoic acid, which is probably the most teratogenic compound in primates (52), remained unchanged. Second, after the intake of 120 mg all-*trans*-β-C, the all-*trans*-retinoic increase remained 43-fold lower than that after the intake of 150 mg retinyl palmitate supplement (53). Finally, the lack of any relationship between the CM β-C response and the plasma all-*trans*-retinoic acid response suggests that the ability to respond to β-C does not affect the amount of retinoic acid produced.

In conclusion, this study highlights that the CM β-C response to a pharmacological dose of β-C is highly variable in humans. It shows that there probably very few or even no true nonresponders to pharmacological doses of β-C in the healthy population, and that the ability to respond to β-C is probably an intrinsic characteristic of the subject. This characteristic is mostly explained by interindividual variations in the efficiency of β-C absorption and in CM metabolism. Note that although it is difficult to extrapolate this result to physiological doses of β-C, the fact that the ability to respond to β-C was related to the β-C status strongly suggests that this characteristic persists for physiological doses of β-C. Finally, it appears that this characteristic affects the apparent provitamin A activity of β-C, but does not affect the amount of retinoic acid secreted in the plasma after a high β-C load.

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