Influence of feeding different vegetables on plasma levels of carotenoids, folate and vitamin C. Effect of disruption of the vegetable matrix

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(Received 18 May 1998 – Revised 17 December 1998 – Accepted 28 April 1999)

Carotenoids, folate and vitamin C may contribute to the observed beneficial effects of increased vegetable intake. Currently, knowledge on the bioavailability of these compounds from vegetables is limited. We compared the efficacy of different vegetables, at the same level of intake (i.e. 300 g/d), in increasing plasma levels of carotenoids, folate and vitamin C and we investigated if disruption of the vegetable matrix would enhance the bioavailability of these micronutrients. In an incomplete block design, sixty-nine volunteers consumed a control meal without vegetables and three out of four vegetable meals (i.e. broccoli, green peas, whole leaf spinach, chopped spinach; containing between 1·7 and 24·6 mg β-carotene, 3·8 and 26 mg lutein, 0·22 and 0·60 mg folate and 26 and 93 mg vitamin C) or a meal supplemented with synthetic β-carotene (33·3 mg). Meals were consumed for 4 d and fasting blood samples were taken at the end of each period. Consumption of the spinach-supplemented meal did not affect plasma levels of β-carotene, although the β-carotene content was 10-fold those of broccoli and green peas, which induced significant increases in plasma β-carotene levels (28 (95 % CI 6·4, 55) % and 26 (95 % CI 2·6, 54) % respectively). The β-carotene-supplemented meal increased plasma concentrations of β-carotene effectively (517 (95 % CI 409, 648) %). All vegetable meals increased the plasma concentrations of lutein and vitamin C significantly. Broccoli and green peas were, when expressed per mg carotenoid consumed, also more effective sources of lutein than spinach. A significant increase in plasma folate concentration was found only after consumption of the spinach-supplemented meal, which provided the highest level of folate. Disruption of the spinach matrix increased the plasma responses to both lutein (14 (95 % CI 3·7, 25) %) and folate (10 (95 % CI 2·2, 18) %), whereas it did not affect the response to β-carotene. We conclude that the bioavailabilities of β-carotene and lutein vary substantially among different vegetables and that the bioavailabilities of lutein and folate from spinach can be improved by disruption of the vegetable matrix.

Folate: Carotenoids: Bioavailability: Vegetable consumption

Many epidemiological studies have indicated that an increased intake of vegetables is associated with a decreased risk of certain cancers (Willett & Trichoupoulos, 1997), cardiovascular disease (Ness & Powles, 1997) and age-related eye diseases (Jacques & Chylack, 1991; Hankinson *et al*. 1992; Seddon *et al*. 1994). This has raised interest in determining the nature and bioavailability of active compounds present in vegetables. Antioxidants, such as carotenoids and vitamin C, may contribute to the beneficial effects of vegetable consumption (Seddon *et al*. 1994; Gey, 1995; Van Poppel & Goldbohm, 1995; Weber *et al*. 1996). In addition, vegetables are a major dietary source of folate (De Bree *et al*. 1997). High intake of folate may be associated with a reduced risk of cancer (Glynn & Albanes, 1994) and there is increasing evidence that folate may reduce the risk of cardiovascular disease by lowering homocysteine levels in plasma (Boushey *et al*. 1995; Verhoef *et al*. 1996).

There are indications that the bioavailability of carotenoids and folate from vegetables is limited compared with supplements (Micozzi *et al*. 1992; De Pee *et al*. 1995; Gregory, 1995). It is plausible that the vegetable matrix plays a major role in determining the bioavailability of these micronutrients. For instance, disruption of the vegetable matrix enhanced the bioavailability of β-carotene from carrots and lycopene from tomatoes (Van Zeben & Hendriks, 1948; Gärtner *et al*. 1997). It is of interest to investigate whether disruption of the matrix also affects the bioavailability of β-carotene from green leafy vegetables, which was found to be low (De Pee *et al*. 1995).

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In addition, there are indications that there may be significant differences between different vegetables as to the bioavailability of carotenoids and folate (Tamura & Stokstad, 1973; Babu & Srikantia, 1976; Micozzi *et al.* 1992). The present study was set up to compare three types of vegetables, i.e. broccoli, green peas and spinach, at the same level of intake (300 g/d), in their effectiveness to increase plasma levels of carotenoids, folate and vitamin C. These vegetables are similar in colour, but clearly distinct with respect to the part of the plant they represent (i.e. flowers, seeds and leaves). In addition, we investigated whether mechanical homogenization of whole leaf spinach would enhance the bioavailability of carotenoids, folate and vitamin C.

Subjects and methods

Volunteers

Seventy-two volunteers were selected for participation in the study. They were recruited from inhabitants of Vlaardingen and its surroundings and employees of Unilever Research Laboratorium in Vlaardingen, The Netherlands, and gave their written informed consent before participation.

Volunteers were eligible if they had a BMI between 19 and 30 kg/m^2 and if they were apparently healthy, as assessed by questionnaire (i.e. they regarded themselves as being healthy, they did not receive medical treatment or use medicines and they reported no chronic gastrointestinal problems). They had not used dietary supplements (e.g. vitamins, minerals, carotenoids) in the month preceding the study and they were not on a slimming diet, did not use excessive amounts of alcohol (less than 21 units/week for females or 28 units/week for males) and smoked a maximum of 15 cigarettes/d. Volunteers who adhered to a vegetarian, macrobiotic or other alternative diet were excluded from participation. Pregnant or lactating women were also excluded.

Study design

The study had an incomplete block design with four experimental periods and with individuals as blocks. All volunteers consumed a control meal during one of the experimental periods and they consumed meals supplemented with vegetables or synthetic β-carotene during the other three periods. The vegetable meals contained 300 g of one of the following vegetables: broccoli, green peas, whole leaf spinach or chopped spinach. The control and the β-carotene-supplemented meals contained no vegetables. The test meals were consumed for four consecutive days, followed by 10 d of wash-out. The duration of the wash-out period was chosen as approximately the half-life of plasma β-carotene *in vivo* (Rock *et al.* 1992). Fasting blood samples were taken at the end of each 4 d experimental period to assess plasma levels of folate (only after control and vegetable-supplemented meals), carotenoids and vitamin C. To determine the effect of the experimental regimen without any vegetables and fruits, a sub-group of twenty-six randomly chosen volunteers supplied an additional fasting blood sample before the start of the experimental period in which they would consume the control meal. The protocol was approved by the Medical-Ethical Committee of Unilever Nederland BV.

Test meals and background diet

The control meal consisted of pasta with ham and a white sauce, and custard for dessert. The vegetables and β-carotene were added to this basic meal. Energy level, fibre content and macronutrient composition were the same for all test meals and similar to those of an average Dutch main meal (Voorlichtingsbureau voor de Voeding, 1993). Differences in fibre content were corrected for by addition of beet fibre (Fibrex; Tefco Food Ingredients BV, Bodegraven, The Netherlands) to the sauce. Synthetic β-carotene (300 g/l microcrystalline suspension in oil, 30 % fluid suspension (E160a), Hoffman-La Roche, Basel, Switzerland) was added to the sauce. The vegetables (300 g/meal: broccoli, Iglo/Mora, Utrecht, The Netherlands; garden peas, Birds Eye Wall's, Walton-on-Thames, Surrey, UK; whole leaf spinach, Sagit, Rome, Italy) were served simultaneously with the basic meal. Broccoli and green peas were cooked conventionally in boiling water for 6 min and 3–4 min respectively. Whole leaf spinach was microwaved (3200 W) for 16 min with a stir after 8 min. For preparation of chopped spinach, whole leaf spinach was minced after 8 min in the microwave (3200 W) and subsequently microwaved for another 8 min.

The hot meals were served at lunch-time and volunteers were instructed not to consume any vegetables or vegetable-containing products, fruits, fruit juices or red sauces (e.g. tomato ketchup) during the rest of the days of the experimental periods. During the wash-out periods the volunteers returned to their habitual diet.

Composition of test meals (Table 1)

Duplicate portions (n 5) of the complete test meals (as consumed by the volunteers) were analysed and found to provide on average 20 (SD 1·5) g fat, 80 (SD 4·7) g carbohydrates, 32 (SD 1·8) g protein and 16 (SD 1·8) g fibre. Carotenoid, folate and vitamin C concentrations were determined in duplicate portions (n 4–8) of the vegetables or sauces (as consumed by the volunteers). After extensive extraction of the vegetables or sauces with *n*-heptane–ether (1 : 1, v/v), ethyl-carotenoate was added as internal standard. ($\alpha+\beta$)-Carotene, lutein and lycopene were separated by straight-phase HPLC using a nucleosil 5CN column (Machery & Nagel, Duren, Germany) and *n*-heptane–isopropanol (1000 : 25, v/v) as mobile phase at a flow rate of 1·0 ml/min and a column temperature of 20°. The eluent was monitored by u.v.-visible detection at 450 nm for ($\alpha+\beta$)-carotene and lutein, and at 470 nm for lycopene. In this system, α-carotene coelutes with β-carotene. As the vegetables and β-carotene supplement contained virtually no α-carotene (i.e. $<$0·04 mg/serving, Mangels *et al.* 1993*b*), the response of ($\alpha+\beta$)-carotene is considered as β-carotene. The vegetables and control sauce were extracted with 0·1 mol/l phosphate buffer (pH 6·1, with sodium ascorbate, 2 g/l) and the filtrate was used for analysis of the folate concentration by microbiological assay with *Lactobacillus*

Table 1. Carotenoid, folate and vitamin C contents (mg/serving*) of the control sauce, vegetables and synthetic β-carotene-supplemented sauce used in the present study†

(Mean values and standard deviations)

Test meal...	Control		Broccoli		Green peas		Whole leaf spinach		Chopped spinach		Synthetic β-carotene	
Variable	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Total folate	ND		0·35	0·029	0·22	0·015	0·60	0·049	0·59	0·030	–	
Monoglutamyl folate	–		–		–		0·17	0·015	0·18	0·0	–	
β-Carotene	ND		2·43	0·2	1·72	0·07	24·6	1·8	23·8	2·4	33·3	1·2
Lutein	ND		3·78	0·4	4·22	0·2	25·0	2·7	26·0	2·8	ND	
Vitamin C	ND		84·6	14·9	25·7	2·1	91·6	10·3	92·6	9·1	ND	

ND, not detectable (< 0·03 mg/serving for folate; < 0·3 mg/serving for carotenoids; < 5 mg/serving for vitamin C); –, not determined.
* Based on analysis of duplicate portions of the prepared vegetables or sauces, as consumed by the volunteers; *n* 4 for folate and vitamin C analysis, *n* 8 for carotenoid analysis.
† The level of lycopene was below detection (< 0·3 mg/serving) in all the meals.

rhamnosus (NCIB 10473), using a commercially obtained assay medium (Merck Folic Acid Medium; Difco Laboratories, Detroit, MI, USA). For total folate content, a portion of the extract was incubated (3 h, pH 4·5, 37°) with human plasma deconjugase (Sigma Chem Co., St Louis, MO, USA) (Finglas *et al*. 1993). This step was omitted for analysis of free folate. After precipitation of the proteins and stabilization with metaphosphoric acid (50 ml/l), the vitamin C content of the vegetables or sauces was determined fluorimetrically as ascorbic acid plus dehydroascorbic acid, as described by Vuilleumier & Keck (1989).

Plasma and serum analyses

Fasting blood samples were taken by venepuncture. Plasma samples were stored at −70° for analysis of carotenoids and TCA-treated plasma was stored at −70° for analysis of vitamin C. For the other analyses, plasma and serum samples were stored at −20°. All analyses were executed within 6 months after the study. Plasma concentrations of β-carotene, lutein and lycopene were determined by straight-phase HPLC as described previously (Weststrate & van het Hof, 1995). β-Carotene and lycopene were

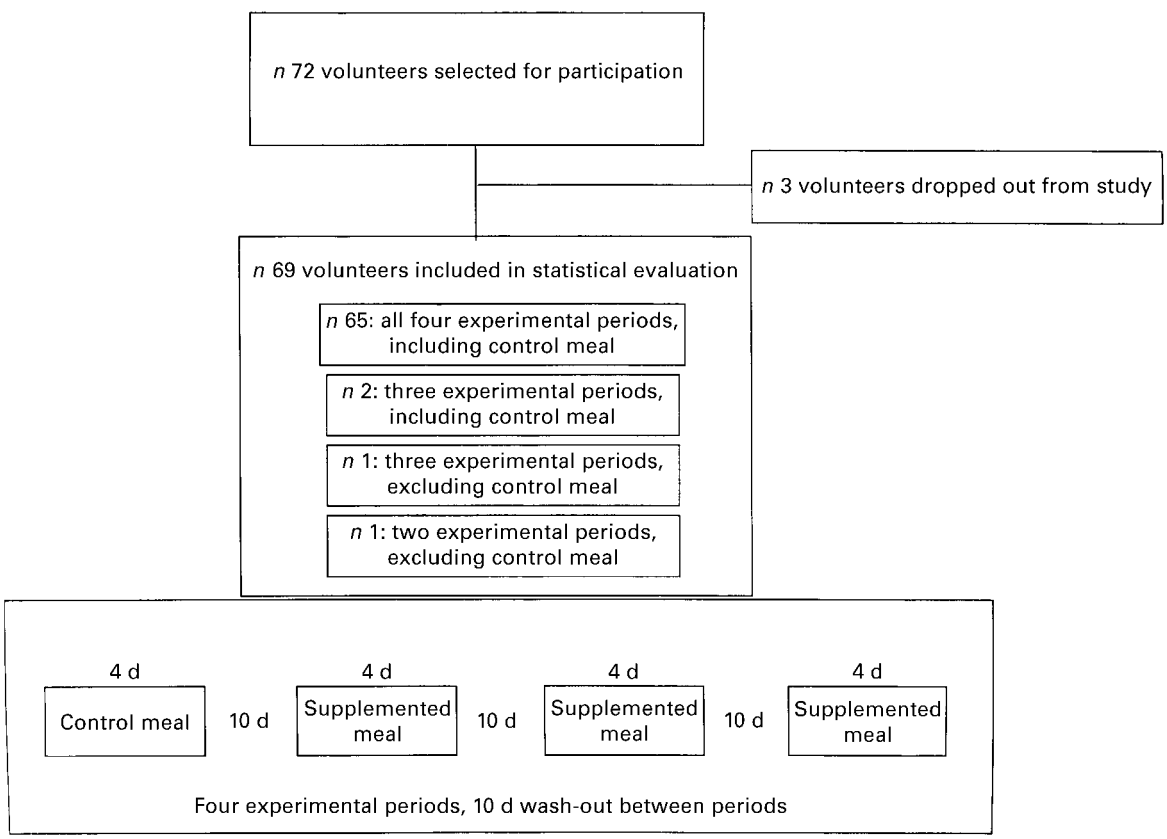

Fig. 1. Experimental design of the study. The control meal contained no vegetables or carotenoids whereas the other test meals were supplemented with broccoli, green peas, whole leaf spinach, chopped spinach or synthetic β-carotene. Volunteers received three of these supplemented meals. The combination of supplemented meals and the order of the test meals were varied among the participants.

Table 2. Plasma levels of carotenoids, folate and vitamin C and serum lipid levels in healthy volunteers before and after 4 d consumption of a control meal and after 4 d consumption of a vegetable- or synthetic β-carotene-supplemented meal†

(Values are least square means with their standard errors; for carotenoids the standard error is expressed as a percentage of the geometric mean)

Test meal...			Control (*n* 67)			Broccoli (*n* 31)			Green peas (*n* 31)			Whole leaf spinach (*n* 26)			Chopped spinach (*n* 26)			β-Carotene (*n* 28)		
	Baseline‡		4 d			4 d			4 d			4 d			4 d			4 d		
Variable	Mean	SE	Mean	SE		Mean	SE	*P*§	Mean	SE	*P*§	Mean	SE	*P*§	Mean	SE	*P*§	Mean	SE	*P*§
Folic acid (nmol/l)	20·9	1·2	21·4	0·4		22·8	0·6	0·23	23·1	0·6	0·087	23·4	0·6	0·048	25·7**	0·7	0·0001	–		–
β-Carotene (μmol/l)‖	0·70	0·09	0·55	4·1		0·71	5·7	0·0019	0·69	6·3	0·017	0·62	6·1	0·46	0·63	6·7	0·34	3·40	5·9	0·0001
Lutein (μmol/l)	0·18	0·02	0·12	2·0		0·28	3·2	0·0001	0·28	3·3	0·0001	0·33	3·5	0·0001	0·37**	3·5	0·0001	0·12	3·4	0·70
Vitamin C (μmol/l)	67·2	1·8	49·6	0·82		63·5	1·3	0·0001	55·3	1·3	0·0018	66·3	1·4	0·0001	66·4	1·5	0·0001	49·9	1·4	1·00
Total cholesterol (mmol/l)	5·4	0·14	5·23	0·037		5·23	0·058	1·0	5·21	0·058	1·0	5·17	0·064	0·9	5·23	0·064	1·0	5·22	0·062	1·0
Triacylglycerol (mmol/l)	1·1	0·084	0·93	0·021		0·96	0·033	1·0	0·89	0·033	0·9	0·90	0·036	1·0	0·98	0·036	0·8	0·97	0·035	0·9

–, Not determined.
Mean values were significantly different from those for whole leaf spinach: $^{**}P = 0{\cdot}01$.
† For details of diets and procedures, see Table 1 and pp. 204–206.
‡ Baseline values are means with their standard errors for twenty-six subjects (*n* 17 for β-carotene).
§ *P* values are based on ANOVA and Dunnett's test for the comparison of the plasma level following consumption of the vegetable- or β-carotene-supplemented meal with that after the control meal.
‖ Due to a carry-over effect in plasma concentrations of β-carotene, some of the results had to be excluded from the statistical evaluation. Control, *n* 43; broccoli, *n* 23; green peas, *n* 19; whole leaf spinach, *n* 20; chopped spinach, *n* 18; β-carotene, *n* 24.

separated on a Nucleosil 5-N $(CH_3)_2$ column with *n*-heptane as mobile phase at a flow rate of 1 ml/min and ethyl-β-apo 8′-carotenoate as internal standard. Lutein was separated on a Nucleosil 5CN column with *n*-heptane–dichloromethane–isopropanol (of 2-propanol) (900 : 100 : 5, by vol.) as mobile phase at a flow rate of 1 ml/min and β-apo-8′-carotenol as internal standard (intra-assay variation: <5·7 %, as determined in control plasma samples with the following average carotenoid concentrations: β-carotene 0·2 μmol/l; lycopene 0·4 μmol/l; lutein 0·15 μmol/l). Plasma folate concentration was assessed by using a chemiluminescence competitive protein-binding test (Magic Lite; Ciba Corning Diagnostics GmbH, Fernwald, Germany) (intra-assay variation; 4·7 %, as determined in plasma samples varying in folate concentration between 10 and 48 nmol/l). Vitamin C was analysed fluorimetrically in TCA-treated plasma as the concentration of ascorbic acid plus dehydroascorbic acid (intra-assay variation: 1·9 %, as determined in control plasma samples with 58 and 283 μmol/l vitamin C) (Vuilleumier & Keck, 1989). Total cholesterol and triacylglycerol concentrations in serum were assessed by using commercially available colorimetric test kits (respectively CHOD-PAP, Boehringer, Mannheim, Germany and GPO-PAP (Roche)/GPO-Trinder (Sigma, St Louis, MO, USA)).

Statistical evaluation

The significance of the changes in plasma carotenoid, folate and vitamin C concentrations during the experimental period in which volunteers consumed the control meal was determined by Student's *t* test for paired data. ANOVA with persons as blocks and sex, smoking habits, period, treatment, treatment × sex and treatment × smoking as factors, was used to compare the plasma and serum values found after consumption of the supplemented meals with those found after consumption of the control meal. Significance of the differences was assessed by Dunnett's test. As sex and smoking had no significant effect, these variables were excluded from the ANOVA model. Differences between the two types of spinach were assessed by orthogonal contrasts.

Plasma carotenoid concentrations were log-transformed to minimize correlation between mean values and standard errors. For these variables, geometric means are presented with the standard error as percentage of the geometric mean. Other variables are shown as least square means with their standard errors. Plasma concentrations of carotenoids, normalized for serum cholesterol and triacylglycerol levels, were also analysed for differences between the treatments (carotenoid concentration/(cholesterol + triacylglycerol concentration)).

All comparisons were at the two-sided 0·05 significance level, except for the difference between chopped and whole leaf spinach which was tested one-sided, based on the hypothesis that disruption of the spinach matrix would enhance the bioavailability of carotenoids, folate and vitamin C.

Results

Three volunteers dropped out of the study before the end of the first experimental period because of lack of time to

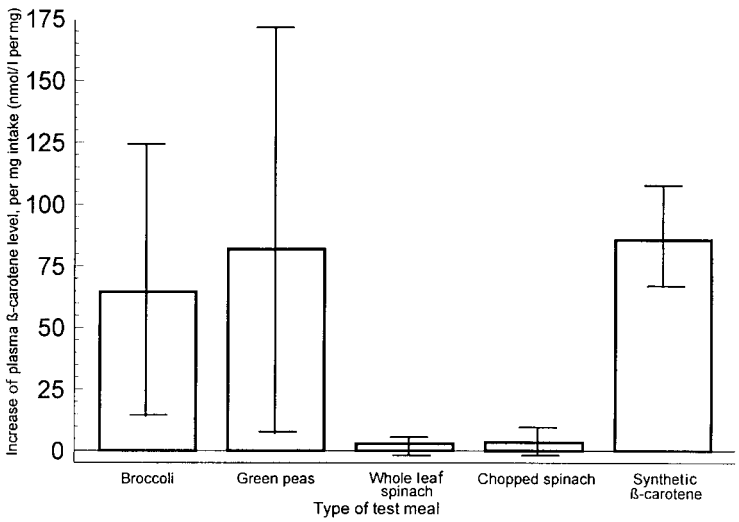

Fig. 2. Increases in β-carotene levels in the plasma of healthy volunteers after 4 d of supplementation with vegetables or β-carotene compared with consumption of a low-carotenoid control meal, expressed as increase per mg β-carotene intake from the vegetable or β-carotene supplemented meal. Values are means for eighteen to twenty-four subjects, with 95 % CI represented by vertical bars. The amounts of β-carotene provided per serving were (mg): broccoli 2·43, green peas 1·72, whole leaf spinach 24·6, chopped spinach 23·8, synthetic all-*trans* β-carotene 33·3. For details of procedures, see pp. 204–206.

participate in the trial and four volunteers were not able to participate in all four experimental periods for various reasons (e.g. illness, business trip) (see Fig. 1). Data for thirty-one males and thirty-eight females were included in the statistical analyses. Sixty-five of these volunteers participated in all four experimental periods whereas three volunteers participated in three experimental periods and one volunteer in only two periods. Two volunteers did not receive the control meal. The average age of the participants was 42 (SD 13) years and their mean BMI was 24·6 (SD 2·3) kg/m^2. Ten of the sixty-nine volunteers were smokers (five females and five males, maximum 15 cigarettes/d).

Table 2 shows the least square means of the plasma concentrations of carotenoids, folate and vitamin C and serum concentrations of total cholesterol and triacylglycerol before and after 4 d of consumption of the control meal without vegetables (low in carotenoids, folate and vitamin C) and after 4 d of the same meal supplemented with vegetables or β-carotene. As no vegetables and fruits were allowed to be consumed during the experimental periods, consumption of the control mean without any vegetables significantly reduced plasma carotenoid and vitamin C levels (β-carotene 0·069 (SE 0·018) μmol/l; lutein 0·038 (SE 0·0034) μmol/l; vitamin C 14 (SE 1·5) μmol/l, $P <$ 0·005), whereas plasma folate concentrations were not significantly affected.

Unfortunately, consumption of the meal supplemented with β-carotene induced a carry-over effect in plasma concentration of β-carotene. The plasma levels of β-carotene found in the first and second test periods following consumption of the β-carotene-supplemented meal were therefore excluded from the statistical evaluation (i.e. using a wash-out period of 38 d). A significant increase in plasma concentration of β-carotene was found after consumption of the β-carotene-supplemented meal (5-fold increase; 95 % CI 409, 648 %) as well as after the broccoli- and green peas-supplemented meals (28 (95 % CI 6·4, 55) % and 26 (95 % CI 2·6, 54) % respectively), whereas consumption of the spinach-supplemented meals had no significant effect compared with consumption of the control meal (Table 2). As the intake of β-carotene was different in each of the test meals, we calculated the plasma response per mg dietary β-carotene supplied per serving. Fig. 2 shows the efficacy of each supplemented meal to raise the plasma level of β-carotene compared with consumption of the control meal. Per mg β-carotene, broccoli and green peas induced similar responses in plasma concentration of β-carotene as consumption of the meal supplemented with synthetic β-carotene.

Consumption of any of the vegetable-supplemented meals resulted in a significantly increased plasma level of lutein compared with consumption of the control meal (1·3-fold (95 % CI 104, 150 %) for broccoli, 1·3-fold (95 % CI 108, 155 %) for green peas, 1·7-fold (95 % CI 139, 197 %) for whole leaf spinach, 2·0-fold (95 % CI 173, 239 %) for chopped spinach) (Table 2). Chopping of spinach enhanced this effect and plasma lutein levels after consumption of chopped spinach were significantly higher than those after whole leaf spinach (difference: 14 (95 % CI 3·7, 25) %). Fig. 3 shows for each vegetable type the plasma lutein response per mg lutein present in the vegetable per serving. Per mg of intake, lutein seems to be more bioavailable from broccoli and green peas than from spinach.

As anticipated, because of the virtual absence of lycopene in the experimental meals, none of the vegetables or the β-carotene-supplemented meals induced a significant change in lycopene levels in plasma (results not shown). No significant differences were found in serum lipid levels

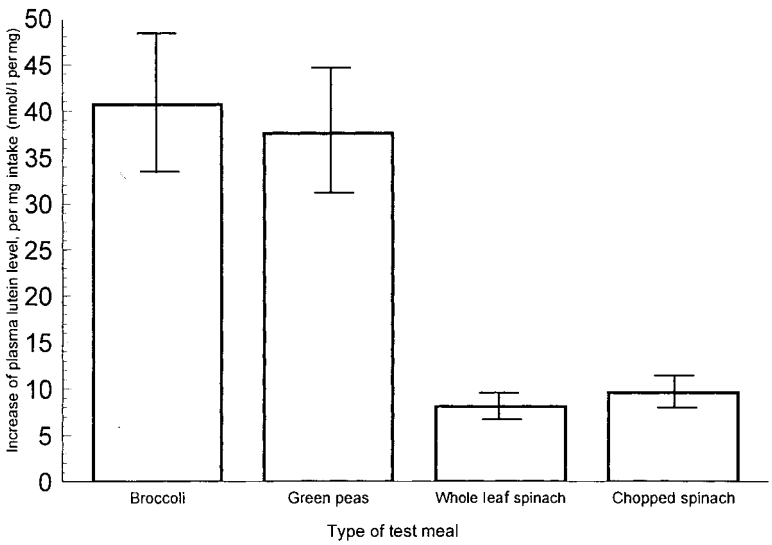

Fig. 3. Increases in lutein levels in the plasma of healthy volunteers after 4 d of supplementation with vegetables compared with consumption of a low-carotenoid control meal, expressed as increase per mg lutein intake from the vegetable supplemented meal. Values are means for twenty-six to thirty-one subjects, with 95 % CI represented by vertical bars. The amounts of lutein provided per serving were (mg): broccoli 3·78, green peas 4·22, whole leaf spinach 25, chopped spinach 26. For details of procedures, see pp. 204–206.

(Table 2) and normalizing plasma concentrations of carotenoids for serum lipids did not alter the results. Also, treatment effects were not significantly different between males and females or between smokers and non-smokers.

Folate levels in plasma were significantly increased by respectively 1·9 (95 % CI 0·021, 3·8) μmol/l and 4·2 (95 % CI 2·3, 6·2) μmol/l after 4 d consumption of the meal supplemented with whole leaf or chopped spinach as compared with the levels found after consumption of the control meal (Table 2). In addition, a significant difference (2·3 (95 % CI 0·8, 3·8) μmol/l) was found between chopped and whole leaf spinach, such that the folate concentration was higher after consumption of the chopped-spinach-supplemented meal. None of the other vegetable-supplemented meals increased folate levels in plasma significantly (Table 2). However, when expressed per mg folate intake,

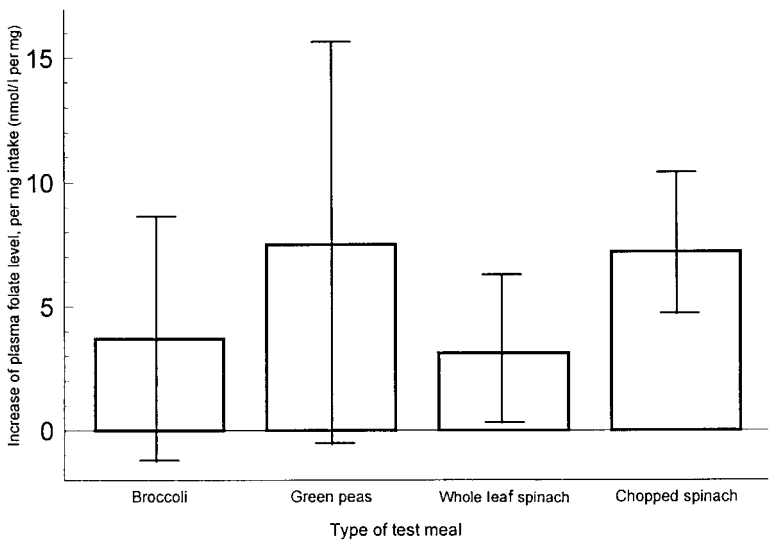

Fig. 4. Increases in folate levels in the plasma of healthy volunteers after 4 d of supplementation with vegetables compared with consumption of a low-carotenoid control meal, expressed as increase per mg folate intake from the vegetable supplemented meal. Values are means for twenty-six to thirty-one subjects, with 95 % CI represented by vertical bars. The amounts of folate provided per serving were (mg): broccoli 0·35, green peas 0·22, whole leaf spinach 0·60, chopped spinach 0·59. For details of procedures, see pp. 204–206.

the plasma response to folate following green peas consumption was larger than that following consumption of broccoli or whole leaf spinach (Fig. 4).

Compared with 4 d of consumption of the control meal, all of the vegetable-supplemented meals increased the plasma concentration of vitamin C significantly, while the synthetic β-carotene-supplemented meal had no effect (Table 2).

Discussion

The present study investigated the effect of 4 d consumption of different vegetables on the plasma status of carotenoids, folate and vitamin C. The 300 g vegetables/d was chosen as a relatively high, but acceptable amount with a reasonable chance of significant effects. In addition, the changes induced by the vegetable-supplemented meals were compared with those following consumption of a vegetable-free diet. Indeed, the results show that 300 g/d was sufficient to increase plasma levels of lutein and vitamin C for all vegetables in such a short time, whereas plasma levels of β-carotene were increased following consumption of broccoli or green peas but not spinach. The plasma concentration of folate was increased after consumption of spinach, which provided the largest amount of folate in the present study. It should be taken into account that the changes induced by vegetable consumption are the sum of the decrease due to exclusion of all vegetables and fruits from the diet and the increase due to consumption of 300 g vegetables/d. Furthermore, although the changes in plasma levels are used as a measure of relative bioavailability, it is not possible to extrapolate these changes into estimates of actually absorbed carotenoids, folate or vitamin C as the underlying kinetics of plasma and tissue distribution have not been assessed.

It is unlikely that 4 d is sufficient to reach new steady-state plasma concentrations for the investigated micronutrients and antioxidants as previous studies have indicated that this would take approximately 2–6 weeks (Micozzi *et al*. 1992; Levine *et al*. 1996; Truswell & Kounnavong, 1997). However, previous studies have shown that differences in plasma levels of carotenoids (Micozzi *et al*. 1992) and folate (IA Brouwer, personal communication) after short-term supplementation are related to the differences found after reaching a new steady state, although the absolute differences may deviate. Such a short-term protocol is advantageous compared with a long-term protocol because it is less labour-intensive and compliance with instructions is easier during a short period. Furthermore, in contrast to a single-dose protocol, it is possible to conduct a study in more realistic circumstances and investigate the effect of the test meals as part of a normal diet.

Effect of vegetable consumption on plasma carotenoid levels

We found a substantial difference between the various vegetables in their efficacy to increase plasma levels of β-carotene. Despite the 10-fold higher β-carotene content of spinach, the total increase in plasma levels induced by broccoli or green peas exceded that of spinach. We calculated the plasma response per mg β-carotene intake as a measure of the efficacy (Fig. 1), assuming a linear dose-response relationship at the dosages provided. When expressed as a percentage of the response induced by 1 mg synthetic β-carotene, the plasma responses to β-carotene after consumption of broccoli or green peas were, per mg β-carotene supplied, 74 % and 96 % of the response following supplementation with β-carotene. Micozzi *et al*. (1992) found that broccoli was 22–24 % as effective in increasing plasma levels of β-carotene compared with encapsulated β-carotene. In their study, the changes in plasma β-carotene levels were compared after 6 weeks intervention. As indicated earlier, the absolute percentages found after short-term supplementation may deviate from those found in steady-state plasma concentration. Both values are, however, higher than the percentage that we found in the present study for spinach, which was only 3–4 % as effective compared with the β-carotene-supplemented meal. De Pee *et al*. (1995) also showed that the relative bioavailability of β-carotene from green leafy vegetables is low. The present findings indicate that this observation is specific for green leafy vegetables and should not be extrapolated to all green vegetables.

There may be several explanations for the observed differences in bioavailability of β-carotene from vegetables. The vegetables contained different amounts of β-carotene and the efficiency of β-carotene absorption or conversion into retinol may decrease with increasing intake. The first phenomenon would imply a relatively lower plasma response following spinach consumption as this vegetable contained more β-carotene than broccoli and green peas. However, the increase induced by broccoli or green peas consumption exceded that of spinach, also without correction for the difference in β-carotene intake (Table 2). A less efficient conversion to vitamin A cannot explain our findings either, as a larger β-carotene response would be expected from a reduced conversion of β-carotene absorbed from spinach.

We used different cooking methods and times and heat treatment is suggested to enhance the bioavailability of carotenoids by loosening their binding to proteins (Erdman *et al*. 1988). However, spinach was heated longer than broccoli and green peas and this difference does therefore not explain the relatively lower availability of β-carotene from spinach. Another explanation might be the interaction of β-carotene with other carotenoids. It has been suggested that the presence of lutein may decrease the bioavailability of β-carotene (Kostic *et al*. 1995; van den Berg *et al*. 1998). However, the ratio β-carotene : lutein was higher, and thus more favourable, for spinach than for broccoli and green peas. Other absorption modifiers, such as fibre (Rock & Swendseid, 1992) may explain the differences. Although the meals were designed to provide the same amount of fibre, the type of fibre may have varied as the ratios hemicellulose : cellulose : lignin are different among broccoli, green peas and spinach (Spiller, 1992). Another factor is probably the intracellular location of carotenoids in vegetables. In plant leaves, carotenoids are present in chloroplasts and have a function in the process of photosynthesis by photoprotection and light collection (Cogdell & Gardiner, 1993). Little is known about the

location of carotenoids in other parts of plants. De Pee *et al.* (1998) recently found that β-carotene from fruits was more effective in increasing plasma levels of β-carotene and retinol than that from green leafy vegetables. If, as in fruits, carotenoids are present in chromoplasts of broccoli (the flower) or green peas (the seeds), this may explain the higher β-carotene response compared with spinach.

For lutein, the difference between spinach and the two other vegetables was less pronounced, although for this carotenoid also, broccoli and green peas were, per mg provided, more effective sources than spinach (Fig. 3). Again, one explanation may be a difference in location of the carotenoids in the plant cells. In addition, the higher β-carotene : lutein ratio in spinach compared with broccoli and green peas may have reduced lutein absorption (Kostic *et al.* 1995). The efficiency of lutein absorption from spinach may also have been reduced due to the larger amount of lutein present in the test meal.

Vegetable processing may improve the bioavailability of carotenoids, as has been indicated for β-carotene from carrots (Van Zeben & Hendriks, 1948; Hussein & El-Tohamy, 1990; Törrönen *et al.* 1996) and lycopene from tomatoes (Stahl & Sies, 1992; Gärtner *et al.* 1997; Porrini *et al.* 1998). Mechanical homogenization of the spinach before consumption resulted in a significantly higher plasma response to lutein. It did not, however, affect the plasma response to β-carotene. Lutein is more hydrophilic than β-carotene and this may have enhanced the release of lutein from the chloroplasts in the cytosol during disruption of the cell structure. The effect was moderate and the release of lutein was still lower than that from broccoli or green peas (Fig. 3). This suggests that a difference in characteristics of the vegetables may be the major determinant.

The differences in relative carotenoid bioavailability between different vegetables are important for the interpretation of health benefits of carotenoid consumption. Most epidemiological studies do not take into account the apparently substantial variation in carotenoid bioavailability from different foods. Giovannucci *et al.* (1995) showed that the association between intake of lycopene-rich foods and risk of prostate cancer varied among different foods. Differences in lycopene bioavailability may have been responsible for this observation (Gärtner *et al.* 1997). The present study shows that for β-carotene and lutein not only should the extent of vegetable processing be taken into account, but also the type of vegetable ingested. This may be an important reason for the rather low correlation coefficients (about 0·50) between carotenoid intake and plasma levels found in cross-sectional studies (Campbell *et al.* 1994; Scott *et al.* 1996; Drewnowski *et al.* 1997).

Effect of vegetable consumption on plasma folate level

A significant increase in plasma concentration of folate was found after 4 d consumption of spinach, which contained the highest amount of folate, whereas the increases following broccoli and green peas consumption almost reached significance. These increases indicate that the different vegetables are valuable sources of folate. When expressed per mg folate intake the investigated vegetables also differed in their folate bioavailability as green peas induced a larger response per mg folate intake than broccoli or whole leaf spinach (Fig. 4). The major part of folate in vegetables is present as polyglutamyl folate, which has to be converted enzymically into monoglutamyl folate before absorption. This conversion is suggested to be one of the limiting steps during the uptake of folate from natural dietary folate sources (Bailey, 1988). Müller (1993) found that 32 % of the total folate content was present as monoglutamyl folate in green peas. The proportion of monoglutamyl folate in the spinach used in the present study was about 30 % (Table 1). This suggests that other characteristics may account for the possible differences in folate bioavailability among the vegetables investigated. Possibly the differences in amount of folate supplied by the different vegetables interfered with their effectiveness to increase plasma folate levels (Truswell & Kounnavong, 1997).

Disruption of the whole leaf matrix of spinach enhanced the bioavailability of folate. However, the finding that chopped spinach induced a larger plasma folate response than whole leaf spinach cannot be attributed to a difference in the mono- : polyglutamyl folate ratio (Table 1). Our present study shows that disruption of the matrix makes folate more accessible for absorption. Apparently, the disruption of the whole leaf spinach matrix in the gastrointestinal tract is not complete and limits the bioavailability of folate.

Effect of vegetable consumption on plasma vitamin C level

Plasma vitamin C levels were increased after consumption of each of the vegetables and the differences in increases between the vegetables were related to differences in their vitamin C content. Due to saturation of plasma levels at about 80 μmol/l, the major factor predicting the plasma response of vitamin C following supplementation appears to be the previous vitamin C status of the volunteers (Levine *et al.* 1996). This concentration was not achieved in our present study, which may explain the dose–response effect of vitamin C we found. Previous studies have shown that the bioavailability of vitamin C from vegetables is similar to that from a supplement (Mangels *et al.* 1993*a*). Therefore, it is not surprising that chopping of whole leaf spinach did not improve the bioavailability of vitamin C. Release of vitamin C from the food matrix is apparently not a limiting step during its absorption.

In conclusion, the present study shows that the bioavailabilities of β-carotene and lutein vary substantially among different types of vegetables. Methods of processing of vegetables, such as mechanical homogenization, can improve the bioavailability of lutein and of folate. This variation in nutrient bioavailability should be considered when the impact of vegetable consumption on health is assessed.

Acknowledgements

We thank Willy Dubelaar, Bert Dubbelman, Marleen Essenberg, Edward Haddenman and other colleagues from the Unilever Nutrition Centre for their help in conducting the study, Jolanda Mathot, Wim van Nielen, Sjaak Sies and Ariette Trom-van den Beukel for analysis of the meals and blood samples, and Tom Wiersma for statistical evaluation

of the results. We acknowledge Professor Dr Clive West (Wageningen Agricultural University) for helpful discussions. We are indebted to the volunteers for their interest and participation in the study.

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