Eur J Nutr (2006) 45 : 29–36
DOI 10.1007/s00394-005-0559-6

ORIGINAL CONTRIBUTION

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GLV supplements increased plasma β-carotene, vitamin C, zinc and hemoglobin in young healthy adults

Summary *Background* Green leafy vegetables (GLV) are rich sources of β-carotene, iron and other micronutrients. Our in vitro studies have demonstrated good antioxidant potential in GLV. Moreover linkages of GLV intakes with plasma retinol and ascorbic acid were seen in apparently healthy Indians. *Aim of the study* To investigate the effect of GLV as a natural fortificant of multiple micronutrients through a prospective human trial. *Methods* Short-term (0–4 h) response (AUC) of single dose of 7.9 mg β-carotene and 130 mg ascorbic acid through a spinach-carrot meal against the standard meal without GLV plus10 mg β-carotene and 150 mg ascorbic acid tablets was studied in two groups of 4 young volunteers each. In the second trial of 3 weeks' supplementation, 5 groups of young adults ($n = 40$) were given either 100 g GLV/day alone or with tablets of vitamin E (100 mg/day), or C (100 mg/day) or more oil (5 g/day) or non-GLV meal with tablet of β-carotene (10 mg/day). Hemoglobin (Hb), plasma β-carotene, zinc, vitamin C, glucose, and triglycerides were measured. *Results* In a postprandial response, AUC were comparable in both GLV and standard meals for β-carotene and ascorbic acid. In case of triglycerides and glucose AUC the GLV meal showed a better recovery to the baseline value after 4 hours than the standard meal. Three weeks' supplementation of GLV with more oil resulted in significant increase of plasma β-carotene (51 %) and Hb (9 %). GLV with vitamin E showed a significant increase in plasma β-carotene (40 %), Hb (8 %) and plasma vitamin C (6 %). Supplementing β-carotene without GLV significantly increased Hb (11 %), plasma zinc (14 %) in addition to β-carotene. Multiple regression analyses weighted for energy intake indicated a significant association of percent increase in Hb with intakes of iron, riboflavin, folic acid, β-carotene, copper, phytate and fiber ($p < 0.01$), percent change in plasma zinc with intakes of zinc, β-carotene, vitamin C, riboflavin, copper, iron, and thiamin ($p < 0.01$), percent change in vitamin C with intakes of vitamin C, vitamin E, niacin, riboflavin, thiamin, β-carotene, zinc, phytate and fiber ($p < 0.05$) and percent change in plasma β-carotene with intakes of β-carotene, thiamin, folic acid, zinc, phytate and tannins ($p < 0.05$). *Conclusion* Using 100 g GLV/day with 10 g oil could be a single moderate strategy for supplementation of iron, β-carotene, ascorbic acid and zinc.

Key words green leafy vegetables – β-carotene – ascorbic acid – hemoglobin – zinc

Received: 18 August 2004
Accepted: 2 February 2005
Published online: 20 April 2005

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Introduction

A variety of green leafy vegetables (GLV) are good sources of β-carotene and many antioxidant micronutrients in addition to their richness in iron contents [1, 2]. There are limited prospective human studies reporting the bioavailability of β-carotene and other micronutrients from GLV-based vegetarian meals [3]. Our recent work has demonstrated i) 3–4 fold improvement in the in vitro bioavailable iron density of cereal-legume meals by inclusion of 100 g GLV [4] and ii) linkage of GLV with the plasma retinol and ascorbic acid in apparently healthy Indians through a cross-sectional study [5].

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Some of the commonly consumed GLV exhibited good antioxidant potential [6] indicating nutraceutical value to GLV. This is also observed through a significant and dose-dependent inverse association between GLV intake and ischemic heart disease risk [7]. Further studies assessing potential of GLV as a food-based intervention for multiple micronutrient enrichment of vegetarian diets are necessary.

β-carotene is important for vegetarians since it is the major source of vitamin A and also a lipid soluble antioxidant. The levels of total and bioavailable β-carotene are likely to be affected by the amount of oil used during cooking of the foods, fiber content and due to interactive effects of other micronutrients. However, systematic human trials to demonstrate effect of added oil in GLV have not been reported. It is likely that addition of antioxidant micronutrients such as vitamin C and E may influence the bioavailability of micronutrients from GLV. Prospective human studies in vegetarians demonstrating beneficial effects of GLV alone or GLV with micronutrients are essential to judge their efficacy as natural fortificants of multiple micronutrients.

Several experimental approaches have been used to study in vivo bioavailability of micronutrients such as β-carotene from foods or formulations [8]. Oral fecal balance is the classical approach but it has several drawbacks. An alternative approach to study absorption is either through short-term plasma responses as area under the curve or blood responses to long-term supplementation. Long-term plasma response after multiple doses of experimental foods to achieve a constant elevated level is of promise to estimate relative bioavailability of different sources [9].

Present study undertook prospective human trials for investigating beneficial effects (as natural fortificant of multiple micronutrients) of GLV alone or GLV plus oil or antioxidant micronutrients. Specific objectives were: i) to compare area under the curve (0 to 4 h) for β-carotene, ascorbic acid, triglycerides and glucose for spinach-carrot meal with non-GLV meal supplemented with β-carotene and vitamin C tablets, ii) to study the efficacy of GLV supplementation for 3 weeks in improving the plasma levels of zinc, ascorbic acid and β-carotene and hemoglobin, iii) to examine the effect of adding 10 g oil; or vitamin C or E to GLV meal on micronutrient status and iv) to examine associations of dietary micronutrient status as influencing factors on changes in hemoglobin and plasma levels of zinc, ascorbic acid and β-carotene.

Methods and materials

Subjects were the students with a diploma in medical laboratory techniques after their graduation in science (20–25 yr). They were selected based upon the criteria as: a) no history of chronic metabolic diseases like diabetes, hypertension, obesity, b) absence of gynecological problems like excessive blood loss for women.

Commonly consumed GLV were chosen for treatment groups. No unusual GLV or invasive procedure was adopted. The subjects participated voluntarily and chose the group under which they should be treated. They were informed about the experimental protocol and written informed consent was taken before the experiment.

Short-term response to a spinach-based meal

An experimental meal containing cereal, spinach and carrot was given to 4 young healthy volunteers and a non-GLV cereal meal along with marketed supplements (150 mg vitamin C and 10 mg β-carotene) was given to other 4 young healthy volunteers. The composition of both the meals is given in Table 1. All eight volunteers were observed in fasting condition and at 1, 2, 3, 4 hours after the meal for their plasma responses. At each time point, 2 ml of blood was drawn in EDTA coated tubes and stored at 4 °C during transport to the laboratory.

Table 1 Composition of meals and nutrient contents for plasma response for 4 hours

Type of meal	Composition	Energy (kcal)	Protein (g)	Fat (g)	CHO (g)	Fiber (g)	β-carotene (μg)	Ascorbic acid (mg)
Standard meal	Plain paratha[a] (200 g), Bottle gourd halva[b] (100 g), Watermelon (100 g)	878	18.8	35.8	120	26	454	9
	Tablets of supplements	0	0	0	0	0	10000	150
Experimental meal	Spinach paratha[a] (200 g), Carrot halva[b] (100 g), Fresh lemon with water (150 ml)	792	18.7	25.9	121	73	7900	133

[a] Plain paratha is a whole wheat pancake roasted with little oil. Spinach paratha is an unleavened layered pancake made of dough containing spinach paste, whole wheat flour and spices roasted with little oil
[b] Halva is a sweet condensed milk-based porridge containing grated bottle gourd or carrot

Plasma was prepared by centrifugation at 5000 RPM at 4 °C. Plasma levels of β-carotene, vitamin C, glucose and triglycerides were measured for all the samples within 4 hours of sample collection. The samples were allowed to attain room temperature (25–30 °C) before analysis.

Long-term responses (3 weeks) of young adults to GLV intake

A total of 50 young adults were allocated randomly to five different groups of treatment. However, considering compliance of the protocol, complete data were available only for 40 subjects. Characteristics of the subjects are given in Table 2. Dietary food intakes of these subjects were recorded by diary method throughout the study period of three weeks. Using our own cooked food nutrient value database, daily nutrient intakes of the subjects were computed (Table 3). The intakes of all the subjects were below the Indian RDA [10] for all the macro- and micronutrients.

At baseline, their blood status of β-carotene, iron and other micronutrients was examined (Table 4). Except iron, the status of other micronutrients was within the

Table 2 Characteristics of the subjects

Group	Age (years)	Weight (kg)	Height (cm)
GLV + N (n = 12)	20.7 ± 1.5	53.1 ± 3.5	157.8 ± 5.8
GLV + M (n = 4)	19.5 ± 0.29	50.7 ± 1.7	156.5 ± 12.0
GLV + E (n = 6)	18.7 ± 0.49	45.3 ± 2.7	163.3 ± 1.7
GLV + C (n = 7)	19.0 ± 0.58	45.2 ± 2.8	158.8 ± 5.4
Other vegetable + β-carotene (n = 11)	20.4 ± 0.69	55.5 ± 5.5	162.5 ± 2.5

Table 3 Mean daily nutrient intakes of the young adults at baseline

Nutrient intake/day	Group (Mean ± S. E.)				
	GLV + N	GLV + M	GLV + E	GLV + C	Other Veg + β-carotene
Energy (kcal)	1496 ± 118	1375 ± 228	1461 ± 236	1413 ± 161	1837 ± 221
Protein (g)	36.3 ± 1.9	32.1 ± 3.2	35.3 ± 7.3	32.7 ± 4.1	45.6 ± 5.6
Fat (g)	42.8 ± 2.6	37.7 ± 6.4	39.0 ± 3.5	41.3 ± 5.9	58.5 ± 8.6
CHO (g)	238 ± 25	242 ± 38	241 ± 43	225 ± 24	282 ± 32
β-carotene (μg)	1997 ± 328	1987 ± 58	1837 ± 383	1944 ± 351	1006 ± 133
Vitamin C (mg)	28.1 ± 2.7	22.9 ± 9.5	26.7 ± 5.1	23.3 ± 2.7	29.0 ± 3.2
Riboflavin (μg)	281 ± 37	181 ± 46	260 ± 65	254 ± 30	278 ± 43
Thiamin (μg)	610 ± 66	365 ± 32	707 ± 227	503 ± 66	753 ± 125
Folic (μg)	73.6 ± 4.9	83.6 ± 2.9	61.2 ± 2.2	68.3 ± 4.6	98.0 ± 5.7
Zinc (mg)	4.8 ± 0.53	5.0 ± 0.3	4.4 ± 0.7	4.3 ± 0.4	6.0 ± 0.4
Copper (mg)	1.71 ± 0.27	1.36 ± 0.28	1.41 ± 0.3	1.57 ± 0.13	2.0 ± 0.2
Iron (mg)	8.4 ± 0.74	7.8 ± 0.9	7.7 ± 1.6	7.6 ± 0.53	10.6 ± 1.0
Phytate (mg)	570 ± 81	650 ± 47	439 ± 32	514 ± 42	816 ± 50
Fiber (g)	30.7 ± 8.6	27.8 ± 2.7	29 ± 5	29 ± 4	39.5 ± 4.4
Tannin (mg)	64.8 ± 8.6	63.5 ± 23.5	72.5 ± 6.2	64.8 ± 3.4	65.3 ± 6.7

Intakes of all the nutrients were found to be far less than their respective RDA

Table 4 Mean blood status of the young adults at baseline

Blood level	Group				
	GLV-N	GLV-M	GLV + E	GLV + C	Other Veg + β-carotene
Hb (g/dl)	12.2 ± 0.3	12.7 ± 0.8	11.5 ± 0.4	11.7 ± 0.4	12.8 ± 0.2
Plasma zinc (mg/l)	0.84 ± 0.06	0.90 ± 0.07	0.93 ± 0.11	0.88 ± 0.06	0.90 ± 0.06
Plasma vitamin C (mg/l)	0.32 ± 0.01	0.30 ± 0.03	0.26 ± 0.02	0.31 ± 0.01	0.32 ± 0.01
Plasma β-carotene (μg/l)	1068 ± 113	918 ± 141	816 ± 79	1083 ± 153	1099 ± 111

Normal range cut offs: ascorbic acid > 0.2 mg/l, Hb ≥ 14.0 g/dl for men and Hb ≥ 12.0 g/dl for women, plasma zinc > 0.7 mg/l, plasma β-carotene > 200 μg/l normal range but on the lower end of the normal range. After the baseline observations, subjects were allocated to any one of the five groups of their choice.

Group 1 (GLV + N):	GLV (100 g/day) cooked with normal oil (5 g oil/100 g GLV/day)
Group 2 (GLV + M):	GLV (100 g/day) cooked with more oil (10 g oil/100 g GLV/day)
Group 3 (GLV + C):	GLV (100 g/day) cooked with normal oil + vitamin C (Celin tablet of 100 mg/day)
Group 4 (GLV + E):	GLV (100 g/day) cooked with normal oil + vitamin E (Evion tablet of 100 mg/day)
Group 5 (control):	Cereal-based diet with other vegetable but no GLV + Parry's β-carotene from spirulina (10 mg) thrice/week

GLVs consumed were: Fenugreek leaves, Amaranth g. tricolor, Amaranthus v., Amaranthus p, spinach, cabbage, colocasia, onion stalks, radish leaves. Nutrient compositions of these GLVs have been given in our earlier study [2]. Subjects consumed 100 g of any of the above-mentioned GLV per day as per their choice. In addition, small amounts (5 g) of coriander/curry leaves were used in the diet for garnishing and taste.

Blood sample collection was done at two time points; at the start and after GLV supplementation for 3 weeks. All the subjects were observed for changes in plasma levels of β-carotene, zinc, vitamin C and hemoglobin.

Plasma levels of β-carotene and vitamin C were measured by spectrophotometry and plasma zinc by atomic absorption spectrometry. Hemoglobin, plasma glucose and triglycerides were assessed by standard kits. The details of the methods of estimation are reported in our previous studies [11].

Statistical methods

All statistical analyses were performed using SPSS version 11.0 under Windows. AUC were computed using the trapezoidal method. In the long-term study, data of blood parameters did not fulfill the assumptions for carrying out ANOVA for comparing different groups. Therefore the nonparametric Wilcoxon test was used for the significance of difference between the paired observations of blood parameters before and after supplementation. To understand the relationship of nutrient intakes with percent change in each of the four study parameters, weighted least square multiple regression analysis was carried out with the percent change after supplementation as the dependent variable and dietary intakes of nutrients, phytate, fiber, tannins as independent variables weighted for calorie intakes. The coefficient of determination R^2 has been given for the adequacy of the model along with level of significance.

Results

Short-term responses to GLV meal vs standard meal

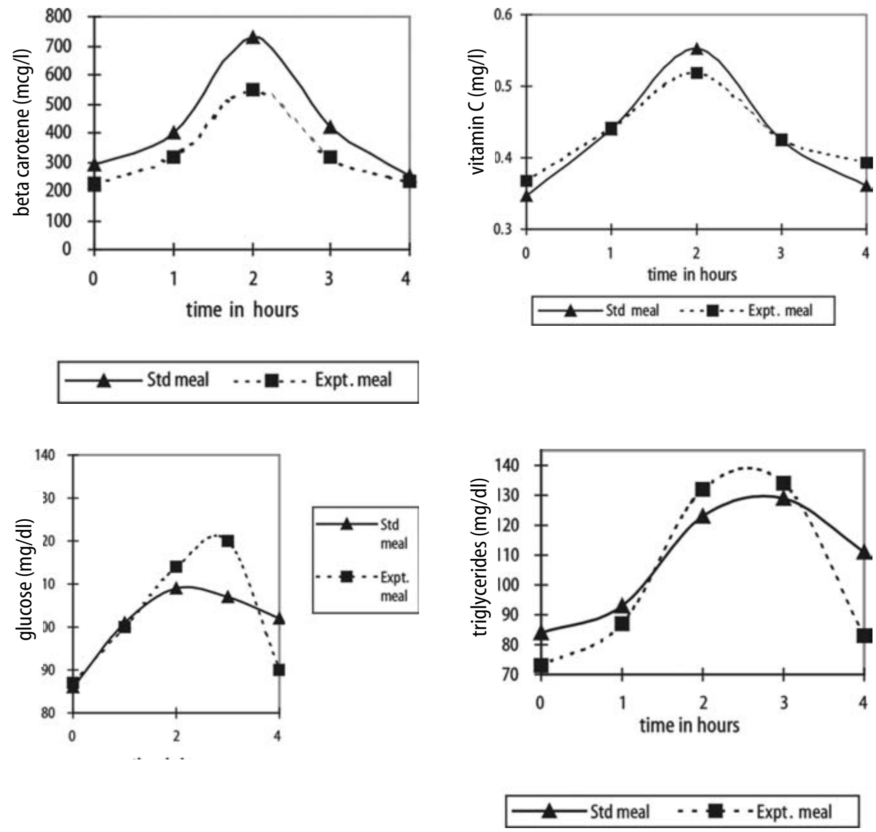
Mean plasma levels of β-carotene and vitamin C at five time points are shown in the Fig. 1a and Fig. 1b. Absorption of β-carotene peaked at 2 hours and returned to base level after 4 h. AUC by trapezoidal method was found to be 463 and 642 μg/L · h for experimental and standard meal, respectively. Although the experimental meal (spinach-carrot) showed lower response than the standard meal (non-GLV with tablet), total increase of β-carotene per unit intake of β-carotene was comparable in both the meals. For vitamin C, the AUC for the experimental and standard meals were 0.26 and 0.34 mg/L · h, respectively. However, when the responses were adjusted for fiber contents, the standard meal showed a three times higher absorption of β-carotene than the experimental meal but not for vitamin C. In the case of triglycerides and glucose response (Fig. 1c and Fig. 1d), the experimental meal showed a better recovery to the baseline value after 4 hours than the standard meal. The trial also showed the feasibility of monitoring the changes in levels of β-carotene and vitamin C after supplementation of GLV.

Long-term responses (3 weeks') to GLV supplementation

Table 4 shows baseline blood status of the subjects in the five groups. At baseline, 62 % of the subjects had hemoglobin (Hb) values within the normal range and 38 % had slightly lower Hb values than the lower cut-off value of the normal range. Plasma β-carotene levels of all subjects were near the lower cut-off for the normal range of 200–1990 μg/l. The plasma zinc levels of three subjects were slightly below the normal level, while plasma vitamin C level of two subjects was lower than the lower cut off for the normal range.

After 3 weeks' supplementation of green leafy vegetable (GLV) with or without oil/micronutrients, there were variable responses between the five groups with respect to hemoglobin, plasma β-carotene, plasma ascorbic acid and plasma zinc. Hemoglobin levels increased significantly in all five treatment groups by 6.4 % to 11.1 % ($p < 0.05$) (Fig. 2a). Plasma zinc levels increased by 2.5 % to 14.5 % (Fig. 2b), with a significant increase in the group with supplementation of a β-carotene tablet ($p = 0.007$) and a marginal increase in the groups GLV + vitamin C and GLV with more oil ($p < 0.1$). Plasma vitamin C levels increased by 2.1 % to 8.2 %

Fig. 1 Comparison of the spinach meal with the standard meal for plasma responses of **a**) β-carotene, **b**) vitamin C, **c**) glucose and **d**) triglycerides over time



(Fig. 2c) with a significant increase in the GLV + vitamin C (p = 0.05), GLV with normal oil (p = 0.002) and marginally in GLV + vitamin E (p = 0.075) groups. There was an increase of 5.7 % to 51 % in plasma β-carotene levels (Fig. 2d), but the increase was significant only in groups with β-carotene tablet (p = 0.033), GLV + vitamin E (p = 0.028) and marginally significant for GLV with more oil (p = 0.05).

To further investigate the linkages of dietary intakes of micronutrients from food plus supplements with plasma responses, multivariate analyses were carried out using micronutrient intakes during the intervention phase adjusted for energy intakes. Weighted regression analysis indicated that intakes of iron, riboflavin, folic acid, β-carotene, copper, phytate and fiber were significantly associated with percent change in hemoglobin ($R^2 = 0.86$, p = 0.001). When percent change in plasma zinc was regressed, intakes of zinc, β-carotene, vitamin C, riboflavin, copper, iron, and thiamin were significant influencing factors ($R^2 = 0.815$, p = 0.004). Percent change in vitamin C was found to have an association with intakes of vitamin C, vitamin E, niacin, riboflavin, thiamin, β-carotene, zinc, phytate and fiber ($R^2 = 0.93$, p = 0.001). Percent change in plasma β-carotene showed an association with intakes of β-carotene, thiamin, folic acid, zinc, phytate and tannins ($R^2 = 0.72$, p = 0.001).

Discussion

GLV is a rich source of many antioxidants like β-carotene, other carotenoids, vitamin C (ascorbic acid), vitamin E (tocopherol), zinc, selenium and polyphenols. As compared to fruit vegetables, root vegetables and fruits, GLV are a cheaper commodity and are also easy to grow in the kitchen gardens in all seasons. During the rainy season, many wild but edible GLV are available in the uncultivated land. Most of the prospective studies related to GLV are mainly on spinach for β-carotene availability. The present study highlights the potential of three more GLV along with spinach as supplements of other micronutrients. Results by Mulokozi G et al. [12] indicated that cooked green leafy vegetables constituted the major source of pro-vitamin A carotenoids, in populations with low intake of yellow/orange fruits and preformed vitamin A. Performance of different GLV in the traditionally cooked form or raw salad form as micronutrient supplements in terms of their contents [4], antioxidant capacity [6] and in vitro dialysability of iron and zinc [2] has been demonstrated. The in vivo present study confirmed the usefulness of GLV as a multiple micronutrient supplement.

Plasma concentration vs. time curves after single or

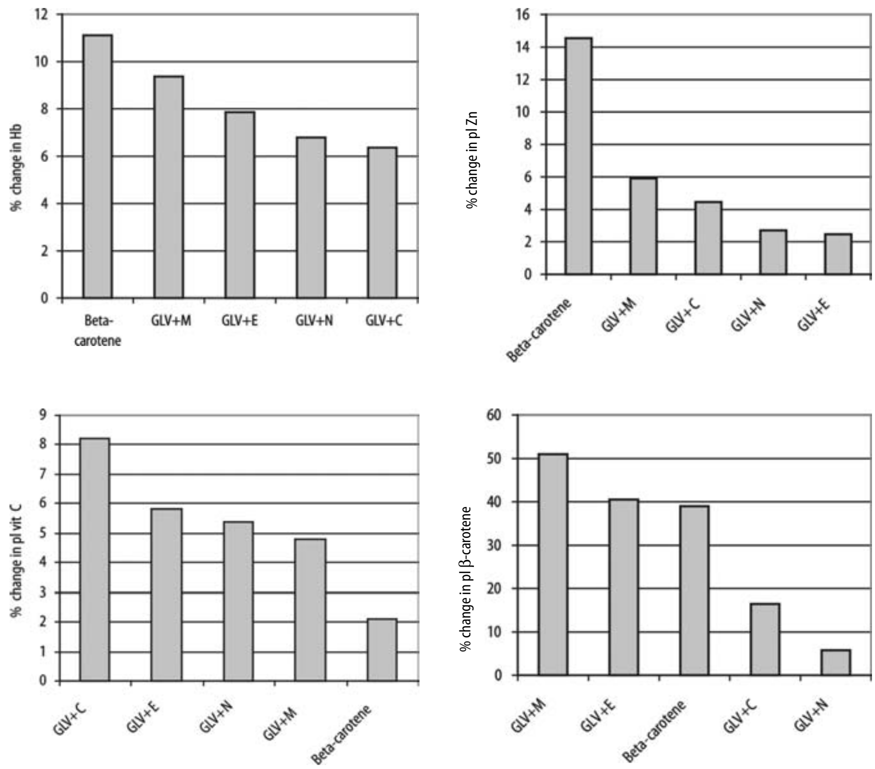


Fig 2 Effect of different supplementation on **a**) hemoglobin, **b**) plasma zinc, **c**) plasma vitamin C and **d**) plasma β-carotene in young adults (bars represent median value)

multiple oral doses have been widely used as an index of carotenoid bioavailability [8]. However the majority of such studies are based on high doses of β-carotene or use pharmacological preparations. Our study based on the simple technique of spectrophotometry has demonstrated the feasibility of using the plasma response curves at lower dosages for the relative comparisons of bioavailability of β-carotene and vitamin C from a natural source of these micronutrients with tablets as the standard source. Furthermore, the fasting and postprandial β-carotene levels observed in the present study are comparable with the reported studies [13].

The present data indicated that the spinach meal was equally good in terms of percent absorption of β-carotene and vitamin C vs. the tablets of β-carotene (Spiruvin) and ascorbic acid (Celin). A similar observation has been reported in the case of children whose absorption of β-carotene from spirulina and GLV and carrots was on the order of 72 to 75 % [14]. Although the peak for glucose and triglycerides was higher for the spinach meal than the non-GLV meal, recovery to baseline levels of plasma glucose and triglycerides was faster in the spinach meal than the non-spinach meal. Furthermore, the beneficial effect of spinach on glycemic response is supported by the fact that some of the marketed ayurvedic formulations for diabetes treatment contain dried spinach powder as one of the ingredients. This suggests the presence of an insulin stimulating factor in spinach, which needs further confirmation, and in depth investigation.

The initial average plasma β-carotene levels of the subjects were between 226 and 290 μg/L, which are at the lower cut-off for the normal range 200–1990 μg/L. This may be one of the reasons for higher absorption of β-carotene by these subjects. The amount of β-carotene in the meals was 7900 and 10000 μg. The corresponding peaks in the plasma levels were at 320 and 440 μg/L, which amounts to 4 % of the intake. In addition, these are in the range of observed peak of 2.23 μmols/L or 1197 μg/L for carotene supplements vs. 0.18 μg/L or 97 μmols/L for a low β-carotene meal [13].

Single micronutrient deficiency is generally not seen but deficiencies of multiple micronutrients co-exist. For example, our previous study on anemia indicated a graded rise in deficiency of vitamin C, vitamin A, riboflavin, folic acid and copper with increasing degree of iron deficiency anemia [14]. In India, deficiencies of iron, vitamin A, zinc and iodine are important issues of concern [15]. Results by Navarro & Wood [16] suggest using multi-micronutrients dietary supplements as a better vehicle to decrease the prevalence of multiple micronutrient deficiencies in healthy adults. Our results in this study corroborate this fact with an increase of 0.9 to 1.5 g/dl in hemoglobin, 220–523 μg/L in plasma β-carotene and marginal increases in zinc and ascorbic acid after GLV supplements. There was a manifold increase in plasma β-carotene when GLV were supplemented with more oil than GLV with normal oil. This finding matches with the study by Brown et al., who have reported a substantially greater absorption of carotenoids when fresh vegetable salads were consumed with full fat than with reduced-fat salad dressing [17]. Kiefer et al. have reported an increase in plasma β-carotene and vitamin C after supplementing mixed fruit and vegetable juice concentrates up to 14 weeks [18]. Our results demonstrated a similar effect in three weeks of supplementing GLV in the normal diet probably because GLV were richer in β-carotene, vitamin C and other trace elements than the fruit vegetable juice.

Supplementing GLV with a tablet of 100 mg ascorbic acid was thought to be a useful strategy for improving hemoglobin through enhancement of iron absorption. The GLV + vitamin C group did show a significant increase of Hb. However, the increase in hemoglobin for this group was lower than the β-carotene group. This could be due to the fact that ascorbic acid is more susceptible to degradation than the fat-soluble micronutrients like β-carotene and alpha tocopherol, and oxidized ascorbic acid is an active and harmful metabolite (strong glycating agent). β-carotene and tocopherol were found to have positive influence on utilization of iron as indicated by the significant increase in hemoglobin.

Plasma ascorbic acid response for the GLV + C group was highest, which was not surprising; this group was followed by the GLV + E group. Vitamin E may be acting as a protective factor against oxidation of ascorbic acid and thus maintaining its plasma level. In case of plasma β-carotene responses, GLV with normal oil group performed the lowest but addition of oil or vitamin E increased the response dramatically. Vitamin E acts as antioxidant in vivo and is known to protect β-carotene from oxidative degradation. The present results suggest a need to either moderately increase the amount of oil or to use vitamin E tablets as an adjunct to GLV supplementation to improve their efficacy. Many of the vegetable oils are good sources of vitamin E and could serve as a common boosting factor for both β-carotene and hemoglobin.

Although the enhancing effect of β-carotene on iron bioavailability is known, the increase of plasma zinc by supplementation of β-carotene is a new finding. Our earlier study showed an association of β-carotene intake with the erythrocyte membrane zinc, an indicator of long-term zinc status (Agte et al. jnpublished). The present data further support the interaction of zinc and β-carotene, but more evidence is necessary through a prospective intervention study on human volunteers to understand the mechanism of its action. Bioavailability of zinc is known to be inhibited by phytic acid, iron and copper [19]. Similar results were obtained in our regression analysis except for phytic acid. This may be due to degradation of phytates by traditional cooking practices [20].

Riboflavin was shown to be a significant enhancer of iron bioavailability in the animal model [21, 22]. In the present study, intakes of riboflavin, iron and vitamin C were also significantly associated with the percent increase in hemoglobin ($p = 0.001$).

Although GLV are promising as natural fortificants of iron, β-carotene and vitamin C in vegetarian diets, children and youngsters may have food preferences and they are quite fussy about consuming green leafy vegetables and fruits thus compromising their intake of micronutrients from important dietary sources. Special efforts are needed to inculcate the habit of eating GLV in this class. It has been emphasized in the WHO guidelines to use existing eating patterns in remedying iron deficiency rather than giving pharmacological preparations [23]. This is evident from the mucosal cell damaging effects of unabsorbed ferrous sulphate reported when giving the highly bioavailable form of iron as 100 mg ferrous sulphate [24]. Considering the wide prevalence of micronutrient deficiencies particularly in vegetarians, GLV offer a moderate but natural source of iron. These are equally rich in protective micronutrients like β-carotene, zinc, ascorbic acid and indicate that consuming 100 g GLV per day could be a single strategy for supplementation of multiple micronutrients viz. iron, β-carotene, ascorbic acid and zinc.

Acknowledgement The authors are grateful to R. D. Birla Smarak Kosh. Bombay Hospital, Mumbai for financial support. Thanks are also due to the volunteers of the study for their co-operation and active participation. Authors wish to thank Dr K M Paknikar for his help in using the atomic absorption spectrometer.

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