Availability of micronutrients from dried, encapsulated fruit and vegetable preparations: a study in healthy volunteers

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Abstract

Background High levels of consumption of fruit and vegetables, which contain antioxidant nutrients including vitamins C and E as well as β-carotene, are associated with reduced rates of atherosclerotic arterial disease and some cancers. Low plasma levels of these micronutrients are associated with an increased risk of chronic degenerative disease. While increasing consumption of fruit and vegetables would be desirable, many factors including cost tend to prevent this. Preparations of the active components of fruit and vegetables encapsulated to achieve convenience may contribute to solving this problem.

Aims This study was designed to determine whether two such supplements prepared from dehydrated fruit and vegetable extracts (Juice Plus+[TM] Fruit and Juice Plus+[TM] Vegetable) would result in elevation in blood concentrations of antioxidants to levels associated with lower disease risk.

Methods Sixteen healthy subjects (eight male, eight female, aged 18–52 years) were screened to exclude disease and to exclude any individual with a serum β-carotene below 0.05 μmol L^{-1}, and were divided into two groups of eight, each to consume either the fruit or vegetable preparation. Four days before administration of the capsules the subjects commenced a low β-carotene diet. Venous blood samples for determination of vitamin C, vitamin E (as α-tocopherol) and β-carotene were taken in the morning on days 0, 2, 4, 6 and 7, after a 10-h fast. Two capsules of either the fruit preparation (containing vitamin E 30 mg, vitamin C 150 mg and β-carotene 6 mg) or vegetable preparation (containing vitamin E 30 mg, vitamin C 50 mg and β-carotene 9 mg) were taken on days 0–6 inclusive with a 610-kcal breakfast (47% energy from fat).

Results Serum β-carotene levels rose significantly by 0.56 μmol L^{-1} and 0.54 μmol L^{-1} after fruit and vegetable preparations, respectively, by day 7, vitamin E levels rose significantly by 3.1 μmol L^{-1} after the fruit preparation but not after the vegetable preparation and vitamin C rose significantly from 28 to 62 μmol L^{-1}, and from 32 to

50 μmol L^{-1} after the fruit and vegetable preparations, respectively. Plasma malondialdehyde, regarded as a general indicator of peroxidation, fell significantly by 19×10^{-5} μmol L^{-1} (about a 40% reduction) in both treatment groups.

Conclusions   These results indicate that concentrated extracts of fruits and vegetables can raise blood antioxidant levels into the ranges associated with reduced risk of disease and that this reduces the concentration of a general measure of lipid peroxidation. Further studies, especially in those with disease characterized by increased oxidative stress, are indicated.

Introduction

A high dietary consumption of fruit and vegetables is associated with reduced rates of certain chronic diseases, notably atherosclerotic arterial disease (Gey *et al.*, 1993; Rimm *et al.*, 1993; Stampfer *et al.*, 1993), cancer (Steinmetz & Potter, 1991; Zeigler, 1991; Block *et al.*, 1992) and neurovascular disease (Acheson & Williams, 1983). Fruit and vegetables contain an abundance of antioxidant nutrients, in particular vitamins C and E as well as β-carotene (McCance & Widdowson, 1994). Suboptimal plasma levels of these micronutrients are inversely correlated with an increased risk of chronic degenerative diseases (Riemersma *et al.*, 1991; Stahelin *et al.*, 1991; Gey *et al.*, 1993). It has been suggested that the antioxidant properties of vitamins C and E and β-carotene, by preventing free radical-induced oxidative damage, protect against the development of these age-related diseases (Diplock, 1991; Halliwell & Chirico, 1993).

Within the population, the intake of fruit and vegetables varies according to a number of factors including sex, age, region and socioeconomic class. In a dietary survey of British adults, it was found that women consumed more fresh fruit and vegetables than men (Gregory *et al.*, 1990), as did older people and those in the higher socioeconomic groups (Colhoun & Prescott-Clarke, 1994). Fewer fruits, salad and green leafy vegetables were consumed in Northern England and Scotland. Intake of dietary vitamins and minerals was lower in men and women in Scotland (Smith *et al.*, 1989) where death rates from coronary heart disease have been consistently and significantly higher in both men and women in all age groups compared to England and Wales in the past half century.

Smokers had lower plasma levels of β-carotene and vitamin C, both of which were positively correlated with dietary intake of these nutrients (Stryker *et al.*, 1988; Schectman *et al.*, 1989; Riemersma *et al.*, 1991), and vitamin C intake (in both smokers and nonsmokers) was seasonal, with the highest consumption in the summer (Gregory *et al.*, 1990).

These variations in the consumption of fruit and vegetables across subgroups in the population highlight the difficulty of establishing a consistent intake of fruit and vegetables. Recent efforts by health agencies, the medical profession and the media encouraging people to increase their intake of these foods have not met with success. During the decade between 1985 and 1994, the family consumption of fruit and vegetables decreased by up to 14% across income groups whilst expenditure on fruit and vegetables rose by an average of 70% (National Food Survey Committee, 1986, 1995). Thus, the trend throughout this period has been for people to spend more but eat less of these foods.

In addition to the cost, the preparation of fruit and vegetables, often involving peeling, chopping and cooking, acts as a disincentive to eat these foods, in particular for people working outside the home. These factors have meant that the health message to eat more fruit and vegetables has fallen largely on deaf ears.

There is therefore considerable merit in looking for alternative convenient methods of administering

the active nutritional components in fruit and vegetables whilst retaining the same proportions of these components as exists in the fresh foods.

The objective of this study was to determine in healthy volunteers of both genders whether serum levels of β-carotene and α-tocopherol and plasma levels of ascorbic acid rise following daily repeat dosing of dried and powdered fruit or vegetable preparations and to measure lipid peroxidation capacity following consumption.

Methods

Design and subjects

A single-centre, open repeat-dose parallel study was undertaken on 16 subjects (eight men and eight women aged 18–52 years and with a body mass index within the range 19–27 kg m^{-2}). The subjects had no history of gastrointestinal surgery or other significant pathology, were nonsmokers, had no history of alcohol or drug abuse, were nondiabetic, were not on a calorie-reduced or vegetarian diet nor were taking antioxidant preparations, had a pretreatment baseline β-carotene level of > 0.05 μmol L^{-1} and, if female, were not pregnant or lactating. No concomitant medication was allowed during the study except the contraceptive pill.

Location, ethical review and independent monitoring

Subjects were recruited from a panel of volunteers and the work was undertaken in the Clinical Unit of BIBRA Toxicology International. The protocol was reviewed and approved by the BIBRA International Ethics Committee and was independently monitored by a scientist from Huntingdon Life Sciences, critical phases being inspected according to a standard QA programme. Good Clinical Practice standards were followed throughout.

Baseline diet

Subjects were randomly allocated to either treatment with two capsules of the vegetable blend for 7 days or two capsules of the fruit blend for 7 days.

Four days before administration of the capsules subjects commenced a low β-carotene diet, designed to ensure that the rapidly turning over pool of β-carotene was low and stable, and continued the diet throughout the study. The diet was based on total avoidance of foods containing high levels of β-carotene (e.g. carrots, cooked red pepper, spinach, mango) and limited consumption of foods containing lower levels of β-carotene (e.g. orange, melon, green pepper).

Preparations

Juice extracts and the residual pulp from freshly picked apples, oranges, pineapples, papaya, cranberries and peaches, and from freshly picked carrots, parsley, beets, broccoli, kale, cabbage, spinach and tomatoes were cryo-evaporated by a proprietary process (Trademark: JuicePlus+^{TM}; NSA International, Memphis, TN, USA) to concentrate and preserve nutrients. The fruit and vegetable powders included *Dunaliella salina* (Henkle Corporation, Le Grange, IL, USA) acerola cherry (Schweizerhall, Piscataway, NJ, USA), and soy derived d-α-tocopherol (Henkle) to provide standardized levels of natural β-carotene, ascorbic acid and α-tocopherol, respectively. The dried juice extracts and pulp blends were encapsulated in hard gelatin capsules to provide 850 mg of fruit powder per fruit capsule and 750 mg of vegetable powder per capsule.

Table 1 shows the micronutrient content of each daily dose.

Dosage and blood sampling regimen

A blood sample for screening of β-carotene was taken 1–2 weeks before commencement of the study and was analysed immediately. Further blood samples were taken just before and 2, 4, 6 and 7 days after commencement of capsule administration, in each case preceded by a 10-h fast from 22:00 hours to 08:00 hours. Two capsules were taken on seven consecutive days, in each case with a high-fat breakfast composed of one cheese sandwich and a drink of semiskimmed milk (approximately 610 kcal, 32 g fat, 52 g carbohydrate, 28 g protein and 47% energy from fat).

Table 1 Micronutrient contents (mg).

Micronutrient	Fruit blend (2 capsules)	Vegetable blend (2 capsules)
Vitamin E	30	30
Vitamin C	150	50
Thiamin	0.4	0.6
Riboflavin	0.3	1.0
Niacin	7	13
Vitamin B6	1	1.5
β-carotene	6	9
Calcium	35	60
Magnesium	25	45
Zinc	1.5	2.5
Manganese	0.3	1.5
Folacin	100 μg	300 μg
Selenium		35 μg
Chromium	18 μg	30 μg

The capsules were swallowed after a quarter of the breakfast had been eaten.

Blood sample processing and biochemical analysis

Venous blood samples (20 mL) were drawn as noted above, were protected from light at all times, and prepared as serum (stored at $-70\,^{\circ}\text{C}$ for measurement of β-carotene and α-tocopherol) and as plasma (stored at $-20\,^{\circ}\text{C}$ for ascorbic acid analysis and analysed immediately for malondialdehyde as a measure of lipid peroxidation).

Analysis was undertaken in the laboratories of BIBRA in accordance with international standards of Good Laboratory Practice. Beta-carotene and α-tocopherol were analysed by HPLC methods based on the methods of Milne & Botnen (1986) and Hatam & Kayden (1979), respectively. Ascorbic acid was measured by a colorimetric method based on the method of Freed (1966). Malondialdehyde was measured by an HPLC method based on the method of Halliwell & Chirico (1993).

Statistical analysis

Serum and plasma levels of measured variables at 7 days were compared with baseline levels using a paired *t*-test.

Results

Sixteen subjects completed the study. During administration, five subjects reported minor symptoms (three upper respiratory tract, one urinary and one musculoskeletal), all of which resolved spontaneously and none of which was deemed likely to have been related to administration of the supplements.

Serum β-carotene levels rose significantly by day 7 after administration of both the fruit and the vegetable preparations ($P < 0.001$ in both cases). The mean rise in the case of the fruit blend was $0.56\ \mu\text{mol L}^{-1}$ whilst in the case of the vegetable blend it was $0.54\ \mu\text{mol L}^{-1}$. In both cases the increase was equivalent to a doubling of the baseline serum level (Fig. 1).

Serum vitamin E levels rose significantly after the fruit preparation by $3.1\ \mu\text{mol L}^{-1}$ ($P < 0.05$), but the rise of $1.2\ \mu\text{mol L}^{-1}$ after the vegetable preparation was not significant (Fig. 2).

Plasma vitamin C levels rose significantly after both the fruit preparation (from a mean of $28\ \mu\text{mol L}^{-1}$ to $62\ \mu\text{mol L}^{-1}$, $P < 0.001$) and
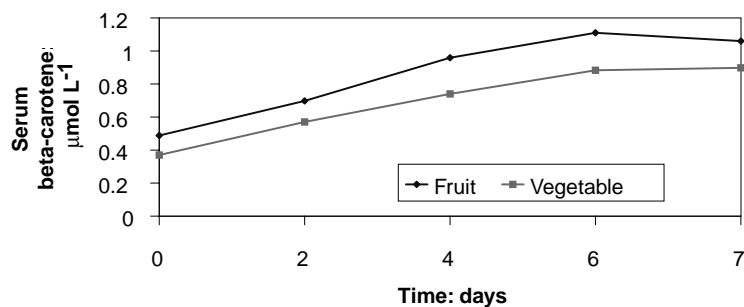

Figure 1 Serum β-carotene (μmol L^{-1}) over 7 days in eight subjects treated with fruit preparation and eight subjects treated with vegetable preparation.
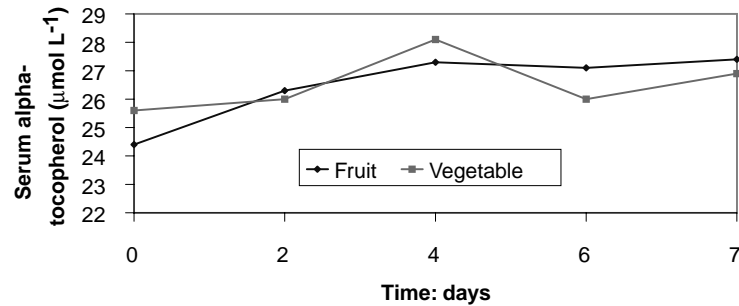
Figure 2 Serum vitamin E (μmol L^{-1}) over 7 days in eight subjects treated with fruit preparation and eight subjects treated with vegetable preparation.

after the vegetable preparation (from a mean of 32 μmol L^{-1} to 50 μmol L^{-1}, $P < 0.001$) (Fig. 3).

Plasma malondialdehyde levels fell significantly by day 7 in both fruit-treated and vegetable-treated subjects by 19×10^{-5} μmol L^{-1} to 29×10^{-5} μmol L^{-1} (fruit preparation, $P < 0.001$) and 32×10^{-5} μmol L^{-1} (vegetable preparation, $P < 0.001$) (Fig. 4).

Discussion

The changes in serum and plasma levels of β-carotene and vitamins E and C shown after 7 days of administration of the fruit and vegetable preparations provide evidence for the bioavailability of these compounds from this type of preparation, though the vitamin E response was not statistically significant after consumption of the vegetable preparation.

The method used for the assessment of lipid peroxidation was the modification of the thiobarbituric acid (TBA) reactive substances test − revised by Halliwell to avoid many of the problems of the original method; the results expressed as malon-

dialdehyde concentrations can be influenced by a number of factors, for example the concentration of antioxidants (especially alpha-tocopherol) in the blood (Halliwell & Chiroco, 1993). The HPLC-based TBA method is regarded as a preliminary general measurement of peroxidation (Halliwell & Chiroco, 1993), and thus in the context reported here the results suggest that the treatment reduced lipid peroxidation.

These results therefore provide evidence that the oral administration of dried and powdered preparations of whole fruits and vegetables can result in changes of blood levels of antioxidant micronutrients and oxidation products strongly suggestive of a potent antioxidative effect, thus demonstrating the bioavailibility of these antioxidants from this type of product. There are many formulated antioxidant products on the market for which there is little published evidence for good bioavailibility.

Many experimental studies have described dynamic synergistic interactions between antioxidants, for example vitamin E recycling by vitamin C, without which the antioxidant status of individual micronutrients may be significantly affected and the
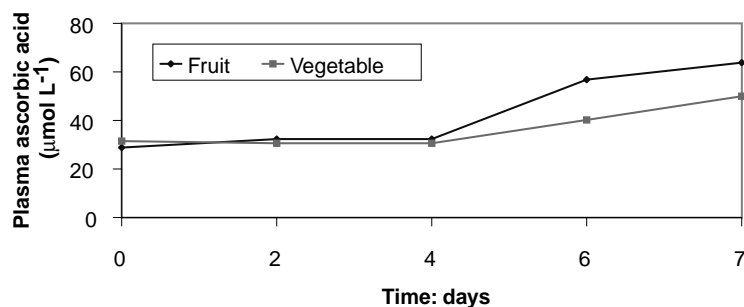

Figure 3 Plasma vitamin C (μmol L^{-1}) over 7 days in eight subjects treated with fruit preparation and eight subjects treated with vegetable preparation.
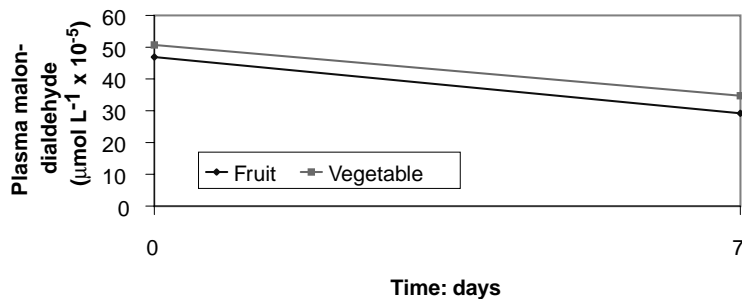
Figure 4 Plasma malondialdehyde (μmol L^{-1} $\times$ 10^{-5}) over 7 days in eight subjects treated with fruit preparation and eight subjects treated with vegetable preparation.

risk of cell damage due to lipid peroxidation increased (Sies, 1991; Gey, 1992).

It has been suggested that to optimize the antioxidant potential for reducing the risk of chronic disease, these micronutrients should be consumed in combination (Gey *et al.*, 1993). In particular, data from cross-cultural epidemiological studies suggest that the risk of ischaemic heart disease may be reduced when plasma concentrations of combined antioxidants have the following values: vitamin E: 27.5–30.0 μmol L^{-1}; β-carotene: 0.4–0.5 μmol L^{-1}; vitamin C: 40–50 μmol L^{-1}. These values are comparable to the mean blood levels of vitamin E (27.3 μmol L^{-1}), and vitamin C (56 μmol L^{-1}) though somewhat lower than the mean blood level of β-carotene (0.98 μmol L^{-1}) after 7 days of treatment with fruit and vegetable preparations reported here. Furthermore, Zino et al. (1997) demonstrated that 44 healthy volunteers who raised their consumption of fruit and vegetables over an 8-week period from an average of 2.4 to 7.1 servings per day showed rises of blood nutrient levels of: α-tocopherol 20.7 μmol L^{-1} to 20.9 μmol L^{-1}, β-carotene 0.34 μmol L^{-1} to 0.52 μmol L^{-1} and vitamin C 33.5 μmol L^{-1} to 57.9 μmol L^{-1}. An analysis of available data provides the basis for proposed daily nutrient intakes of 150 mg vitamin C, 30 mg vitamin E and 3 mg β-carotene (Blumberg, 1995). The daily doses of vitamin E in both fruit and vegetable preparations and of vitamin C in the fruit preparation are identical to Blumberg's proposed daily intakes; however, the β-carotene contents of both preparations are higher than Blumberg has suggested but well within the ranges (up to 15 mg daily) suggested by other authors and within the range used to correct evidence of oxidative stress,

for example in children with cystic fibrosis given 13.2 mg per day (Lepage *et al.*, 1996).

In view of the evidence for abnormal oxidative stress in some disease states, e.g. in cystic fibrosis (Brown & Kelly, 1994) and in hyperlipidaemia (Chen *et al.*, 1994) and in smokers (Bridges *et al.*, 1993), further investigation of the use of this type of preparation in these conditions and in healthy subjects over longer periods of time is indicated.

Acknowledgments

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