

Antioxidant vitamin status in high exposure to oxidative stress in competitive athletes

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We conducted a cross-sectional study in 118 well-trained athletes to investigate 'high exposure' to sub-deficient antioxidant status, and consequently to oxidative damage, in relation to estimated daily energy expenditure (EE) and dietary antioxidant intake. Subjects completed 7 d food and activity records. Blood samples were obtained on day 8. Of the athletes 81, 60 and 43 % had intakes of vitamins E, C and β -carotene below two-thirds of the French RDA respectively, which is adjusted for EE (FRDAa). The deficit in vitamin E intake was positively correlated with EE (r 0.51, $P < 0.0001$). All the athletes had normal plasma vitamins E and C and 14 % had marginal plasma β -carotene. Plasma thiobarbituric acid-reactive substances (TBARS) did not increase with increased EE. As evidenced by ANOVA, EE-induced vitamin C intakes increased and consequently led to increased plasma ascorbic acid concentrations. In male athletes, plasma total carotenoids were negatively correlated with plasma TBARS concentrations (r -0.31, $P < 0.006$). The relationship between vitamin C intakes and plasma concentrations was logarithmic (r 0.59, $P < 0.0001$). To summarize, it is not clear whether vitamin E requirements are overestimated with reference to EE in the FRDAa. Daily requirements for vitamin C do not exceed 200 mg. Our present results could be interpreted as meaning that carotenoids play a protective role as exogenous antioxidants. Carotenoid intakes in athletes must be considered carefully.

Dietary assessment: Antioxidant vitamins: Carotenoids: Oxidative stress: Physical activity

Competitive athletes are thought to have a much greater likelihood of antioxidant vitamin sub-deficiency status as a result of an increased O₂ utilization. This antioxidant vitamin deficiency is thought to cause an increase in highly reactive oxygen molecules and hence oxidative damage (Ji, 1999). Athletes therefore represent a population potentially exposed to oxidative damage. In addition, alterations of the antioxidant status in athletes could also be related to specific deficiencies resulting from inadequate and unbalanced dietary intakes. Indeed, in competitive athletes the decrease in antioxidant densities in the diet reported in industrialized countries (Hercberg *et al.* 1991), as well as the adoption of unbalanced nutritional habits (e.g. high-carbohydrate diet) because of high daily physical energy expenditure (EE; Blundell & King, 1999), occurs frequently. Thus, the intake of antioxidant supplements in athletes has generated interest. Because antioxidant supplementation has been shown in some studies to have a favourable effect against lipoperoxidative damage in highly trained (Rokitzki *et al.* 1994) or in overloaded (Itoh *et al.* 1999) subjects, it has been suggested that recommendations for exogenous antioxidant requirements

should be increased for those with high EE. The French RDA for athletes explicitly includes EE in its definition (Guillard *et al.* 2001). It has also been suggested that when diet fails to meet the antioxidant requirement, dietary supplements should be recommended. However, many studies (Urso & Clarkson, 2003) have shown no benefit of antioxidant supplementation (vitamins E, C and β -carotene) in athletes. Moreover, regarding biological status, few studies have evidenced a low antioxidant vitamin status or a high basal lipoperoxidation index in athletes (Marzatico *et al.* 1997; Balakrishnan & Anuradha, 1998; Schröder *et al.* 2000). However, a better antioxidant status was shown in soccer, basketball and rugby players compared with sedentary subjects (Brites *et al.* 1999; Pincemail *et al.* 2000; Evelson *et al.* 2002). This led us to focus on the justification of the French RDA, knowing that EE, an indicator of training load, could modulate endogenous antioxidant adaptation in response to aerobic training (Powers *et al.* 1994). Generally studies have been confined to only one category of athletes and have had an insufficient number of subjects to investigate the extent or source of individual variability (e.g. EE, gender,

Abbreviations: EE, energy expenditure; FRDAa, French recommended dietary allowance adjusted for EE; TBARS, thiobarbituric acid-reactive substance.

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type of activity performed) in plasma antioxidant status response to dietary intakes. Thus, we aimed to verify the hypothesis of the 'high exposure' to sub-deficient antioxidant status (vitamins C, E and carotenoids) in athletes and consequently to high lipoperoxidation marker concentrations in relation to estimated daily EE and dietary antioxidant intakes.

Methods

Subjects

Competitive athletes (eighty-four male, thirty-four female) and sedentary subjects (eight male, eight female) participated. Only athletes with a regional to international level of competition, with a minimum of three training sessions per week, more than 3 h of training per week and with each training session lasting > 1 h were selected. As a control group sedentary subjects with no regular physical activity for a minimum of 3 years were selected. Excluded from the present study were smokers, regular alcohol users, subjects with hypo- or hyper-energetic diets, subjects with a BMI > 35, subjects with a family history of CHD and subjects treated by anti-inflammatory drugs or by any therapeutic agent containing antioxidants or modifying food behaviour. Participants were all asked to maintain their normal behaviour during the 7 d follow-up. Female subjects started their survey between the seventh and the fourteenth day of their menstrual cycle. The protocol was developed in agreement with the declaration of Helsinki of 1975 reviewed in 1989. The survey received the approval of the Protective Committee of People in Biomedical Research (no. 02.002). Subjects were informed of the nature and the progress of the experimentation before giving their formal consent.

Subjects with the highest EE (> 13.5 MJ/d) were mostly male and mostly involved in aerobic activities. Therefore, the 134 subjects were divided into four groups according to the main energy pathway used for the activity performed and gender (sedentary (male *n* 8, female *n* 8), anaerobic (male *n* 9, female *n* 4), aerobic (male *n* 48, female *n* 21), intermittent (male *n* 27, female *n* 9)).

Experimental procedures

Food and activity records (7 d) were completed by each subject to quantify antioxidant vitamin intakes and daily EE respectively. Venous blood samples were collected in basal conditions on day 8.

Diet and activity record

A 7 d food record and 7 d activity diary representative of typical training days were completed in a notebook by each subject. At the start, a standardized individual information session gave each subject instructions on how to record their daily food intake. Food quantities were estimated, specifying the number of units and the code of the size of the corresponding portion, using a validated portion guideline book (Le Moullec *et al.* 1996).

The activities to be recorded in the activity diary were divided into the following groups: (1) personal activities

(toilet, meal); (2) home activities (housework, 'pottering', gardening) and leisure (disco, cinema); (3) activities outside of work (walking, driving, biking); (4) professional activities, or school or voluntary activities; (5) physical training. A scale of intensity was also included. Subjects reported their body mass each morning in the same conditions to ensure stability of energy balance. After the 7 d recording, diet and activity records were reviewed individually by an expert to ensure completeness. Diet and activity recalls (24 h) were carried out face-to-face by trained interviewers (all experts in nutrition) and lasted on average 30 min. The average dietary intakes of vitamin E, vitamin C and β -carotene were calculated using Regal Micro[®] software (version 1.2; Max Feinberg, Paris, France), which uses the CIQUAL nutrient database (French version). Because no data were available for other carotenoids (lutein, zeaxanthin, lycopene, β -cryptoxanthin and α -carotene), the quantity of intakes was estimated by the use of the European carotenoids database (O'Neill *et al.* 2001). The estimated vitamin E, vitamin C and β -carotene intakes were compared with the French RDA for physically active people (FRDAa) based on additional needs for daily EE exceeding 9.21 MJ for men and exceeding 7.72 MJ for women; these RDA have recently been established by the French Agency for Food and Health (Guillard *et al.* 2001).

The compendium of physical activities (Ainsworth *et al.* 2000) was used to provide the energy cost of physical activity, expressed as metabolic equivalents. The appropriate metabolic equivalents values, based on the subject's report of the type and intensity of activity, were assigned. EE was calculated using a computer program specifically designed for this task. Activities not found in the database were carefully evaluated to determine the best-suited corresponding activity.

Blood sampling

Blood samples were collected from the antecubital vein in Vacutainer tubes (Becton Dickinson, Le Pont de Claix, France) between 08.00 and 10.00 hours after an overnight fast and at least 12 h after the last exercise session. On the day before the blood samples were taken subjects were permitted only light training. Blood samples were collected in heparinized tubes protected from light. Tubes were centrifuged immediately after the blood sampling at room temperature for 10 min at 3000 g. The plasma fraction was then transferred to cryotubes, which were kept at -80°C until analysis 6 months later.

Biological analysis

Index of lipoperoxidation. Plasma TBARS were determined as described by Richard *et al.* (1992b) using the fluorimetric determination of malondialdehyde–thiobarbituric acid complex after extraction with *n*-butanol. Since the fluorimetric determination of plasma malondialdehyde may also cause the formation of a variety of chromogens other than the malondialdehyde–thiobarbituric acid adduct by reacting with substances such as amino acids or sugars, we verified the results from every fifth sample using the thiobarbituric acid test followed by HPLC separation as described by Richard *et al.* (1992a).

Table 1. Estimation of daily energy expenditure and antioxidant vitamin intakes in all subjects† (Mean values and standard deviations)

	Male subjects (n 92)						Female subjects (n 42)									
	SED (n 8)		ANA (n 9)		AE (n 48)		INT (n 27)		SED (n 8)		ANA (n 4)		AE (n 21)		INT (n 9)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
EE (MJ/d)	9.76	1.30	12.64	2.24	14.81	2.76	13.36	1.99	8.34	1.26	10.08	0.94	11.56	1.38	11.85	1.50
Vitamin C (mg/d)	87*	58	97*	47	160*	72	114*	92	80*	42	119*	24	130*	73	84*	50
Vitamin E (mg/d)	8.0	3.2	12.2	5.3	13.9	5.3	11.7	5.6	9.3	5.0	9.3	4.5	9.8	4.2	6.5	3.3
β-Carotene (mg/d)	1.55	1.07	1.59	0.46	3.90	3.22	2.58	1.74	2.62	2.25	1.81	0.87	4.08	3.00	2.14	2.76
Lutein (+zeaxanthin) (mg/d)	0.55	0.31	1.01	0.69	1.36	0.77	1.34	0.90	0.87	0.59	1.24	0.85	1.16	0.64	0.77	0.62
Lycopene (mg/d)	2.54	1.29	4.15	2.46	5.17	4.45	2.54	1.29	2.75	1.84	3.12	2.88	2.37	1.74	2.16	1.45
α-Carotene (mg/d)	0.23	0.20	0.08	0.06	0.94	1.10	0.41	0.60	0.49	0.61	0.50	0.21	0.65	0.49	1.72	0.77
β-Cryptoxanthin (mg/d)	0.37	0.73	0.65	0.60	0.43	0.62	0.51	1.02	0.46	0.52	1.04	1.11	0.63	0.51	0.34	0.53
Total carotenoids (mg/d)‡	5.29	2.85	7.45	3.31	12.02	7.04	11.59	4.77	7.18	4.66	7.70	2.49	8.53	4.17	6.76	6.70

SED, sedentary control group; ANA, anaerobic group; AE, aerobic group; INT, intermittent group; EE, energy expenditure.

* $P < 0.05$; effect of EE covariate on vitamin C intake.

† For details of subjects and procedures, see p. 462.

‡ Total carotenoids = β-carotene + lutein + lycopene + α-carotene + β-cryptoxanthin.

Vitamin determination. Vitamin C concentration was measured by fluorimetry using an automated method in plasma after stabilization and extraction with a metaphosphoric acid solution (50 g/l) according to Speek *et al.* (1984). α-Tocopherol and carotenoid concentrations were determined by HPLC as described by Arnaud *et al.* (1991).

Statistical analysis

Factorial ANOVA tests were performed for comparisons between groups (gender and type of activity performed). EE was introduced as a covariate. Combined effects of EE and antioxidant intakes (vitamin C, vitamin E or carotenoids) on plasma antioxidant concentrations (ascorbic acid, α-tocopherol or carotenoids) or isolated effects of EE on plasma antioxidant concentrations (ascorbic acid, α-tocopherol or carotenoids) were analysed in relation to gender. In this analysis, EE and antioxidant intakes were introduced as covariates.

If significant differences were found, bivariate associations between continuous variables (EE, vitamins C, E and carotenoid intakes, and plasma ascorbic acid, α-tocopherol and carotenoid concentrations) were assessed by linear or polynomial regression analysis.

Analysis was performed with StatView Abacus Concept, version 5 software (Cary, NC, USA) on a personal computer. All results are presented as means values and standard deviations with significance accepted when $P < 0.05$.

Results

Mean values for age, BMI and plasma total cholesterol concentrations were 26.8 (SD 6.8) years, 22.4 (SD 2.5) kg/m² and 11.1 (SD 3.5) mg/l respectively and were not related to EE, activity performed or gender. The mean training loads were 13.0 (range 4.5–26.0) h per week for male subjects and 9.5 (3.0–16.0) h per week for female subjects.

Antioxidant vitamin intakes

Athletes had vitamin C and E intakes significantly below the FRDAa. However, vitamin C intakes were well above the recommendation for French moderately physically active people (110 mg vitamin C/d) but only 40% of athletes reached the FRDAa. For vitamin E, 81% of the athletes and 68.7% of the sedentary subjects had intakes below two-thirds of the FRDAa. β-Carotene intakes were below two-thirds of the FRDAa for 43% of the athletes and for 56% of the sedentary subjects.

The deficit in vitamin E intake, calculated as the FRDAa for vitamin E minus vitamin E intake, was positively correlated with EE (r 0.51, $P < 0.0001$) in athletes. This relationship was not verified for deficits calculated for vitamin C and β-carotene intakes.

Using the ANOVA test, and with allowance made for possible confounding or interacting factors (gender and type of activity performed), we found a main effect of EE on vitamin C intakes ($P < 0.05$) (Table 1). A positive polynomial association between vitamin C intakes and EE by regression analysis in athletes (n 118) was observed (r 0.59, $P < 0.0001$) with a breaking slope near 14.6 MJ EE/d (Fig. 1). In contrast,

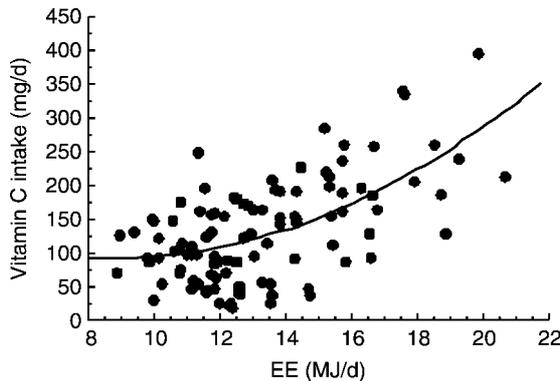


Fig. 1. Polynomial regression of daily vitamin C intake *v.* daily energy expenditure (EE) in athletes (n 118; $y = 84.75 - 9.63x + x^2$; r 0.59, $P < 0.0001$). For details of subjects and procedures, see p. 462.

we did not find any relationship between vitamin E or β -carotene and other carotenoid intakes and EE.

Antioxidant vitamin status

None of the subjects had plasma ascorbic acid and α -tocopherol concentrations below the cut-off point for deficiency (<20 and <9.3 $\mu\text{mol/l}$ respectively). For β -carotene, 14% of the athletes and 31% of the sedentary subjects had plasma concentrations <0.30 $\mu\text{mol/l}$.

Plasma ascorbic acid concentrations were significantly related to the combined covariates (EE, vitamin C intakes) and factor (gender) effect (Table 2). A significant logarithmic relationship was observed between plasma ascorbic acid concentrations and vitamin C intakes in male athletes (n 84; r 0.45, $P < 0.0001$) (Fig. 2). Plasma ascorbic acid concentrations were closely linked with vitamin C intakes when intakes were <100 mg/d. A minimal increase in plasma ascorbic acid concentration was observed when the intake of vitamin C was >200 mg, corresponding to a plasma ascorbic acid concentration of 65 $\mu\text{mol/l}$. This intake was mostly obtained by athletes whose EE exceeded 16.5 MJ/d (Fig. 1). Intake by female subjects was mostly

>100 mg/d. This could explain why the dose–effect logarithmic relationship was not significant for female subjects.

EE did not account for the relationship between dietary intakes and plasma vitamin E concentrations or for the major plasma carotenoids (β -carotene, lutein, zeaxanthin, β -cryptoxanthin, lycopene and α -carotene). However, an interaction between EE, gender and total carotenoid intake was shown after considering the sum of plasma carotenoids (β -carotene, lutein, zeaxanthin, β -cryptoxanthin, lycopene and α -carotene) (Table 2).

Lipoperoxidative damage indices

Plasma TBARS concentrations are shown in Table 3. No difference between groups (gender and type of activity performed) and no effect of EE was found by ANOVA.

Correlation between plasma TBARS concentration and plasma total carotenoid concentration was negative in male athletes (r -0.31 , $P < 0.006$) (Fig. 3).

Discussion

The focus of the present study was antioxidant vitamin status and lipoperoxidative damage in competitive athletes in relation to their dietary intakes and EE. The large number of athletes involved in the present study (n 118) allowed use of a factorial analysis taking into account the potential confounding or interacting factors such as gender and type of activity performed.

We found that many athletes did not meet the FRDAa for vitamin C (60%), β -carotene (43%) and vitamin E (81%). Moreover, the deficit in vitamin E intakes increased with the increased daily EE. No study has reported this deficit in vitamin E intake for athletes in energy balance. This is probably due to the fact that none of these studies has referred to the need for the maintenance of vitamin E density in food (Linseisen *et al.* 1993; Singh *et al.* 1993). It has been shown that the susceptibility of tissues to oxidative stress is related to the vitamin E level per unit mitochondrial activity (Gohil *et al.* 1987),

Table 2. Status of plasma antioxidants in athletes† (Mean values and standard deviations)

	Male athletes (n 84)		Female athletes (n 34)		Statistical significance of effect: EE \times vitamin intake \times gender
	Mean	SD	Mean	SD	
EE (MJ/d)	14.13	2.61	11.46	1.44	
Ascorbic acid ($\mu\text{mol/l}$)	59.3	13.7	65.6*	13.5	$P < 0.05$
α -Tocopherol ($\mu\text{mol/l}$)	25.8	5.2	27.7	6.0	NS
β -Carotene ($\mu\text{mol/l}$)	0.56	0.28	0.73	0.43	NS
Lutein ($\mu\text{mol/l}$)	0.31	0.13	0.39	0.21	NS
Zeaxanthin ($\mu\text{mol/l}$)	0.10	0.06	0.067	0.011	NS
Lycopene ($\mu\text{mol/l}$)	0.68	0.34	0.68	0.35	NS
α -Carotene ($\mu\text{mol/l}$)	0.15	0.10	0.22	0.16	NS
β -Cryptoxanthin ($\mu\text{mol/l}$)	0.28	0.15	0.43	0.37	NS
Total carotenoid ($\mu\text{mol/l}$)‡	2.09	0.61	2.61*	1.13	$P < 0.05$

EE, energy expenditure.

Mean value was significantly different from that of the male athletes (protected least squares difference Fisher *post hoc* test): * $P < 0.05$.

† For details of subjects and procedures, see pp. 462–463.

‡ Sum of carotenoids quantified (β -carotene, lutein, zeaxanthin, lycopene, α -carotene and β -cryptoxanthin).

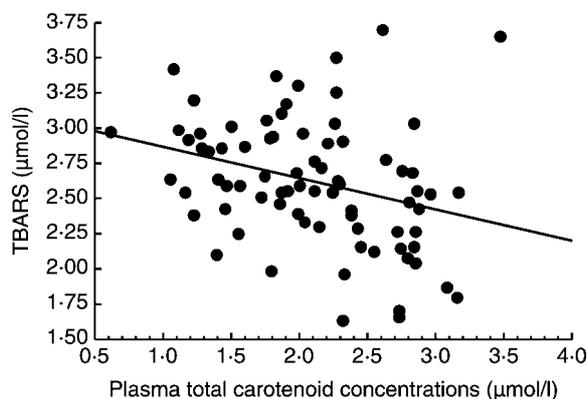


Fig. 3. Linear regression of plasma thiobarbituric acid-reactive substances (TBARS) *v.* plasma total carotenoid concentration in male athletes (n 84; $y = 3.09 - 0.23x$; $r = -0.31$, $P = 0.006$). For details of subjects and procedures, see p. 462.

to believe that they are not at high risk of deficiencies, given the adequacy of their intakes. This seems to be true not only for vitamin E but also for the other antioxidant vitamin requirements. Like vitamin E, carotenoids are fat-soluble compounds and occur naturally in fruits and vegetables. So far, no recommendation has been made on the intake of carotenoids, except for β -carotene, which has been widely studied (Guillard *et al.* 2001). The quality of the data on the carotenoid contents of foods is considered to be limited (O'Neill *et al.* 2001). Because these compounds may also be subject to oxidation, it has been suggested that the EE should be considered in the interpretation of carotenoid status. Plasma concentrations of major carotenoids show a greater inter-individual variability, mainly reflecting different dietary patterns among subjects, rather than their level of physical activity reflected by EE. The concentrations of different carotenoids observed in our present study gave valuable information on the physiological ranges achieved under habitual dietary conditions in athletes, given that to our knowledge no data have been published on the carotenoid status in athletes (except for β -carotene and to a lesser extent lycopene and β -cryptoxanthin; Aguilo *et al.* 2003). Plasma α -tocopherol and carotenoid concentrations observed in the athletes in the present study are similar to those reported in previous studies in healthy French subjects (Olmedilla *et al.* 2001). The body pool of lipophilic compounds is probably determined by factors such as the cholesterol-rich lipoprotein content, which is not systematically related to daily EE.

With regard to hydrophilic antioxidants, training has been shown in some studies to improve plasma ascorbic acid concentration (Bergholm *et al.* 1999; Evelson *et al.* 2002) and serum total antioxidant capacity (Child *et al.* 1999), but antioxidant intakes were not quantified. In contrast, in our present study the determination of daily vitamin C intakes showed that plasma ascorbic acid concentrations were significantly related to a combination of the effects of EE, vitamin C intake and gender. In fact, the relationship between vitamin C intake and plasma ascorbic acid concentration was logarithmic for male athletes only. Plasma ascorbic acid concentration

was closely linked to vitamin C intakes < 100 mg/d, but only a minimal increase in plasma ascorbic acid concentration was observed above this dose. This minimal increase was mainly observed in women whose intakes were > 100 mg/d. As a consequence, the increase in plasma ascorbic acid concentrations seems not to be related to vitamin C intakes in women. Moreover, subjects with greater vitamin C intake:EE ratio had high EE (> 14.6 MJ/d). This could be explained by the fact that these athletes were very concerned about their diets and increased their daily dietary nutritional density without taking supplements. Thus, we postulate that higher plasma ascorbic acid concentrations observed in athletes is directly induced by higher vitamin C intakes. This relationship has been already shown in male non-athletes (Levine *et al.* 1996) and women non-athletes (Levine *et al.* 2001). In athletes, the optimal bioavailability of vitamin C seems to be reached at 200 mg/d, an intake that corresponds to a plasma ascorbic acid concentration of $65 \mu\text{mol/l}$. This relationship, combined with the fact that no relationship of vitamin C nutrition with plasma TBARS was observed, does not support the assumption that athletes need additional vitamin C over 200 mg in relation to their EE.

The results of the present study indicate that phytochemical nutrients other than vitamin E and vitamin C, such as the carotenoids (β -carotene, lutein, zeaxanthin, β -cryptoxanthin, lycopene and α -carotene), may be responsible at least in part for the prevention of lipoperoxidation in French male athletes. Carotenoid-radical interactions have been shown *in vitro* (Krinsky & Yeum, 2003), but the exact mechanism by which carotenoids prevent lipid peroxidation *in vivo* is unknown. The total carotenoid concentration in plasma reflects short-term carotenoid intake and may be considered as a biomarker of fruit and vegetable intakes (Olmedilla *et al.* 2001).

The present results provide evidence to support the fact that the combined action of multiple compounds derived from a diet rich in fruits and vegetables cannot be replaced by supplementation with a single or a combination of antioxidants. It is known that carotenoids interact directly or indirectly with other phytonutrients that may enter the overall pool of antioxidants, which fuel recycling reaction (Prior, 2003).

Conclusion

Vitamin E requirements may be overestimated with reference to EE in FRDAa. An improved ascorbic acid status was observed with an increase in daily EE in athletes by increasing dietary vitamin C intake. A vitamin C intake of 200 mg/d appears to correspond to an optimal intake in well-trained subjects. The subgroup of athletes expending < 14.6 MJ/d could be at higher risk of deficit, since these athletes frequently have poor diets. Inter-individual variation in antioxidant intake and status requires recommendation of intakes on an individual basis. However, the present results for total carotenoids suggest a synergistic beneficial antioxidant action of multiple phytonutrients derived from a diet rich in fruits and vegetables.

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