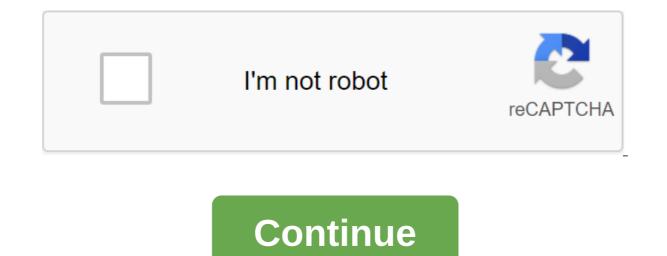
Beta carotene antioxidant mechanism pdf



PDF Split View article Content Figures and Tables Video Audio Additional data in the vitamin A-abound population, increased concentration of serum carotenoids have been associated with a reduced risk of degenerative diseases. The mechanism of action of carotenoids in determining antioxidant activity is largely unknown. The aim of the study was to study the effect of carotenoids of supplementation and spinach consumption on the activity of antioxidants in serum or plasma, as well as concentrations of oxidically damaged amino acids in plasma. Subjects received for 3 wk basic diet (n No 10), a basic diet with carotenoid supplement (n No 12) or with spinach product (n No 12 per group), i.e. whole leaf, minced meat, liquefied or liquefied spinach plus added dietary fiber. After 3 wk dietary interventions, changes in serum or plasma concentrations of ascorbic acid, αtocopherol, FRAP (ferrick reducing plasma capacity) and uric acid and red blood cell enzyme activity were evaluated, and differences between experimental groups were tested. Consumption of spinach has led to a greater (P zlt; 0.01) activity of red blood cells glutathione and a decrease (P zlt; 0.05) of the activity of red blood cell catalysis and serum concentration α-tocoferol compared to the control group. Consumption of carotenoids supplement led to a decrease in α-tocopherol responses (P 0.02) compared to the main diet only. Our data suggest that short-term changes in the activity of red blood cells glutathione and serum concentration α-tocopherol can be explained by increased consumption of carotenoids (lutein and zeaxanthin), but β-carotene is unlikely to be a causal factor. The decrease in the activity of red blood cell catalase after intervention with spinach products may be due to other spinach components such as flavonoids. carotenoids, antioxidants, α-tocopherol, humans, spinach Carotenoids are found in plasma and human tissues. The main carotenoids are β, lutein, α carotene, zeaxanthin, cryptoxanthin and lycopene. A number of carotenoids are precursors to retinol and retinoids, but carotenoids also have a number of other functions in the human body, including protection against oxidation by hardening singlet oxygen (Stahl et al. 1997). In addition, β chemically reacts with peroxil radicals to produce epoxy and apocarotin products (Canfield 1992). Low levels of carotenoids are associated with poor cognitive performance (Berr et al. 1998), and higher plasma levels β -carotene are associated with better memory performance in older adults (Perrig et al. 1997). Based on consumption or biomarkers consumption, carotenoids have been postulated to play a protective role in angina (Riemersma al. 1991), сердечно-сосудисты заболеванияя (Gaziano et al. 1995, Cardinal et al. 1993, Knekt et al. 1994, Street et al. 1994), and cancer (Dartigues et al. 1990) and stomach (Chen et al. 1992). However, interventional studies did not confirm this (The ATBC Cancer Prevention Study Group 1994, Blot et al. 1993, Blot 1997, Greenberg et al. 1996, Omenn et al. 1996, Rapola et al. 1997). These intervention studies ß supplements rather than a mixture of carotenoids as is present in fruits and vegetables. The positive effect on health postulated for carotenoids has been attributed to a large extent to their antioxidant actions. However, studies linking higher carotenoid intake with better antioxidant protection and reducing oxidative damage in the body are very few, and are generally used indirect methods (Esterbauer 1996, Miller and Rice-Evans 1997, Puhl et al 1994). Therefore, we conducted a dietary controlled intervention study to study the effects of carotenoide consumption dissolved in oil and three different processed spinach products on a number of enzymatic and non-natimatic antioxidant parameters in human blood. Separately, the effect of consumption of processed spinach products on the bioavailability of carotenoids (Castenmiller et al. 1999) has been published. The main purpose of this study was to assess whether spinach affects antioxidant markers and whether this effect can be explained by the carotenoids present in spinach. SUBJECT and METHOD Subjects. The subjects were 72 healthy, non-smokers, normolipidemic volunteers; there were 42 women and 30 men between the ages of 18 and 58. These subjects, students at Wageningen Agricultural University and other residents of the Wageningen district, were recruited through local advertisements. None of the subjects take oral medications, except oral contraceptives, or supplements of any kind during the last 3 mo before the study or during the study. Screening procedures included a test for elevated glucose and protein levels in the urine and a test for abnormal hematologies or low concentrations of haemoglobin. All subjects completed a medical and general questionnaire in addition to the food frequency questionnaires to assess their energy intake, as well as carotenoids and vitamin A. The subjects had normal body weight (18-28 kg/m2); their serum cholesterol concentrations of 6.5 mmol/L and triacilglycerol concentrations were 2.8 mmol/L. In the study, two men declined to participate in the study for personal reasons. Characteristics of the subjects see the Protocol of this study was approved by the Medical and Ethical Committee of the Wageningen Agricultural University, and all subjects gave written informed consent. TABLE 1 Characteristics of subjects involved in the study to determine effect of carotenoids consumption on antioxidant activity in human blood1. Group. Management. Carotenoid supplement. Unced spinach. Liquefied spinach Vegetarians, n 3 1 3 7 3 3 Age, y 21 (18, 25) 2 21 (18, 24) 20 (18, 58) 21 (18, 42) 21 (18, 38) 20 (18, 54) Energy (estimated), MJ/d 11.4 ± 2.3 Serum cholesterol, mmol/L 4.14 ± 0.65 4.07 ± 0.61 4.11 ± 0.79 4.23 ± 0.74 3.93 ± 0.47 4.17 ± 0.87 Serum triacylglycerol, mmol/L $0.88 \pm 0.41 \ 0.87 \pm 0.32 \ 0.92 \pm 0.32 \ 1.00 \pm 0.39 \ 0.89 \pm 0.27 \ 1.00 \pm 0.34$ Body mass index, kg/m2 21 ± 2.1 22 ± 2.3 22 ± 2.0 22 ± 2.4 23 ± 2.1 22 ± 1.8 Study design. The study began in January 1997 with a 3-WK run during which subjects chose their own diets, but were tasked with avoiding foods rich in carotenoids and retinol. The subjects then stratified according to age, gender, cholesterol concentration and energy consumption and were assigned to six experimental groups. Six treatment groups fed the same basic diet throughout the study and the menu was changed daily on a weekly cycle. The main diet does not include fruits and vegetables with moderate or high amounts of carotenoids and meets the requirements of the Dutch Recommended Daily Allowance (Netherlands Food and Nutrition Council 1992). In addition to the basic diet, four groups received a daily spinach product; one group received a suspension in vegetable oil of microcrystal β (40 g/kg; Hoffmann-La Roche, Basel, Switzerland) and crystalline lutein and zeaxanthin derived from marigold flowers (60 g/kg and 3 g/kg, respectively; FloraGLO, courtesy supplied by Kemin Industries, Des Moines, IA). The carotinoid supplement was suspended in sunflower oil; For a group of carotenoids supplement, some of the sunflower oil used in salad sauce is served to control and spinach groups have been replaced by a carotenoids pendant supplement. Spinach groups have been replaced by a carotenoids pendant supplement. spinach products were produced from the same batch and were provided, prepared and subsequently frozen by Langnes-Iglo (Wunstorg, Germany) for Unilever Research (Vlaardingen, Netherlands). Spinach from whole leaf washed and blanched for 90 s and guickly cooled; the stuffed spinach was crushed to 5mm after blanching. An ensimatic drug with pectinase, hemicellase and cellulose (Rapidase LI, courtesy of Gist-Brocades, Seclin, France) was used to liquefy minutes. Enzymes, One group received liquefied spinach plus fiber made from beet pulp (10) g/kg, Fibrex 600, courtesy of TEFCO Food Ingredients b.v., Bodegraven, Netherlands). The energy content of the carotenoids group was adjusted to the spinach group content due to the additional number of relevant foods. All frozen spinach is defrosted and heated by microwave before consumption. Spinach products did not contain measurable nitrate intake (FAO/WHO Joint Expert Committee on Food Supplements 1995). Microbiological calculations have shown normal values and confirmed that spinach products are safe for human consumption. Subjects were provided with a full diet, except for a limited selection of free foods (~10 energy). They received the same foods as non-vegetarian subjects (n No.50), with the exception of meat, which was replaced by a vegetarian substitute with a similar nutritional composition. The daily selection of free-choice foods was recorded in the diary and the nutrient content was calculated (NEVO Foundation 1995). Individual body weights throughout the study were maintained at ± 2 kg. d 0, 1, 8, 15, 21 and 22 periods of intervention to analyze serum carotenoids and concentrations of αtocopherol, on d 0, 8, 15 and 22 for plasma analysis of ferricoponosizing ability (FRAP), 3 concentrations of uric acid and vitamin C, as well as d 0 and 22 to analyze erythrocytes enzyme activity and oxidically modified plasma amino acids in the protein. Carotenoids and α tocopherol, Samples to which no anticoagulant was added were left on the clot; for 1 hour after they were drawn, they were centrifuged and stored at 80 degrees Celsius. Serum carotenoids α anticoferol have been measured by HPLC. To avoid day-to-day analytical variations, all samples from humans were analyzed as a set. After ethanol precipitation, extraction was accompanied by hexagonal samples evaporated under nitrogen and were injected into the HPLC (Craft and Wise 1992) system. All samples of preparation and under muted yellow light with minimal minimum Oxygen. Intra-assid summary for analysis of serum α -carotene, β carotene, lutein and zeaxanthin in control pools averages 7.4, 3.9, 3.6 and 8.7% respectively. Erythrocytes are antioxidant enzymes. Antioxidant enzymes. Antioxidant enzymes are antioxidant enzymes. Antioxidant enzymes are antioxidant enzymes. blood cells are rinsed twice in 4 vol by sterile physiological buffered saline solution, resuspended in 1 vol of sterile, deionized water for the lick and immediately frozen at 80 degrees Celsius. Automated analyzers were conducted on the Cobas Mira analyzer (Roche, Basel, Switzerland) to determine the activity of antioxidant enzymes glutathione peroxidase (GPx), glutathione reductase (GR), superoxide dismutase (SOD) and catalase (CAT). The activity of enzymes was associated with the amount of hemoglobin in blood hemolates. SOD (Randox cat. no. SD 125, Ardmore, United Kingdom), GPx (Randox cat. no. RS 05) and haemoglobin (Randox cat. no. HG 980) were identified using commercially available kits. The activities of GR and PPC were determined in accordance with the methods described earlier (Wheeler et al. 1990). All analyses were conducted within 20 d. The intraday summary of SOD, CAT, GR and GPx on average is 1.9 1.4, 3.1 and 1.9%, respectively. NADPH, glutathione, FAD, purpald and potash periodate were purchased from Sigma Chemical (St. Louis, MO). Definition of adipic semi-aldehyde (AAS). The oxidizingly modified amino acids in plasma proteins were analyzed by HPLC (Daneshvar et al. 1997). Protein fractions reacted from fluoresceinamine to hydrolysis, and decarboxylated derivatives of fluoresceinamine adipic and glutamic semi-aldehids were measured by HPLC using a diode array detector. The intraday resume for this definition was 5.2%. FRAP, uric acid and vitamin C. The antioxidant activity of the samples to which EDTA was added as an anticoagulant was assessed as their FRAP (Benzie and Strain 1996). The concentration of uric acid was measured in plasma samples using ensimatic colorimetric methods (Boehringer Mannheim, Germany). The concentration of vitamin C in plasma treated with trichloroacetic acid was determined fluorimetrically as ascorbatic plus dehydroacscorbat (Vuilleumier and Keck 1996). Intra-assai inter-day resume for plasma analysis of FRAP, uric acid and vitamin C was 3.5, 2.0 and 5.6%, respectively. Food measurements. The duplicate portions of the daily food intake of one item collected during the study were carefully mixed into weekly portions; Subsequently, the combined samples were stored at 20 degrees Celsius before analysis. The humidity and ash content in each weekly serving were determined by a vacuum furnace 85 degrees Celsius and mouflic furnace at 550 degrees Celsius. The concentration of protein was determined by the Kjeldal method using a conversion rate of 6.25. The Folch et al. method (1957) was used to extract fat. Fat. Fibers were analyzed according to AOAC (1996) Official Method 992.16 for common dietary fiber. Assimilated carbohydrates were calculated on the difference. Carotenoids were extracted from the moist material after homogenization using tetrahydrofuran (THF), remade into THF/methanol (1:1 v/v) and introduced into the HPLC system (Hulshof et al. 1997). Summary within the mileages of α-carotene, β-carotene and lutein in control pools averaged 5.7, 6.8 and 8.8%, respectively. Samples to determine vitamin C, to which metaphosphoric acid was added, were collected from a number of daily homogenized duplicative parts and immediately frozen. Vitamin C was defined fluorimetrically, after extraction of metaphosphoric acid/acetic acid (60:80, wt/v) as ascorbat plus dehydroascorbat (CV was 5.6%, Vuilleumier and Keck 1996). Statistical analysis. Responses to dietary intervention were averaged for carotenoids and α -tocopherol for d 0 and 1 and for d 21 and 22 for each item. All other variables were measured once at the beginning and end of the dietary intervention period. The response to treatment was calculated for each person, as a change in serum or plasma concentration or enzyme activity from the beginning to the end of the dietary intervention period. ANOVA has been used to test the equality of middle elevation for different treatment groups, thereby controlling several factors and covariables (SPSS Advanced Statistics, Chicago, Illinois). When all significant antioxidant predictors are included in the model, it can be shown whether a particular variable is an independent predictor of the reaction after treatment. For example, for the ferricononic ability of plasma (FRAP), uric acid should be included as covariable, as the concentration of uric acid determines ~60% of the antioxidant activity of plasma (Benzie and Strain 1996). To assess the effect of one of the enzyme activity reactions were included in the model as covariable. Since the initial values may have influenced the magnitude of the changes, the effects of treatment were also assessed after adjusting for baseline values by analyzing the covarians where applicable. Significant F-tests were followed by Tukey student trials of a range of pair differences (LSD) tests to assess the differences between the four remedies each treatment. Pearson's correlations were calculated for the answers of several variables. The P-values of 0.05 were regarded as significant. Values are a means of ± sd. RESULTS Results of analyses of various red blood cells of enzymatic activity and nonenzymatic, antioxidant concentrations in serum or plasma are described in Table 3 for control, carotenoids supplement and combined spinach groups. On (wk 0) there were no significant differences red blood cells are antioxidant enzymes activity or serum or plasma antioxidant concentrations of various experimental groups, with the exception of REDrocytes SOD and GR activities. The results of consumption of various processed spinach products by bioavailability of carotenoids are described elsewhere (Castenmiller et al. 1999). Serum β carotene concentration increased from 0.253 ± 0.130 to 2,608 ± 1.06 mm/L in the car group of additives and from .283 ± 0.141 to .445 ± 0.172 mmol/l in spinach groups. For lutein, the increase was 0.218 ± 0.064 to 0.983 ± .287 mcmol/L in the carotenoids group and from .216 ± 0.072 to 0.915 ± 0.272 mmol/l in spinach groups. The response to serum zeaxanthin was higher in the carotenoids group than in the control group (P 0.006) or in spinach groups. The response to serum zeaxanthin was higher in the carotenoids group than in the control group (P 0.006) or in spinach groups. 0.001; independent test t), but did not differ between the combined spinach and control groups (P No. 0.07). Concentrations of triacillycylol and serum cholesterol have not changed during the experimental period or among experimental groups (data are not shown). TABLE 2To daily energy intake and nutrients subjects involved in the study to study the effects of consumption of carotenoids supplement or spinach products on antioxidant activity in human blood1. Group. Management. Carotenoid supplement. Carotenoid supplement. Carotenoid supplement. Carotenoid supplement. Spinach products. Energy, MJ/d 10.6 10.7 10.8 β-carotene, mg/d 0.5 9.8 9.3 lutein, mg/d 0.5 6.6 11.5 zeaxanthin, mg/d 0.1 0.3

0.1 retinol, mg/d 0.4 0.3 0.4 vitamin C.2 mg/d 100 83 63 Dietary fiber, 3 g/d 27.4 25.2 31.1 TABLE 3Variable antioxidant activity in human blood: concentration and activity in human blood: concentration and activity in human blood: concentration and activity control, carotenoids supplement and combined spinach groups at a basic level (wk 0), after 3 WK dietary intervention (wk 3) and responses from the beginning to the end of the period of dietary intervention1. Group. Management. Carotenoid supplement. Pool spinach. n 10 12 48 Adipic semialdehyde, pmol/mg protein Wk 0 23.8 \pm 3.9 25.7 \pm 4.0 24.8 \pm 4.5 Wk 3 27.3 \pm 4.3 28.1 \pm 8.8 27.2 \pm 4.6 Response 3.5 \pm 6.5 2.4 \pm 9.1 2.3 \pm 6.1 Glutathione peroxidase, U/g Hb Wk $0.61.3 \pm 27.754.6 \pm 8.551.7 \pm 10.8$ Wk $3.61.8 \pm 26.559.2 \pm 9.254.3 \pm 9.7$ Response $0.5 \pm 8.94.6 \pm 7.92.5 \pm 9.3$ Glutathione reductase, U/g Hb Wk $0.9.7 \pm 2.17.7 \pm 1.48.5 \pm 1.7$ Wk $3.88 \pm 1.88.1 \pm 1.98.7 \pm 2.1$ Response $-0.9 \pm 1.30.4 \pm 1.620.2 \pm 1.93$ Superoxide dismutase, U/g Hb Wk $0.1299 \pm 3121082 \pm 155$ 1152 ± 180 Wk 3 1295 ± 248 1143 ± 101 1190 ± 130 Response -4 ± 181 61 ± 133 38 ± 162 Catalase, U/g Hb Wk 0 17.2 ± 3.1 17.5 ± 8.5 16.2 ± 3.3 Wk 3 22.1 ± 9.0 20.1 ± 5.5 18.1 ± 4.5 Response 4.8 ± 7.1 2.6 ± 5.0 1.9 ± 5.23 FRAP, mmol/l Vk 0 1.01 0.134 1.03 ± 0.22 1.06 ± 0.17 Wk 3 1.02 ± 0.28 1.01 ± 0.19 1.05 ± 1.01 0.12 Response $0.02 \pm 0.22 - 0.02 \pm 0.10 - 0.01 \pm 0.12$ Uric acid, mmol/L Wk $0.242 \pm 424253 \pm 70266 \pm 55$ Wk $3.247 \pm 102244 \pm 63254 \pm 43$ Response $5 \pm 73 - 9 \pm 21 - 12 \pm 36$ Vitamin C, µmol/L Wk $0.57.0 \pm 11.558.3 \pm 14.560.5 \pm 14.6$ Wk $3.67.8 \pm 4.069.8 \pm 12.770.2 \pm 10.3$ Response $11.8 \pm 11.911.4 \pm 15.29.7 \pm 102244 \pm 63254 \pm 43$ Response $5 \pm 73 - 9 \pm 21 - 12 \pm 36$ Vitamin C, µmol/L Wk $0.57.0 \pm 11.558.3 \pm 14.560.5 \pm 14.6$ Wk $3.67.8 \pm 4.069.8 \pm 12.770.2 \pm 10.3$ Response $11.8 \pm 11.911.4 \pm 15.29.7 \pm 102244 \pm 63254 \pm 43$ Response $5 \pm 73 - 9 \pm 21 - 12 \pm 36$ Vitamin C, µmol/L Wk $0.57.0 \pm 11.558.3 \pm 14.560.5 \pm 14.6$ Wk $3.67.8 \pm 4.069.8 \pm 12.770.2 \pm 10.3$ Response $11.8 \pm 11.911.4 \pm 15.29.7 \pm 102244 \pm 63254 \pm 102244 \pm 63254 \pm 102244 \pm$ 13.6 α-Tocopherol, µmol/L Wk 0 18.8 ± 3.9 21.1 ± 4.5 20.5 ± 4.2 Wk 3 21.9 ± 4.5 22.6 ± 4.1 21.5 ± 4.4 Response 3.1 ± 2.5 1.5 ± 2.12 1.0 ± 2.93 Diets. Duplicate portions of food provided to participants were analyzed as combined weekly; the results of the chemical analysis are shown in Table 2. For macronutrients β carotene measured concentrations were consistent among treatment groups and for 3 wk; lutein intake in the carotenoids group (0.6 mg/MJ). Vitamin C content in the diet with carotenoid supplement, whole leaf spinach, chopped spinach, liquefied spinach and liquefied spinach plus added dietary fibers were 83, 75, 61, 68 and 48%, respectively, in the control group. The α of tocoferol would be similar for all treatment groups, but may have been different for a vegetarian diet in which the meat was replaced by a vegetarian product. Therefore, when analyzing the α responses to tocopherol, an adjustment of the factor of vegetarian diet was made. Foods from spinach and carotenoid supplement against a control diet. The grthocyte activity response, adjusted for SOD and CAT responses, was higher (P zlt; 0.01), while CAT, adjusted for GR, GPx and SOD responses, and α-tocopherol responses adjusted for the vegetarian diet (P No. 0.02 and P No. 0.04, respectively), were lower in the spinach group. The reaction of GR red blood cells, adjusted for CPR and SOD reactions, was higher (P 0.02), while the serum response α-tocopherol was lower (P 0.02) in the carotenoids group than in the supplement control group. By introducing the base value into the model, the difference between control and spinach groups remained significant. The change in serum concentration α tocopherol in the control group, which had the lowest average baseline (wk 0) serum α concentrations to cholesterol and triacsilcellsel can provide a better reflection of dietary (Willett et al. 1983). The relative concentration of α tocopherol in the serum. The relative reactions of serum α-tocopherol have also shown significant differences between control and combined spinach groups (P It; 0.001) and tend to differ between the control and carotenoid supplementation group (P No. 0.06; P 0.006 when adjusted for a vegetarian diet). Analysis of corarians with baseline values (wk 0) and vegetarian diet as covariaable and serum concentration in wk 3 as a dependent variable showed that the difference between the spinach group and the control group was no longer significant. This study could not demonstrate any effect of spinach or carotenoids intake on the concentration of AAS plasma, vitamin C, FRAP or uric acid. Carotinoid supplement against spinach products. Although the bioavailability of β -carotene from the carotenoide supplement was much higher than that of spinach products, no significant differences can be found for any of the responses in the enzyme red blood cell activity, plasma AAS, FRAP, uric acid and vitamin C concentration or serum α-tocoferol concentrations between carocinoids supplementation and merged spinach groups. Results among different spinach products. Differences between the four spinach groups are usually different (P 0.07) only for uric acid reactions (see table 4). Whole leaf spinach received a higher response to uric acid than the consumption of whole leaf spinach, which led to a decrease in serum carotenoide response than consumption of other spinach products, may increase oxidative protection more than consumption of liquefied spinach. TABLE 4Variables of antioxidant activity at a basic level (wk 0), after 3 wk dietary intervention (wk 3) and responses from the beginning to the end of the period of dietary intervention for each spinach group1. Group. Whole leaf spinach. Liquefied spinach. Liquefied spinach. Liquefied spinach, Liquefied spinach plus dietary fiber. Adjpic semialdehyde, pmol/mg protein Wk 0 25.5 ± 4.8 25.2 ± 4.2 23.8 ± 4.6 24.8 ± 4.7 Wk 3 27.3 ± 4.4 27.0 ± 4.7 25.3 ± 3.9 29.0 ± 5.2 Response 1.8 ± 7.3 1.8 ± 5.8 1.6 ± 6.0 4.2 ± 5.6 Glutathione peroxidase, U/g Hb Wk 0 50.1 ± 8.5 50.6 ± 13.2 52.5 ± 13.4 53.7 ± 8.0 Wk 3 52.5 ± 10.4 52.2 ± 9.6 57.3 ± 8.0 55.0 ± 11.0 Response 2.4 ± 6.8 1.6 ± 9.1 4.8 ± 10.6 1.3 ± 11.1 Glutathione reductase, U/g Hb Wk 0 9.4 ± 1.6 8.2 ± 1.2 7.6 ± 1.8 8.7 ± 2.0 Wk 3 9.9 ± 2.9 8.4 ± 1.5 8.0 ± 1.2 8.4 ± ± 2.6 0.2 ± 1.3 0.4 ± 1.8 -0.3 ± 1.8 -0.3 ± 1.2 8.4 ± 1.4 -0.3 ± 1.4 -0.4 ± 1.4 -0.4 ± 1.4 -0.4 ± 1.4 -0.4 ± 1.4 -0.4 ± 1.4 -0.4 ± 1.4 -0.4 ± 1.4 +0.4 \pm 1.4 \pm 1.4 +0.4 \pm 1.4 1.8 Superoxide dismutase, U/g Hb Wk 0 1167 ± 150 1131 ± 194 1096 ± 187 1213 ± 187 Wk 3 1173 ± 86 1211 ± 180 1133 ± 120 1242 ± 102 Response 6 ± 182 80 ± 170 37 ± 172 29 ± 132 Catalase, U/g Hb Wk 0 16.5 ± 3.2 16.7 ± 3.2 15.1 ± 2.7 16.6 ± 4.0 Wk 3 20.5 ± 6.5 16.3 ± 2.6 17.3 ± 3.7 18.2 ± 3.7 Response 4.0 ± 100 123 ± 120 1242 ± 102 Response 6 ± 182 80 ± 170 37 ± 172 29 ± 132 Catalase, U/g Hb Wk 0 16.5 ± 3.2 16.7 ± 3.2 $6.6 - 0.4 \pm 1.12 2.3 \pm 4.0 1.6 \pm 5.7$ FRAP, mmol/L Wk 0 1.04 ± 0.14 1.02 ± 0.21 1.11 ± 0.16 1.07 \pm 0.17 Wk 3 1.07 ± 0.12 1.04 ± 0.12 1.05 ± 0.10 0.02 ± 0.13 - 0.06 ± 0.12 - 0.04 ± 0.12 Uric acid, mmol/L Wk 0 264 ± 46 249 ± 63 284 ± 50 268 ± 61 Wk 3 268 ± 43 244 ± 45 252 ± 40 253 ± 40 25 46 Response 5 ± 34 -5 ± 32 -32 ± 312 -15 ± 39 Vitamin C, mkmol/I Vk 0 67.3 ± 15.2 60.4 ± 14.9 54.7 ± 12.0 59.6 ± 15.0 Wk 3 72.4 ± 9.0 71.4 ± 15.0 11.2 66.4 ± 7.9 70.8 ± 12.8 Reply 5.0 ± 14.3 11.0 ± 12.3 11.7 ± 12.7 11.2 ± 15.6 α -Tokoferol, mkmol/I WC 0 21.0 ± 4.7 21.1 ± 4.5 20.4 ± 3.4 19.8 ± 4.3 W 3 22.6 ± 5.4 21.3 <2> <0> 4.3 W 3 22.6 ± 5.4 21.3 ± 4.3 W are calculated to significantly correlate reactions between antioxidant activity variables and concentrations. Significant correlations (r zgt; 0.30, P zlt; 0.05) were observed among the reactions of red blood cells of the enzyme antioxidant activity GPx, GR, SOD and CAT (all positive), except for the correlation between SOD and CAT. There is a positive correlation between frap plasma reactions and uric acid, FRAP plasma responses and α-tocopherol α concentrations, plasma AAS and vitamin C responses. There was no significant correlation between β reaction to carotene and the reaction of any of the other measured. markers of antioxidant status. DISCUSSION This study found that spinach consumption led to a greater response of red blood cells GR activity and a decrease in blood cells GR activity and reduced α the reaction of micro-tocopherol compared to the control group. However, the evidence for α-tocopherol response in the carotenoids supplement group is not definitive. Because the diets supplied to the group of carotenoids supplement and control group were similar For carotenoids supplement, the differences between these groups, as found for responses of red blood cells GR activity and serum α-tocopherol, can be attributed to the intake of β-carotene, lutein and a small amount of zeaxanthin as carotenoids of supplementation dissolve in oil. It should be noted that lutein is derived from a natural source (calendulas) and thus may contain some flavonoids and other phytochemicals. There was a small but significant correlation to GR activity and the concentrations of lutein in the serum (Pearson correlation ratio: 0.29; Assuming that higher GR reactions in carotenoids and spinach groups compared to the control group were associated with a greater response to the concentration of lutein in the serum. The antioxidant reactions in the group of combined spinach were not significantly different from those in the carotenoids supplement group. This suggests that the observed changes in antioxidant activity or concentration were not caused by increased absorption of β -carotene, because the β -carotene response, but not lutein, was much higher in the carotenoids supplement group than in spinach groups. Where significant differences between the combined spinach and control group were present, but not between the carotenoids supplement and the control group, as we found to respond to in red blood cell CAT activity, the difference is probably due to components other than carotenoids present in spinach (Dragsted et al. 1997). Antioxidant enzyme activity. GR activity increased after 3 wk dietary intervention with carotenoids. The decrease in degradation of antioxidant enzymes due to the protective role of carotenoids, known to deactivate singlet oxygen, may explain the relative increase in enzyme activity in carotenoids supplementation and spinach groups compared to the control group. In addition, carotenoids can act by causing enzymes. Although induction of the antioxidant enzyme theoretically cannot occur in red blood cells, induction, it is reported that the activity of enzymes located in red blood cells changes within a few hours of eating (Saghir et al. 1997). GPx catalyzes the degradation of peroxide with the collateral oxidation of glutathione. If GR and NADPH are present, the oxidized glutathione is immediately converted into a reduced form. So it's not surprising that we found that GPx and GR activities were correlated. SOD catalyzes the dismutation of superoxide anion radicals and catalyses the reduction of hydrogen peroxide into the water. From a kinetic point of view, CAT and GPx are capable of destroying hydrogen peroxide than PPC, suggesting that hydrogen peroxide degrades mainly on GPx under normal conditions (Delmas-Beauvieux et al. a study conducted in France, elderly hospitalized subjects were provided daily with a placebo; 20 mg of zinc plus 100 micrograms of selenium (mineral group); 120 mg of vitamin C and 6 mg ß carotene and 15 mg of vitamin E (vitamin group); or 20 mg of zinc, 100 micrograms of selenium, 120 mg of vitamin C, 6 mg β-carotene and 15 mg of vitamin group). After 6 mo supplements (Monget et al. 1996), significant effects of vitamin group). After 6 mo supplements (Monget et al. 1997), a significant increase was observed in GPx activity in groups receiving minerals alone or in combination with vitamins, but there was no effect on SOD or thiobarbrication activity. Omaye and the staff (1996) fed nine women a low carotenod diet, followed by the same diet supplemented with 15 mg of β -carotene daily for 28 d and found a positive correlation between CPR and GPx activity; they came to β that carotene deficiency does affect the antioxidant status of red blood cells. Our study also found a correlation between CAT activity and GPx. Dixon et al. (1994) reported that RED blood cell activity SOD was suppressed in women emaciated with carotene, but it recovered with a resplendent. In a later study (Delmas-Beauvieux et al. 1996), supplementation of 60 mg of β -carotene daily for 1 in does not result in a significant difference in SOD activity compared to baseline levels, while GPx activity increased slightly, and a significant increase in glutathione status values was observed after 12 m compared to baseline. Thus, there is some previous evidence β-carotene can affect the activity of antioxidant enzymes. Our study shows that not only β, but also lutein affects the antioxidant activity of enzymes. Antioxidant vitamins. After 3 WK dietary interventions, serum α-tocopherol response was lower in groups, given carotenoids supplement or spinach products, than in the control groups. A lower α-tocopherol response may reflect its increased use as an antioxidant, combined with a lower vitamin C content in diets in spinach groups. In addition, a significant correlation was found between α reaction to tocopherol and zeaxanthin. Xi and his colleagues (1992) supplied the subjects with a placebo or 15-60 mg of β-carotene daily for 9 mo and found that all doses of β-carotene led to a similar decrease in plasma levels α-tocoferol after 6 mo. On the other hand, the CARET study, after up to 6 years of supplementation, found a small but significant increase in serum concentration α-tocopherol in participants taking 30 mg of β-carotene and 7.5 mg palmitata (as retinol) per day (Goodman et al. 1994). 1994). studies have shown that oral β-carotene supplements do not change serum levels α-tocopherol. The Polyps Prevention Team (Nierenberg et al. 1994) supplied 505 patients with placebo or 25 mg of β carotene daily and found that serum vitamin E concentrations did not change after 9 m. The Alpha Tocopherol Cancer Prevention Group (Albanes et al. 1997) studied 491 men in Helsinki. After averaging 6.7 u, the group supplemented with 20 mg of β-carotene/d did not have a different α-tocopherol than the control group. Nierenberg et al. (1997) conducted a supplementary study in which subjects received a placebo (n No. 54) or 25 mg β-carotene daily (n No. 54) for 4 y and found that β-carotene supplementation given orally did not alter serum concentrations α-tocoferol. In the course of the experiment in New zealand (cino et al. 1997), the increase in dietary intake of fruits and vegetables in the intervention group by 8 v did not change the concentration of α-tocoferol. We have concluded that most studies β synthetic carotene is given as a supplement there has no effect on the concentration of α-tocopherol. Our study now suggests a link between serum zeaxanthin α responses to tocopherol. Other antioxidants. It should be noted a higher response of plasma uric acid in the group of spinach with whole leaf compared to the group of liquefied spinach. Urat not only behaves like a radical scavenger, but also stabilizes ascorba in bodily fluids, such as in a human serum. This effect is largely associated with iron chelation by urate. Unlike radical-cleaning reactions, this protective effect of the ura is not associated with its depletion, as a stable, non-catholic urat-iron complex (Sevanian et al. 1991) is formed. This phenomenon may explain why we have not found changes in plasma vitamin C responses. Antioxidative effects of spinach and carotenoids cannot be observed in FRAP measurements. A study of the effect of juice intervention on antioxidant status markers showed that after 1 wk intervention with an increase in the amount of blackcurrants and apple juice, only GPx activity increased significantly with the dose and FRAP remained unchanged (Young et al. 1999). Conclusions. Consumption of carotene additives, ß lutein and zeaxanthin, as well as spinach products, has led to changes in the enzyme activity of red blood cells GR and PPC and a concentrations of microcoferol. Antioxidants and antioxidant enzyme systems play a key role in protecting biological membranes. systems are very complex, and changes in one antioxidant can lead to changes in the concentration of other antioxidants or red blood cells of enzymes. We have documented the changes that occur after taking pure carotenoide supplement, and lutein, and a small amount of zeaxanthin, during a 3-WK controlled human dietary study intervention. The results were compared with the results obtained after eating spinach products in the 3-wk study. Consumption of red blood cells GR activity and a decrease in red blood cells CPR and serum a reactions to tocopherol. Consumption of carotenoids supplemented by increased activity of red blood cells GR and lowered the α reaction to tocopherol. Our data show that the consumption of carotene, is positively associated with the activity of REDrocytes GR and negatively associated with the concentration of α-tocoferol, respectively. The effect of spinach consumption on PPC activity is most likely not related to its carotenoids. If analytical analyses are improved in the near future and more information about the antioxidant activity of enzymes becomes available, we will be able to achieve a better understanding of the kinetics and mechanisms of complex antioxidant protection systems. We thank all the participants for their invaluable contribution to the study: Hanneke Reitsma for experimental research on spinach liquefaction; Jerg Kramer (Langnese-Iglo GmbH, Wunstorf, Germany) for spinach production; Saskia Meiboum, Karin Rusemalen, Els Sibelink and Jean de Vries for the dietary aspects of the study; Joke Barendse, Peter van de Bovenkamp, Jan Garrivan, Robert Hovenier, Paul Hulshof, Truus Kosmeijer, Frans Schouten, Marga van der Steen, Peter Versloot and Johan de Wolff for drawing blood and chemical blood tests and food. 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