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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 of the red blood cell enzyme, concentration of non-rimatic 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,  $\alpha$ -tocopherol, 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 catalase and serum concentration  $\alpha$ -tocopherol compared to the control group. Consumption of carotenoids supplement led to a decrease in  $\alpha$ -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  $\alpha$ -tocopherol can be explained by increased consumption of carotenoids (lutein and zeaxanthin), but  $\beta$ -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,  $\alpha$ -tocopherol, humans, spinach Carotenoids are natural compounds in plants, but only a limited amount of carotenoids are found in plasma and human tissues. The main carotenoids are  $\beta$ , lutein,  $\alpha$  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,  $\beta$  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  $\beta$ -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 заболевания (Gaziano et al. 1995, Cardinal et al. 1993, Knekt et al. 1994, Street et al. 1994) and cancer (Stahelin et al. 1991, van Poppel 1996), in particular lung cancer (Dartigues et al. 1990, Knekt 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  $\beta$  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 carotenoid 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/m<sup>2</sup>); their serum cholesterol concentrations of 6.5 mmol/L and triacylglycerol 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. Whole leaf spinach. Minced spinach. Liquefied spinach. Liquefied spinach plus dietary fiber. n 10 12 12 12 12 Men, women; n, n 3, 7 5, 7 5, 7 5, 7 5, 7 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  $\pm$  2.6 12.8  $\pm$  3.4 11.5  $\pm$  2.3 11.3  $\pm$  2.1 11.4  $\pm$  2.1 11.4  $\pm$  2.3 Serum cholesterol, mmol/L 4.14  $\pm$  0.65 4.07  $\pm$  0.61 4.11  $\pm$  0.79 4.23  $\pm$  0.74 3.93  $\pm$  0.47 4.17  $\pm$  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/m<sup>2</sup> 21  $\pm$  2.1 22  $\pm$  2.3 22  $\pm$  2.0 22  $\pm$  2.4 23  $\pm$  2.1 22  $\pm$  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  $\beta$  (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 received 20 g/MJ whole leaf spinach, minced spinach, liquefied spinach or liquefied spinach, to which food fiber was added. All 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 quickly cooled; the stuffed spinach was crushed to 5mm after blanching. An enzymatic drug with pectinase, hemicellulase and cellulose (Rapidase LI, courtesy of Gist-Brocades, Seclin, France) was used to liquefy minced meat. After the enzyme treatment, the spinach was cooked for 5-10 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 diet of the control group and 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 nitrite and 1000 mg of nitrate/kg, thus ensuring that nitrate intake by subjects was lower than permissible daily 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  $\pm$  2 kg. d 0, 1, 8, 15, 21 and 22 periods of intervention to analyze serum carotenoids and concentrations of  $\alpha$ -tocopherol, on d 0, 8, 15 and 22 for plasma analysis of ferricoposizing 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  $\alpha$  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  $\alpha$  anticofeol 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 extraction were conducted in duplication and under muted yellow light with minimal minimum Oxygen. Intra-assid summary for analysis of serum  $\alpha$ -carotene,  $\beta$ -carotene, lutein and zeaxanthin in control pools averages 7.4, 3.9, 3.6 and 8.7% respectively. Erythrocytes are antioxidant enzymes. Antioxidant enzyme activity has been identified in red blood cell leaf cells. Heparinized blood samples were centrifuged at 1,500  $\times$  g for 10 minutes and the plasma was removed. The red 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-aldehydes 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 enzymatic colorimetric methods (Boehringer Mannheim, Germany). The concentration of vitamin C in plasma treated with trichloroacetic acid was determined fluorimetrically as ascorbic plus dehydroascorbat (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 mouflif 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  $\alpha$ -carotene,  $\beta$ -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, w/v) as ascorbat plus dehydroascorbat (CV was 5.6%, Vuilleumier and Keck 1996). Statistical analysis. Responses to dietary intervention were averaged for carotenoids and  $\alpha$ -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 ferriconic 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 red blood cells, all other 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 and the least significant 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  $\pm$  sd. RESULTS Results of the composition of the diet are presented in Table 2 and the 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  $\beta$  carotene concentration increased from 0.253  $\pm$  0.130 to 2.608  $\pm$  1.06 mm/L in the car group of additives and from .283  $\pm$  0.141 to .445  $\pm$  0.172 mmol/l in spinach groups. For lutein, the increase was 0.218  $\pm$  0.064 to 0.983  $\pm$  .287 mcml/L in the carotenoids group and from .216  $\pm$  0.072 to 0.915  $\pm$  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 (P lt; 0.001; independent test t), but did not differ between the combined spinach and control groups (P No. 0.07). Concentrations of triacylcylol 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. Spinach products. Energy, MJ/d 10.6 10.7 10.8  $\beta$ -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

