Supplementation of a Diet Low in Carotenoids with Tomato or Carrot Juice Does Not Affect Lipid Peroxidation in Plasma and Feces of Healthy Men

(Manuscript received 10 November 2003. Initial review completed 8 December 2003. Revision accepted 24 February 2004.)

Karlis Briviba,2 Kerstin Schnäbele,* Gerhard Rechkemmer,* and Achim Bub

Institute of Nutritional Physiology, Federal Research Centre for Nutrition and Food, Karlsruhe, Germany and *Chair of Biofunctionality of Food, Department of Food and Nutrition, Life and Food Science Center Weihenstephan, Technical University of Munich, Freising-Weihenstephan, Germany

ABSTRACT Antioxidant properties of carotenoids are thought to be at least partly responsible for the protective effects of fruits and vegetables rich in carotenoids against colon cancer. There are large amounts of in vitro data supporting this hypothesis. But there is little known about the antioxidant effects of carotenoid-rich food in vivo particularly in the gastrointestinal tract. In a randomized, crossover trial, healthy men (n = 22) who were consuming a low-carotenoid diet drank 330 mL/d tomato juice or carrot juice for 2 wk. Antioxidant capacity was assessed by the "lag time" of ex vivo LDL oxidation induced by copper and lipid peroxidation as determined by measurements of malondialdehyde (MDA) in plasma and feces using HPLC with fluorescence detection. Although consumption of both carotenoid-rich juices for 2 wk increased the carotenoid level in plasma and feces (P < 0.001), the antioxidant capacity of LDL tended to be increased by only ~4.5% (P = 0.08), and lipid peroxidation in the men's plasma and feces was not affected. Thus, processes other than lipid peroxidation could be responsible for the preventive effects of tomatoes and carrots against colon cancer. J. Nutr. 134: 1081–1083, 2004.

KEY WORDS: • tomato • carrot • lipid peroxidation • malondialdehyde

The molecular mechanisms of the anticancer effect of diets rich in fruits and vegetables remain unclear. It was suggested that the antioxidant properties of some phytochemicals such as carotenoids and tocopherols may be responsible for these effects. However, intervention with β-carotene and α-tocopherol did not affect colon carcinogenesis in several clinical trials (1,2). It was proposed that the complex mixture of different phytochemicals in fruits and vegetables can exhibit synergistic beneficial health effects including antioxidant activities. This hypothesis is supported by in vitro experiments showing that mixtures of different carotenoids and mixtures of carotenoids with α-tocopherol or with some polyphenols had synergistic effects on the prevention of lipid peroxidation (3,4).

There are a number of biologically relevant reactive oxygen and nitrogen species (ROS/RNS)3 such as superoxide, hydrogen peroxide, hydroxyl radical, nitric oxide, peroxynitrite, and singlet oxygen and prooxidants such as copper and iron ions that may play a role in colon carcinogenesis (5). These reactive species are able to induce lipid peroxidation during which alkoxyl, peroxyl radicals, and hydroperoxides of unsaturated fatty acids can be formed. The reactivity of these ROS/RNS varies, but they can cause severe DNA damage and induce formation of mutations. ROS/RNS can also induce oxidation of LDL. LDL oxidation can be inhibited by antioxidants, such as carotenoids, which are transported by the LDL particles in plasma. Malondialdehyde (MDA) is an end product of lipid peroxidation and can be used as a marker for in vivo lipid peroxidation and also for formation of the above-mentioned reactive species. There are some doubts whether MDA is a specific in vivo biomarker for lipid peroxidation measured by spectrophotometric methods. However, the use of HPLC with fluorescent detection increases the sensitivity and specificity of MDA measurement and makes this sensitive marker informative. Further, MDA is a frequently used measure and allows us to compare our data with other findings.

Absorption of carotenoids in the gastrointestinal tract is <50%; thus, they reach the colon and are excreted with feces (6). It is possible that carotenoids and other unabsorbed compounds from tomato and carrot juices can modulate oxidative processes in the colon (7). Therefore, we investigated whether carrot or tomato juice consumption (330 mL/d) for 2 wk could affect lipid peroxidation in feces in healthy men (3).

SUBJECTS AND METHODS

After a screening history and medical examination, healthy men (n = 22) who did not smoke or take supplements were selected for the intervention trial. The study was approved by the Medical Ethical Committee of the Landesärztekammer Baden-Württemberg and all participants gave their written consent. The study was designed as a randomized, crossover trial, consisting of two 2-wk treatment periods with tomato juice or carrot juice (each 330 mL/d, Schoenenberger). Intervention periods were preceded by washout phases of a low-carotenoid diet for 2 wk. After the second treatment period, there was a third 2-wk washout period, resulting in a study period of 10 wk.

3 Abbreviations used: MDA, malondialdehyde; RNS, reactive nitrogen species; ROS, reactive oxygen species.
Subjects were told to consume the juices with their main meals. Their daily diet was not restricted, but the subjects were instructed to avoid fruits and vegetables high in carotenoids throughout study. The complete 48-h stool of 12 volunteers was collected the last 2 d of each 2-wk period, directly frozen, and stored at −80°C. During the 2 d of stool sampling and the 2 preceding days, all 12 volunteers received the same energy-adjusted diet to minimize diet-related differences in stool composition. The number of volunteers in the feces sample group was smaller due to the limited capacity of the human nutrition ward and sample facilities.

**Food samples.** Carotenoids from juices were extracted as described previously (8) using diethyl ether ethanol and analyzed by HPLC (see below). The tomato juice (330 mL) provided 37 mg lycopene and 1.6 mg β-carotene; 330 mL of the carrot juice contained 27 mg β-carotene and 13 mg α-carotene.

**Chemicals.** Unless otherwise stated, all chemicals were purchased from Sigma-Aldrich. Organic solvents were purchased from Merck. Carotenoids for HPLC standard solutions were obtained from Carl Roth.

**Preparation of fecal samples.** The frozen complete feces were thawed and homogenized at 4°C (Stomacher 400, Stomacher Laboratory Systems). PBS (10 mL) containing 0.22 mg BHT was added to aliquots of homogenized fecal samples (10 ± 0.1 g) to inhibit lipid peroxidation and to stabilize the carotenoids. Fecal samples were then frozen quickly with nitrogen and stored at −80°C until analysis. On the day of analysis, the fecal samples were thawed, diluted with distilled water, and thoroughly mixed. An aliquot was used for MDA analysis. Carotenoids were extracted using diethyl ether. The organic phase was dried under a stream of nitrogen gas and redissolved in HPLC mobile phase A (methanol:acetonitrile:2-propanol, 45:52:3, vol). HPLC mobile phase B (methanol:acetonitrile:water, 48:52:0, vol, 10 mL/L acetic acid).

**HPLC analysis of MDA and carotenoids.** MDA was measured using a method previously described (9) with minor modifications. To a 0.1-mL sample, 0.68 mL dH2O, 20 μL of 50 g/L BHT (dissolved in ethanol), 70 μL trifluoroacetic acid, and 0.5 mL of 14 g/L thiobarbituric acid were added. The oxides were incubated at 95°C for 45 min. Following a 10-min centrifugation (1000 × g), the samples were analyzed using HPLC on a 4 × 250 mm Vydac RP C18 column with fluorescence detection (excitation and emission wavelengths, 515 and 550 nm, respectively). The mobile phase was potassium phosphate (0.05 mol/L; pH 5.5) and methanol (65:35, v:v). The flow rate was 0.75 mL/min. Standard solutions of MDA were used for calibration. Carotenoids were determined by reversed-phase HPLC using a Supelcosil LC-18 column (5 μm, 250 × 4.6 mm, Supelco) and detected with a Shimadzu photodiode array detector at 450 nm as described previously (10).

**LDL isolation and oxidation.** LDL were isolated by a short-run ultracentrifugation method based on nonequilibrium density-gradient ultracentrifugation (236,000 × g for 2 h at 15°C) (11). Purity of the LDL fraction was confirmed by agarose gel electrophoresis. EDTA and salts were removed from LDL by gel filtration. LDL oxidation was assayed on the day of preparation.

The in vitro oxidation of LDL was performed using a modification of the procedure described by Esterbauer et al. (12). The LDL concentration in the PBS solution was determined by measuring total cholesterol with the CHOD-PAP enzymatic test kit (Boehringer Mannheim) and adjusted for the oxidation assay to 0.1 μmol/L LDL (=0.204 mmol/L cholesterol). The LDL oxidation process was followed by recording the conjugated diene absorption at 234 nm in a Perkin Elmer spectrophotometer (Lambda 15). The instrument was equipped with a water-heated auto-cell holder for simultaneous measurement of 6 samples. Oxidation was started by adding CuCl2; to a final concentration of 20 μmol/L. The recording of the 234-nm absorption was started immediately after the addition of CuCl2 and continued at 3 min for ≤4 h. Intra- and interassay CV were <5% and <8%, respectively.

**Statistical analyses.** Results are given as mean ± SD. Logarithmic transformation was performed, where appropriate, to achieve uniformity of variance. Repeated-measures ANOVA was used to test differences in the variables over time within treatment groups (tomato and carrot juice intervention). A Bonferroni multiple comparison test was used to compare the baseline ("before intervention") with the intervention periods ("after intervention" and "2 wk after intervention"). All statistical calculations were performed with the StatView program (SAS Institute). Differences were considered significant at P ≤ 0.05.

**RESULTS**

All subjects complied with the study protocol and completed both dietary treatments.

**Plasma.** Tomato and carrot juice consumption significantly increased the plasma concentration of the major carotenoids of the corresponding juice (lycopene in tomato juice; α- and β-carotene in carrot juice) (Table 1). After the subsequent 2-wk washout period, concentrations of carotenoids in plasma declined almost to initial values (Table 1).

Consumption of the diet low in carotenoids for 2 wk did not affect (P = 0.36) the plasma concentration of malondialdehyde (MDA) (data not shown). In contrast to the pronounced plasma carotenoid response after intervention with tomato or carrot juice, plasma levels of MDA were not affected by consumption of either juice for 2 wk (Table 1). However, consumption of either vegetable juice tended to increase (P = 0.08) the lag time during ex vivo LDL oxidation by ~4.5%.

**Feces.** Intervention with tomato and carrot juice resulted in significantly increased fecal levels of lycopene and α- and β-carotene, respectively (Table 1). After the subsequent washout period (diet low in carotenoids), the concentrations of carotenoids in feces declined almost to initial values (Table 1). Similar to the data for plasma, supplementation with tomato or carrot juice did not affect MDA concentration in feces (Table 1).

**DISCUSSION**

Although supplementation of a diet low in carotenoids with carrot or tomato juice significantly increased antioxidative carotenoid concentrations in plasma and feces, markers of lipid peroxidation such as ex vivo LDL oxidizability and MDA in plasma and feces were not affected. This could mean that the equilibrium between the prooxidants relevant to lipid peroxidation and endogenous and exogenous antioxidants under normal physiologic conditions followig a 2-wk diet low in carotenoids cannot be affected by supplementation with tomato or carrot juice. Lipid peroxidation is only a part of the complex reduction/oxidation processes in the human body. We cannot exclude that oxidative processes other than lipid peroxidation were affected by supplementation of these vegetable juices.

The intervention with tomato and carrot juice resulted in fecal concentrations of the major carotenoids of ~300 nmol/g dry weight. Concentrations of carotenoids such as lycopene, α-, and β-carotene are able to completely inhibit lipid peroxidation in various in vitro systems (3). It is possible that carotenoids were not solubilized in the feces and were not able to interact with reactive species at the sites of lipid peroxidation.

The increase in carotenoids in plasma after intervention with tomato and carrot juice for 2 wk was significant and agreed with previous reports (13). Despite the increase in plasma carotenoid concentrations, LDL oxidizability only tended to be reduced after vegetable juice consumption (P = 0.08), which might be explained by the short study period and/or the small number of study participants. Previous reports on carotenoids, carotenoid-rich food, and lipid peroxidation
are inconsistent [for a review see (14)]. It was reported that consumption of a carotenoid-deficient diet for 60 d significantly increased plasma levels of MDA (15). We did not observe any changes in plasma MDA levels after the men consumed a diet low in carotenoids for 2 wk. Furthermore, an increase in carotenoid concentration was not associated with changes in plasma MDA concentration. This result indicates that neither carotenoids nor other compounds (e.g., polyphenols) from these juices affect this marker of lipid peroxidation in the blood of healthy men during a 2-wk intervention. This again could be explained by the low level of reactive species able to induce lipid peroxidation in blood or by sufficiently high antioxidant defense against lipid peroxidation even in the absence of antioxidants from carotenoid-rich fruit and vegetables in healthy men.

In conclusion, a 2-wk dietary intervention with a diet low or rich in carotenoids strongly affected the concentration of carotenoids in feces and plasma, but did not affect lipid peroxidation in feces or plasma of healthy men. Consequently, processes other than lipid peroxidation could be responsible for the preventive effects of carrots and tomatoes against colon cancer.

ACKNOWLEDGMENTS

We gratefully acknowledge the excellent technical assistance of C. Driesner, S. Demirel, and T. Gadau.

LITERATURE CITED


