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Colonic Mucosal Bacteria Are Associated with Inter-Individual Variability in Serum Carotenoid Concentrations

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ABSTRACT

Background Relatively high serum carotenoid levels are associated with reduced risks of chronic diseases, but inter-individual variability in serum carotenoid concentrations is modestly explained by diet. The bacterial community in the colon could contribute to the bioaccessibility of carotenoids by completing digestion of plant cells walls and by modulating intestinal permeability.

Objective To evaluate whether colonic bacterial composition is associated with serum and colon carotenoid concentrations.

Design The study was a randomized dietary intervention trial in healthy individuals who were at increased risk of colon cancer. Colon mucosal biopsy samples were obtained before and after 6 months of intervention without prior preparation of the bowels.

Participants/setting Participants were recruited from Ann Arbor, MI, and nearby areas from July 2007 to November 2010. Biopsy data were available from 88 participants at baseline and 82 participants after 6 months.

Methods Study participants were randomized to counseling for either a Mediterranean diet or a Healthy Eating diet for 6 months.

Results At baseline, bacterial communities in biopsy samples from study participants in the highest vs the lowest tertile of total serum carotenoid levels differed by several parameters. Linear discriminant analysis effect size identified 11 operational taxonomic units that were significantly associated with higher serum carotenoid levels. In linear regression analyses, three of these accounted for an additional 12% of the variance in serum total carotenoid concentrations after including body mass index, smoking, and dietary intakes in the model. These factors together explained 36% of the inter-individual variance in serum total carotenoid concentrations. The bacterial community in the colonic mucosa, however, was resistant to change after dietary intervention with either a Mediterranean diet or Healthy Eating diet, each of which doubled fruit and vegetable intakes.

Conclusions The colonic mucosal bacterial community was associated with serum carotenoid concentrations at baseline but was not appreciably changed by dietary intervention.

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AMONG THE BENEFICIAL ASPECTS OF A MEDITERranean diet is its high content of fruits and vegetables. Relatively high dietary carotenoid intakes are linked to decreased risks of several cancer types.[1] Although the data linking colon cancer risk with dietary intakes of carotenoids are equivocal, high serum carotenoid concentrations were significantly associated with lower rates of colon polyp recurrence and of colon cancer risk when repeated measures of beta carotene concentrations were analyzed.[2,3] A caveat is that in those studies, dietary fat was concomitantly being reduced. In an intervention trial, the Polyp Prevention Trial, individuals who had the best compliance with the low-fat, high-dietary fiber, high-fruit, and -vegetable eating pattern had lower polyp recurrence rates.[4]

Inter-individual variation in serum carotenoid concentrations is generally large and stems from many factors.[5] In the Healthy Eating Study, from which colon biopsy samples were obtained for the present study, the coefficient of variation for total serum carotenoid concentrations was about 65%.[6] Carotenoids are obtained from fruits and vegetables, but the correlation coefficients between serum carotenoids and dietary intakes are typically not >0.5.[7,8] Behavioral and metabolic factors, therefore, might be important in governing serum concentrations of carotenoids. Many polymorphisms have been identified that either

influence carotenoid uptake (eg, lipoproteins that transport carotenoids) or metabolism, including the beta carotene oxygenases.[9] Demographic factors associated with relatively low serum carotenoid concentrations include male gender, high body mass index (BMI), smoking, and, possibly, alcohol intake, although the latter does not display consistent associations with serum carotenoid concentrations across population groups and/or types of carotenoids.[10-12] In a middle-aged French population, age, diet, alcohol intake, serum cholesterol, BMI, and smoking status explained 15.2% of the variance of beta carotene in men and 13.9% in women.[12] In another study of European middle-aged adults, gender, BMI, smoking, age, education, alcohol consumption, season, population center, and supplement use explained 15% to 25% of the variance in individual serum carotenoid concentrations.[11] Concurrent intake of food components other than carotenoids is also important: dietary fiber can inhibit carotenoid absorption by interfering with micelle formation and micelle interactions with enterocytes, and a small amount of dietary fat is needed for complete absorption.[13-15]

Carotenoid bioavailability from foods also varies. In a review of studies, the bioavailability of beta carotene from vegetables compared with purified beta carotene ranged between 3% and 24%, depending on the vegetable and method of preparation.[13] Breakdown of plant cells walls appears important, and the bioavailability of carotenoids is improved by heat treatment of foods.[16,17] It is also possible that undigested plant cells trap carotenoids and other phytochemicals that are then released upon microbial digestion in the colon. Several studies show that intestinal bacteria have a role in maximizing bioaccessibility of phytochemicals by degrading plant cell walls.[13] In vitro, colonic fermentation increases release of carotenoids from the food matrix.[18] Polyphenols in whole plant foods, as opposed to polyphenols in juices, are estimated to be more bioaccessible in the colon than in the small intestine,[19] which also may be the case for carotenoids.

Thus far, the vast majority of human gut microbiome studies have been based on fecal bacteria, but we postulate that mucosal bacteria play a more direct role in mediating availability and absorption of dietary carotenoids. Certain bacteria, such as *Akkermansia*, are known to reside in the mucin of the mucosal surface, but their physiological roles are not yet well defined and this is the subject of ongoing research.[20]

In the present study, colonic biopsy samples were available from individuals with an elevated risk of colon cancer who were enrolled in a dietary intervention trial.[21] The trial randomized participants to receive dietary counseling for either a Healthy Eating diet or a Mediterranean diet for 6 months. Blood, colon biopsy samples, and dietary data were obtained before and after intervention. The primary goal of the intervention was to reduce proinflammatory eicosanoids in the colon. The present study evaluated whether the relative abundance of the bacterial populations adhering to the mucosal surface are associated with inter-individual variation in carotenoid concentrations in serum. In addition, changes in the relative abundance of specific bacterial taxa were evaluated after intervention with either a Healthy Eating diet or Mediterranean diet for 6 months.

METHODS

Participants and Samples

Details of the Healthy Eating Study have been published previously.[6,21] The study was approved by the University of Michigan Institutional Review Board (HUM00007622) and registered at ClinicalTrials.gov (registration no. NCT00475722). Briefly, a total of 120 individuals at increased risk of colon cancer were enrolled from Ann Arbor, MI, and surrounding areas from July 2007 to November 2010. Study participants provided signed, informed consent and were randomized to a Healthy Eating diet or Mediterranean diet. A total of 93 participants completed 6 months of study. Increased risk was defined as a family history of colon cancer in a first-degree relative or two second-degree relatives, or a personal history of an adenoma or colon cancer. The primary goal of the study was to evaluate changes in colonic eicosanoid concentrations. Dietary intakes of carotenoids, from analysis of food records, roughly doubled in both study arms.[6]

Fasting blood samples were obtained in ethylenediaminetetraacetic acid tubes, and plasma was stored at −80°C before analysis as described.[6,21] C-reactive protein, lipopolysaccharide (LPS)-binding protein, cholesterol, high-density lipoprotein (HDL), low-density lipoprotein, and triglyceride levels were measured using commercial kits as previously published.[22] The homeostasis model of assessment for insulin resistance was calculated from fasting C-peptide and glucose using an online calculator from the University of Oxford (The HOMA Calculator version 2.2).[23] Eight colon biopsy samples were obtained without prior preparation of the bowels by flexible sigmoidoscopy from each participant at each time point. The biopsy samples were all collected in the colon 20 to 25 cm from the anal sphincter. Biopsy samples were flash frozen in liquid nitrogen exactly 5 seconds after harvesting and were frozen at −70°C until analysis. After biopsy samples were used for the primary study end points, one colon biopsy sample was available for microbiome analysis from 94 participants at baseline and from 85 participants after dietary intervention (179 biopsy samples of the 212 biopsy samples originally collected for the study).

Dietary Intervention

Subjects were randomized to receive counseling with a registered dietitian nutritionist for either a Healthy Eating or a Mediterranean diet for 6 months. The Healthy Eating arm had goals for consuming at least $5^1/_2$-cup servings/day of fruit and vegetables (including at least a single serving that is a dark green or dark orange fruit or vegetables), at least 3 servings/day from whole grains, and <10% of calories from saturated fat. The Mediterranean arm goals were to maintain 30% of calories from fat while achieving a polyunsaturated to saturated to monounsaturated fatty acids ratio of 1:2:5. Other goals were to consume foods high in n-3 fatty acids at least twice a week, at least 3 servings/day from whole grains, at least 7 to $9^1/_2$-cup servings of fruits and vegetables per day, depending on energy intake, and to include both culinary herbs and allium vegetables daily. The counseling was done mainly by telephone. Details of the interventions, including adherence, have been published previously and indicated that the main difference between arms after 6 months was in