hypothesis group antigens. Recent studies (9) showed the contribution of host genetic factors (the FUT2 genotype in particular) on differences in the gut microbiota between patients with Crohn disease and healthy individuals. Thus, an intimate interaction between host genetics and bioactive components is expected and should be linked to both infant microbiota colonization and immune system development.

The process of microbial colonization of the infant’s gut plays a pivotal role in the metabolic and immunologic development of the child. Deviations from the characteristic microbiota of the healthy breastfed infant are associated with an increased risk of allergic and inflammatory conditions as well as obesity. The transfer of microbiota via breast milk from mothers to infants is influenced by maternal allergic and obesity status (10, 11). Therefore, the different species of bifidobacteria and lactobacillus bacteria present in the breast milk of allergic and obese mothers are expected to influence infant gut colonization through a constant daily inoculum during breastfeeding. Thus, modulation of maternal gut microbiota during pregnancy and lactation could have a direct effect on infant health, and microbes present in milk should be characterized to evaluate if this microbiota could be a source for future and specific probiotic strains.

We hope that our article stimulates further work to determine the role of host factors in modulating the composition of the human microbiota, enabling new applications in the field of personalized nutrition and medicine.

The authors did not declare any conflicts of interest.

Raul Cabrera-Rubio
Alex Mira

Joint Unit of Research in Genomics and Health Centre for Public Health Research (CSISP) Cavanilles Institute for Biodiversity and Evolutionary Biology University of Valencia Valencia Spain

Seppo Salminen

Functional Foods Forum University of Turku Turku Finland

Erika Isolauri

Department of Pediatrics Turku University Hospital and University of Turku Turku Finland

M Carmen Collado

Institute of Agrochemistry and Food Technology, Spanish National Research Council (IATA-CSIC) Av Agustin Escardino 7 49860, Paterna Spain
E-mail: mcolam@iata.csic.es

REFERENCES


Nutrient biomarkers are not always simple markers of nutrient intake

Dear Sir:

In a recent meta-analysis (1), blood measurements of carotenoids were more strongly associated with lower breast cancer risk than were dietary measurements. When results from studies based on nutrient biomarkers provide a stronger association than those based on dietary assessments, the frequently accepted explanation for the discrepancy is that the true relative risk is attenuated from measurement error by using dietary questionnaires. Assuming that a causal association exists, the blood measurement may more directly assess the relevant exposure. However, potential limitations of nutrient biomarkers as representing a causal association need to be considered. A causal association here assumes that altering the nutritional will directly affect disease risk. I will briefly describe 5 examples where results from nutrient biomarkers are confounded on the basis of nondietary determinants of the concentration of the
nutrient biomarker. Some of these examples are relevant for carotenoids, but these can be considered generally for nutritional biomarkers.

First, absorption of the nutrient in the gastrointestinal tract is one factor that contributes to a lower correlation between a dietary questionnaire assessment and a circulating biomarker. A biomarker taking into account bioavailability could potentially help strengthen an association between the nutrient and an outcome, but it might also exhibit confounding. For example, circulating vitamin B-12 concentrations are strongly influenced by absorption, and especially in older populations vitamin B-12 concentrations may largely reflect chronic gastritis and low production of stomach acid and intrinsic factor (2). Thus, any association between circulating vitamin B-12 concentration and an outcome could be confounded by determinants of gastritis. For example, low vitamin B-12 would likely be a risk factor for gastric cancer, but this association merely reflects confounding by gastritis, the causal factor. Whereas this may represent an extreme example, absorption of some nutrients varies widely among individuals, often due to poorly understood factors.

Second, blood concentrations of the nutrient may be influenced by exogenous and endogenous factors, which could potentially be causal factors of the disease of interest. For example, blood carotenoid concentrations are lowered by smoking and alcohol intake (3, 4). Although we can control statistically for smoking and alcohol, the biomarker will also be influenced by genetic and other factors that modify the smoking and alcohol exposure, so controlling for smoking and alcohol behaviors would not entirely control for confounding. For example, if 2 individuals have the same alcohol intake, but differ in metabolism due to genetics, their alcohol “exposure” will differ despite similar intakes. The person who metabolizes alcohol more slowly (ie, higher alcohol “exposure”) will have lower carotenoid concentrations, all else being equal. Thus, carotenoid concentrations inevitably have some degree of residual confounding from alcohol metabolites.

Third, pathophysiologic processes that may influence both the biomarker concentration and the disease of interest may confound the association between the nutrient biomarker and the disease. For example, inflammation is known to decrease circulating pyridoxal 5'-phosphate (PLP) concentrations (the biomarker of vitamin B-6) (5, 6). In a meta-analysis, colorectal cancer risk decreased significantly by 49% for every 100-pmol/mL increase in blood PLP concentration (7). However, a recent study of vitamin B-6 intake did not show an association with colorectal cancer risk, even though intake predicted a large difference in circulating PLP (for men, PLP was 183.2 and 91.5 pmol/mL in those in the high and low quintiles of intake, respectively) (8). Extrapolating the dose-response from the meta-analysis, the dietary exposure measure should have been precise enough to detect a substantial association with colorectal cancer, albeit with some attenuation. This finding suggests that nondietary determinants of plasma PLP may influence risk. For example, if PLP is associated with risk of colorectal cancer because low concentrations correspond to an inflammatory state (5), increasing PLP concentrations through diet may not necessarily lower risk of colorectal cancer.

Fourth, the biomarker may not reflect the etiologically relevant metabolite of the nutrient. For example, the metabolite of folate is complex, and various forms of folate may have differential effects on cancer. What is typically measured in the blood, 5-methyltetrahydrofolate (THF), is a reasonable surrogate of overall folate status, but some evidence suggests that 5,10-methyleneTHF is the relevant protective form of folate (9). For example, the methyleneTHF reductase (MTHFR) C677T polymorphism (rs1801133), which increases 5,10-methyleneTHF concentrations at the expense of 5-methylTHF, appears to be protective for colorectal cancer (10). Because this variant tends to drive folate intracellularly in the form of 5,10-methyleneTHF, 5-methylTHF concentrations in the blood are slightly lowered. In a recent study of plasma folate status, higher plasma folate was associated with a slightly higher risk of colorectal cancer, but when variation in plasma folate was partitioned into that related to intake and that related to MTHFR, variation due to intake was inversely associated with risk and variation due to MTHFR C677T was positively associated with risk (9). A possible interpretation is that folate may be a true protective factor, but high circulating 5-methylTHF reflects both high folate intake and low intracellular 5,10-methyleneTHF due to the genetic variant. The association of circulating folate (ie, 5-methylTHF) and cancer risk may thus be unpredictable because this marker may not always reflect intracellular 5,10-methyleneTHF.

Finally, the nutrient biomarker may have correlations with other circulating factors, which may be confounders. The reasons for these correlations may not always be apparent. For example, carotenoid concentrations have sizable positive correlations with plasma cholesterol and inverse correlations with plasma triglyceride concentrations; the reasons for these correlations are not entirely clear and may reflect how carotenoids partition in different lipid compartments (4). Whatever the reason, these lipids may have associations with disease that could potentially cause confounding between carotenoid concentrations and the disease.

Nutritional biomarkers can be useful in our understanding of a nutrient-disease relation. Nonetheless, variation in concentrations of biomarkers may often reflect other exposures (eg, alcohol, tobacco) and metabolic, genetic, physiologic, or pathophysiologic processes. If these factors are directly related or predictive of disease, they may produce an association between the biomarker and the disease. If so, altering the concentration of the biomarker through modifying intake of the nutrient would not affect risk of the outcome.

The author declares no conflict of interest.

Edward Giovannucci
Department of Nutrition
Harvard School of Public Health
665 Huntington Avenue
Boston, MA 02115
E-mail: egiovann@hsph.harvard.edu

REFERENCES


doi: 10.3945/ajcn.112.053769.

Reply to E Giovannucci

Dear Sir:

We thank Giovannucci for his thoughtful comments related to our article comparing dietary intake and blood concentrations of carotenoids in relation to breast cancer risk (1). Giovannucci expands some of the points and indeed several of the limitations that we mentioned in our discussion, albeit somewhat briefly due to space constraints.

As for the first point that Giovannucci raises that circulating biomarkers may reflect confounding by diseases and/or interindividual variation in absorption, we stated in our article’s Discussion, “The interpretation of our results was also complicated because dietary assessment of carotenoid intake may not reflect bioavailability as their absorption may be influenced by several factors including degree of processing or cooking of foods, the lipid content of the diet, degree of fermentation in the colon, menstrual cycle and hormonal factors, and possibly genetic factors. In addition, the metabolism of carotenoids can affect their blood concentrations and reduce their correlation with dietary intake. Carotenoids can be metabolized to retinol, particularly in subjects with low vitamin A status; in addition, smoking and high alcohol consumption may reduce blood concentrations of carotenoids.”

As for the second point that carotenoids may be influenced by exogenous and endogenous factors that could potentially be causal factors of the disease of interest, such as smoking and alcohol intake, we stated in the third paragraph of the Discussion, “We cannot exclude the possibility that the observed inverse association between dietary β-carotene intake or blood concentrations of carotenoids and breast cancer risk could be a result of unmeasured or residual confounding. Persons with higher carotenoid exposures may have higher levels of physical activity, lower prevalence of overweight and obesity, and lower intakes of alcohol and dietary fat. Many, but not all of the studies included in this meta-analysis adjusted for these and other potential confounders. In subgroup and meta-regression analyses, no evidence of between-subgroup heterogeneity in the dietary analyses was found, but there was no association in the subgroup analyses of blood concentrations of carotenoids that were adjusted for BMI, physical activity, and energy intake, although the number of studies was small in some of these analyses. Any further studies might want to assess whether adjustment for more confounders has any influence on the risk estimates.”

The variability in bioavailability and absorption of carotenoids is difficult to assess in large epidemiologic studies. A large study of determinants of blood concentrations of carotenoids in men and women from 16 geographical regions in Europe showed that region (most likely reflecting differences in diet) is the most important determinant of plasma carotenoids concentrations in the general population (2). BMI explained part of the variability in plasma carotenoid concentrations, with partial $R^2$ values in the range of 0.8–4.2%, but may simply reflect a lower fruit and vegetable intake among persons with a high BMI. Although smoking and alcohol intake are thought to influence carotenoid concentrations in blood, both smoking and alcohol contributed little to the variability in carotenoid concentrations in this study (partial $R^2$ for alcohol intake ranged between 0.2% and 0.6% and between 0.9% and 2.6% for smoking), even though there was variability in smoking habits and alcohol intake across regions (2). In contrast, dietary intake of fruit and vegetables explained a larger part of the variability in plasma carotenoid concentrations in a separate analysis from the same study (partial $R^2$ for fruit and vegetables ranged between 1.6% and 15.8%) (3). Specific types of vegetables also explained a larger part of the variation in plasma concentrations of specific carotenoids. For example, tomatoes and tomato products and carrots explained 13.8% and 13.4% of the variation in lycopene and β-carotene concentrations, respectively, whereas total fruit and citrus fruit explained 17.2% and 12.9% of the variation in β-cryptoxanthin concentrations, respectively. These results are largely in agreement with another study in older people across Europe (4).

As for the third point that pathophysiologic processes may influence both the biomarker concentration and the disease of interest may confound the findings, we agree that this is a possibility, but we are not aware of any direct examples relating to carotenoids and breast cancer.

As for the fourth point that the biomarker may not reflect the etiologically relevant metabolite of the nutrient, we agree. Although we provided some potential mechanisms by which carotenoids may influence cancer risk in the discussion, we found no association between supplemental β-carotene and breast cancer risk, and we also clearly stated that there may well be other correlated constituents that may be the relevant agents: “However, the inverse associations between blood concentrations of carotenoids and breast cancer risk we observed may not be solely a result of the effect of single antioxidants. Blood concentrations of carotenoids are biomarkers of intake of fruit and vegetables, which contain a myriad of bioactive compounds, including fiber, flavonoids, and other antioxidants, that may act synergistically to reduce breast cancer risk.”

As for the fifth point with regard to the possibility of confounding by other circulating factors, such as cholesterol and plasma triglyceride concentrations, we are not aware that there is an established association between these factors and breast cancer risk. However, we have conducted additional analyses of blood concentrations of selected carotenoids stratified by adjustment for plasma cholesterol concentrations (only one of the studies adjusted for plasma triglyceride concentrations). There was no evidence of significant heterogeneity between subgroups in these additional stratified analyses, although a somewhat stronger inverse association was observed in the analysis of blood concentrations of β-cryptoxanthin that was adjusted for plasma cholesterol ($P$-heterogeneity = 0.09, Table 1).

The dietary estimation of carotenoid intake is prone to measurement error and may not reflect the actual carotenoid bioavailability. Given the few dietary risk factors that have been established for breast cancer (5), we think further well-conducted studies of blood concentrations of carotenoids, which is an integrated measure of intake and absorption, in relation to breast cancer risk may clarify inconsistent results between dietary intake and breast cancer risk.