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**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 α-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.
Neither of the authors had a conflict of interest to disclose.

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REFERENCES


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### TABLE 1

<table>
<thead>
<tr>
<th>Adjustment for plasma cholesterol</th>
<th>Total carotenoids, per 100 µg/dL</th>
<th>β-Carotene, per 50 µg/dL</th>
<th>α-Carotene, per 10 µg/dL</th>
<th>β-Cryptoxanthin, per 15 µg/dL</th>
<th>Lycopene, per 25 µg/dL</th>
<th>Lutein, per 25 µg/dL</th>
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<tr>
<td></td>
<td>n1</td>
<td>RR (95% CI)</td>
<td>n</td>
<td>RR (95% CI)</td>
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<tr>
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<td>0.52 (0.33, 0.82)</td>
<td>5</td>
<td>0.82 (0.40, 1.70)</td>
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<td>0.83 (0.67, 1.04)</td>
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<td>0.82 (0.70, 0.97)</td>
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<td>P-heterogeneity2</td>
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1 n, number of studies
2 Between subgroups.