Author’s response to reviews

Title: Concurrent validity of skin carotenoid status as a concentration biomarker of vegetable and fruit intake compared to multiple 24-hour recalls and plasma carotenoid concentrations across one year: a cohort study

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Response to Reviewers

Concurrent validity of skin carotenoid status as a concentration biomarker of vegetable and fruit intake compared to multiple 24-hour recalls and plasma carotenoid concentrations across one year: a cohort study

Lisa Jahns, PhD, RD; LuAnn K. Johnson; Zach Conrad; Michael Bukowski; Susan K. Raatz; Stephanie Jilcott Pitts; Youfa Wang; Igor Ermakov; Werner Gellermann Nutrition Journal

Reviewer #1:

General remarks
The authors present a manuscript on the correlation between measurements related to fruit and vegetable intake, as assessed via analyzing carotenoid concentrations. More specifically, fruit and vegetable intake (VF multiple 24 h recalls) are compared with carotenoid concentrations in plasma and by skin carotenoid status (SCS) as measured via either RAMAN (RRS) or reflection spectroscopy (RS), as measured during a 1-year period in a longitudinal study on 40-60 year old women.

It is found that RS and RRS strongly correlated, and RS correlated well with carotenoids at baseline and reasonably over the year. VF intake did not or only weakly correlate well with RRS and RS.

The study appears well-conducted, even though it was not originally designed to have SCS as the primary outcome. It is well presented, and results are of much interest, as it is important to find good proxy markers for VF intake, due to the associated health benefits. Specific suggestions are made below.

Thank you for your review and helpful suggestions.

Specific points

1. Line 10 - unclear how many 24 h recalls were carried out per group?

Each participant in the two cohorts completed 36 recalls over the year. We have added the following to the abstract (line 11):

“…total 1,866 recalls…”

2. Line 22 - perhaps "SCS as measured by RS and RRS is…”

Thank you, we have added the above text (Ln 22).

3. Line 38 - which tools are these - perhaps give a few examples.

We have amended the sentence to read (Ins 38-39):

“Accurately measuring VF intake is crucial for population surveillance and for evaluating the efficacy of interventions, but a key dilemma in this pursuit has been inherent bias and error in self-reported measurement tools such as food frequency questionnaires, food records, and 24-hour recalls [6].”

4. Line 42 - what is meant by "the substance"?

We have clarified by changing the term “substance” to “marker of interest” (Ln 43).

5. Line 96 - "all equally to this frequency…” - can you add a reference here?
The reviewer is correct, phytoene and phytofluene, UV-absorbing carotenoids, do not contribute to the signal. We have amended the text on lines 99-100 and added a reference:

“Since almost all carotenoid subspecies contribute almost equally to this frequency (excluding phytoene and phytofluene) [11],” …

6. Lin 88ff - which instrument was used in this study?

We used devices built by two of the authors, Igor Ermakov and Werner Gellermann. We have added to the text on lines 92-93:

“The two devices used were built by the authors (IE and WG) for use with nutrition studies.”

7. Line 113 - which food-database is used here - the USDA one?

We have included the following text to Ins 122-125 to clarify where the data originated:

“Provided reports included servings of VF derived from the USDA Food and Nutrient Database for Dietary Studies 4.0 [27] and the USDA MyPyramid Equivalents Database 2.0 [28], and dietary carotenoids (mg) estimated using data from the USDA Standard Reference 22 [29] database.”

8. Line 125 - it is unclear which carotenoids were measured finally?

Our apologies, we are not sure to what the reviewer is referring? We list the carotenoids used in this research on lines 133-134.

9. Line 125 - which were qualifying, which quantifier-ions measured? Which mode of the instrument was used - please give more information here. This is important, in order to understand better the obtained correlation results. For example, were colorless carotenoids such as phytoene and phytofluene also measured?

We report the total of α- and β-carotene, β-cryptoxanthin, lycopene, and lutein and zeaxanthin (coeluted). As these are detected by MRM, we have only three points to go on: 1) The precursor ion mass, 2) the product ion mass, and 3) the retention time. Not all carotenoid standards are available or relevant. For example, some authors use eichenone, which is a naturally occurring carotenoid in sea urchin. We aren’t reporting astaxanthin or canthaxanthin but that is more an indictment of the American diet, low in fatty fish and shell fish. We also do not include phytoene and phytofluene, the so-called “colorless carotenoids” as these are not reflected in skin carotenoid measurements by RS or RRS (please see response to Q5).

10. Line 185 - was it attempted to stratify for BMI - or taking this into account via creating regression models - also perhaps checking on other personal parameters?

The reviewer is absolutely correct that we should have tested for BMI as a potential covariate. In our previous studies, we did not find BMI to be significant, although one would expect it to be.
We added BMI to the model and found a slight decrease in both the between person correlation (0.78 to 0.76, \(P < 0.001\)), and within-person correlation \([0.32 \ (P = 0.02) \text{ to } 0.30 \ (P = 0.03)]\).

We have corrected the methods, figure, the figure legend, and the results reported. Age was not significant in the model. We did not test other personal parameters as this sample was meant to be homogenous, e.g. all women between 40-60, almost all non-Hispanic white, living in same small town.


It was an oversight to not include this important synthesis and we have included it as indicated. Thank you for bringing it to our attention.

12. Line 245 - add animal foods as an important source also.

Thank you, we have added the following sentence to lines 257:

“Animal foods, such as eggs (a rich source of lutein), shellfish and salmon contain carotenoids.”

13. Table 1, total plasma carotenoids: This is geometric mean plus minus SEM then ?

Yes, the plasma values were skewed and so transformed for normality prior to analysis, then back-transformed (please see methods section).

Reviewer #2: Thank you for your well-written submission, which aims to assess the utility and validity of spectroscopy-based quantification of skin carotenoid levels compared to plasma carotenoid concentrations and self-reported dietary carotenoid intake. Multiple measurements were taken over one year to attempt to consider seasonal changes.

Thank you for your kind assessment of our manuscript.

A few comments:

1) The introduction reads very well, but the reader requires some additional explanation about the relationship between carotenoid intake and skin carotenoid deposition - I assume that circulating carotenoid levels reflect very recent V&amp;F intake, but what is SCS is measuring? As a reader who is not a carotenoid expert, I assume skin deposition of carotenoids might not reflect short-term V&amp;F intake, and might instead reflect habitual, longer-term carotenoid intake, with additional influence by exposure to free radicals generated during cigarette smoking, illness and UV radiation etc. How long can carotenoids remain in the skin, and are they longer-lived in the skin compared to in the bloodstream? I realise this is what you are trying to determine with this research, but the reader requires more of an explanation about the
physiological relationship between dietary carotenoid intake, plasma carotenoid levels and skin carotenoids concentration, and the potential limitations of these optical methods.

As the reviewer states in Q5 that we largely address the concerns above, we added text to the introduction as follows on lines 55-56:

“…and found to be representative of longer-term intake than blood carotenoid concentrations, perhaps by 1-2 weeks.”

2) Methods: The research is methodologically sound. I would recommend you include the percentage of women participating in the study who were smokers (even if reported elsewhere, it needs to be mentioned in this article).

We have added the following to line 86:

“None of the participants reported current smoking”.

3) Methods: What is the intra-individual percentage error for these SCS techniques? This is especially important for the prototype RS instrument used.

This is an important point, and the reviewer brings up a good question. We have added the following to the methods section on lines 103 and 112-113:

“The intraindividual variability between the 3 scans is 5% on this instrument.”

“The intra-individual variability of the prototype instrument varies between 0.5% and 14%, depending on the individual.

4) Please report the percentage of 24-hr recalls that were not completed during the study. Over time, participants may have been less compliant with the completion of these recalls (a potential limitation?)

We actually had a 92% response rate of individuals completing all 36 recalls, (range 33-37). We have added to the methods the following (ln 126):

…with a 92% response rate for all 36 recalls (range 33-37).

5) The discussion is well-written as it clearly describes the strengths and limitations of the study, does not oversell the findings and to a large extent addresses my first comment at 1).

Thank you.