Validity and Reliability of Field Resonance Raman Spectroscopy for Assessing Carotenoid Status

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(Received March 1, 2016)

Summary Carotenoids in fruit and vegetables are important for health, yet determining dietary intake is challenging. This study aimed to establish the validity and reliability of a portable field Resonance Raman Spectroscopy (RRS) in reflecting human carotenoid status. A diagnostic accuracy study involving 81 healthy adults was conducted. The RRS was the index test. Serum carotenoids (β-carotene, lycopene, lutein, and zeaxanthin) and consumption of fruit and vegetables were primary and secondary reference standards respectively. Data were collected in two seasons. Validity was determined by the correlation between the RRS score and the two reference standards, and by diagnostic statistics comparing dichotomised RRS scores and serum β-carotene. Reliability was assessed by intra-class correlation from repeated observations within subjects and within repeated measurements using three devices. The RRS score was significantly correlated with the individual and summed serum carotenoids (r range 0.45 to 0.78; p always <0.001), and with fruit and vegetable intake (season one: r=0.38, p=0.016; season two: r=0.42, p<0.001). Sensitivity: 87.5%; specificity: 75.5%; positive and negative predictive values: 35.0% and 97.6% respectively. Within- and between-device reliability was high (r=0.98, p=0.004 and r=0.97, p=0.009 respectively). The RRS field model achieved criterion validity for assessing carotenoid status and fruit and vegetable intake, and also demonstrated reliability. It thus holds promise for the screening of carotenoid status and fruit and vegetable intake.

Key Words Resonance Raman Spectroscopy, fruit and vegetable screener, skin carotenoid testing, validity, reliability

Fruit and vegetable consumption forms a cornerstone of good nutrition, partly stemming from the presence of carotenoids such as β-carotene, lycopene, lutein and zeaxanthin. Intakes in South Africa are below international recommendations and a recent revision of the national food-based dietary guidelines stresses increased intakes of fruit and vegetables (1). A diet rich in carotenoids is associated with eye and skin health, and prevention of heart disease, stroke and several cancers (2–4). Measuring carotenoid status and fruit and vegetable intake relies on biochemical analysis of body tissues and dietary assessment, which have practical (e.g. cost, invasiveness, time, expertise) and scientific (e.g. recall bias) limitations (5, 6). Recent research has focused on the assessment of carotenoids in the skin, one of the storage sites of these substances. Carotenoid levels in the skin were related to serum concentrations (7) and carotenoid levels in skin biopsies as assessed by Resonance Raman Spectroscopy (RRS) were positively correlated to assessment by high performance liquid chromatography (HPLC) (8). Manufacturer-sponsored studies of a portable field model of the RRS indicated that scores obtained with this device were related to fruit and vegetable consumption (9–11) and that a correlation exists between serum and skin carotenoids when measured by HPLC and field RRS respectively (10, 12, 13). No South African data are available, yet the field RRS is used in some clinical practices.

The aim of this study was to perform a diagnostic accuracy study where, first, the validity of a field RRS was determined relative to serum carotenoids as a primary reference standard and to dietary intake of fruit and vegetables as a secondary reference standard. Second, the study aimed to establish the reliability of the device. Validity was defined in terms of criterion validity, meaning that the field RRS was compared to one or more external criteria that intend to measure the same attribute (14). Reliability referred to intra- and inter-repeatability of the field RRS score. Beta-carotene, lycopene, lutein and zeaxanthin (four of the six most abundant carotenoids) in human serum were taken to represent carotenoid status.

MATERIALS AND METHODS

Subjects. Eighty-seven healthy South African volunteers were recruited by referrals from RRS operators, through word of mouth and a health awareness website. Factors previously shown to affect serum carotenoid levels were used as exclusion criteria. These factors were self-reported smoking, moderate to heavy alcohol consumption (10 g or more daily), underlying chronic diseases like cancer, daily sunlight exposure...
exceeding 3 h and current use of antibiotics, oral contraceptives and carotenoid-containing supplements (9, 10, 12). People with measured body mass index (BMI) over 30 kg/m² were also excluded. Data collection took place during seven testing events in two sites in Pretoria, South Africa. The first three events (season one) took place from August to October and the remaining events (season two) produced all the participants with blood samples (April to May). Approval to conduct the study was obtained from the Research Ethics Committee of the Faculty of Health Sciences, University of Pretoria. Informed consent was obtained from each participant.

**RRS.** The Biophotonic Scanner (NuSkin Enterprises, Provo, Utah) was the RRS device used. For the spectroscopic examination the participant placed the palm of his/her hand against the laser port of the device where the skin of the palm was exposed to a non-invasive blue laser light. The score was obtained after 3 min via a connected notebook computer. To test the intra-device repeatability of the spectroscope a second measurement was taken after about 5 min from a group of 57 participants using the same device, the second test being performed after the spectroscope had been recalibrated. To test the inter-device repeatability of the spectroscope two measurements were taken from a group of 48 participants, using a different device for the second test immediately after the first test had been taken. This second spectroscope malfunctioned after 24 scans and a
Anthropometric data were measured following standard procedures ([96%]), one as Indian and two as African. The mean age was 40.6 (SD 12.2) and 42.8 (SD 12.0) y for males and females respectively. For 61 subjects RRS and serum carotenoid as well as dietary data were available. Mean BMI was 25 (SD 2.2) and 23.7 (SD 2.7) kg/m² for males and females respectively.

Validity

The correlations between the RRS score and each of the carotenoids and the sum of the carotenoids was highly significant. Figure 1 and Table 1 illustrate this point. $\beta$-Carotene had the strongest correlation with the RRS score ($r=0.78$; $p<0.001$). When $\beta$-carotene was used as a reference standard, the RRS had a sensitivity of 87.5%, a specificity of 75.5%, a negative predictive value of 97.6% and a positive predictive value of 35.0%. $R^2$ was 0.61, meaning that 61% of variation in $\beta$-carotene can be explained by variation in Raman scores.

The correlations between the RRS scores and the fruit and vegetable intake scores were statistically significant in both seasons.

As can be seen from Table 2 and Fig. 1 the correlation coefficients between serum carotenoid levels and the fruit and vegetable intake scores were relatively low yet

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### Table 1. Raman score versus serum carotenoid and retinol concentrations, and fruit and vegetable intake score.

<table>
<thead>
<tr>
<th>Serum carotenoids</th>
<th>n</th>
<th>Mean (SD)</th>
<th>$\mu$g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sum of carotenoids</td>
<td>61</td>
<td>3.470 (1.824)</td>
<td></td>
</tr>
<tr>
<td>$\beta$-Carotene</td>
<td>61</td>
<td>1.224 (893)</td>
<td></td>
</tr>
<tr>
<td>Lycopene</td>
<td>61</td>
<td>80 (44)</td>
<td></td>
</tr>
<tr>
<td>Lutein/zeaxanthin</td>
<td>61</td>
<td>2.146 (1.224)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Retinol</th>
<th>61</th>
<th>504 (102)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fruit and vegetable intake score</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Season 1</td>
<td>38</td>
<td>6.7 (3.4)</td>
</tr>
<tr>
<td>Season 2</td>
<td>62</td>
<td>6.9 (4.3)</td>
</tr>
</tbody>
</table>

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### Table 2. Serum carotenoid concentrations versus fruit and vegetable intake scores ($n=61$).

<table>
<thead>
<tr>
<th>Serum carotenoids</th>
<th>r</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sum of carotenoids</td>
<td>0.24</td>
<td>0.059</td>
</tr>
<tr>
<td>$\beta$-Carotene</td>
<td>0.29</td>
<td>0.016</td>
</tr>
<tr>
<td>Lycopene</td>
<td>0.30</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lutein/zeaxanthin</td>
<td>0.14</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Retinol</td>
<td>0.12</td>
<td>0.355</td>
</tr>
</tbody>
</table>

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Six of the recruited subjects did not meet all the inclusion criteria, resulting in 81 remaining participants (male: $n=19$ [23%]; female: $n=62$ [77%]). Most participants classified themselves as Caucasian ($n=78$ [96%]), denoted as the fruit and vegetable intake score. The BMI was calculated as weight (kg) divided by height squared (m²). Pearson’s product-moment correlation was used for determining the relationship between the field RRS score and (1) the individual and summed serum carotenoid concentrations and (2) the fruit and vegetable intake score. Using a cut-off of 25,000 on the field RRS score and categorising serum $\beta$-carotene as low and high (lowest range for normality used by the laboratory, i.e. 644 $\mu$g/L and 483 $\mu$g/L for males and females respectively) diagnostic statistics (sensitivity, specificity, positive predictive value, negative predictive value) were calculated. Intra- and inter-device repeatability were determined using the intra-class correlations from a mixed model approach. Testing was done at the 0.05 level of significance. STATA Release 10 statistical software was used for the analysis.

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### RESULTS

The correlations between the RRS score and each of the carotenoids and the sum of the carotenoids was highly significant. Figure 1 and Table 1 illustrate this point. $\beta$-Carotene had the strongest correlation with the RRS score ($r=0.78$; $p<0.001$). When $\beta$-carotene was used as a reference standard, the RRS had a sensitivity of 87.5%, a specificity of 75.5%, a negative predictive value of 97.6% and a positive predictive value of 35.0%. The $R^2$ was 0.61, meaning that 61% of variation in $\beta$-carotene can be explained by variation in Raman scores.

The correlations between the RRS scores and the fruit and vegetable intake scores were statistically significant in both seasons.

As can be seen from Table 2 and Fig. 1 the correlation coefficients between serum carotenoid levels and the fruit and vegetable intake scores were relatively low yet
statistically significant for the individual carotenoids. Serum retinol was not significantly related to either the RRS score (r=0.16; p=0.226) or the fruit and vegetable intake score (Table 2).

The correlations between the individual items on the fruit and vegetable screener and both the RRS-score and carotenoids are given in Table 3. The items that emerged with statistically significant positive associations with the RRS score and at least one serum carotenoid were consumption of lettuce salad, fruit in the morning, vegetables at lunch and vegetables at supper (bold type in Table 3).

**Reliability**

The intra-device repeatability coefficient was 0.98 (p=0.004) and the inter-device repeatability was 0.97 (p=0.009).

**DISCUSSION**

The findings of this study show a strong and significant positive correlation between the field RRS score and serum carotenoids, in particular \( \beta \)-carotene (Fig. 1). These results are similar to those published by the manufacturer of the RRS (10, 12, 13). The high sensitivity indicates that over 87% of participants with low serum \( \beta \)-carotene levels were correctly identified by the field RRS. Equally, about 75% of those with adequate serum \( \beta \)-carotene levels were correctly identified as such by the field RRS. Dissimilarly, the low positive predictive value of 35% suggests that among those that were identified by the field RRS as being low (that is below 25,000 units) many were false positives. False negatives, were, however, highly unlikely. The lower correlations for lycopene and lutein/zeaxanthin can possibly be explained by the fact that the field RRS utilised in this study had 473 nm excitation, which focuses on the more abundant fractions of carotenoids like \( \alpha \)- and \( \beta \)-carotene and \( \beta \)-cryptoxanthin (absorption value of 488 nm) and not lycopene (absorption value of 514 nm). It thus produced correlation results similar to manufacturer trials for total carotenoids of \( r=0.78 \) (17). In two more recent studies this problem was overcome using a customised field RRS to measure total carotenoids at 488 nm with a blue argon laser and simultaneously scan in the 514 nm range with a green argon laser, in this way producing more accurate results for lycopene (18, 19). The absence of a significant correlation between RRS scores and serum retinol confirms that the field RRS should not be considered when serum retinol levels are of interest, as retinol detection occurs at a completely different wavelength, and lycopene, lutein and zeaxanthin do not have provitamin A activity (5).

The relationship between the field RRS and fruit and vegetable consumption in this study was in step with the work of Mayne and co-workers, who reported a correlation coefficient of 0.39 (p<0.001) for fruit and vegetable intake versus skin RRS measurement (18). In the comparison of the fruit and vegetable screener to the serum carotenoid concentrations, the correlation coefficients were highest for \( \beta \)-carotene and lycopene. The absorbance of \( \beta \)-carotene (absorption value of 486 nm) and \( \beta \)-cryptoxanthin (absorption value of 488 nm) and not lycopene (absorption value of 514 nm). It thus produced correlation results similar to manufacturer trials for total carotenoids of \( r=0.78 \) (17). In two more recent studies this problem was overcome using a customised field RRS to measure total carotenoids at 488 nm with a blue argon laser and simultaneously scan in the 514 nm range with a green argon laser, in this way producing more accurate results for lycopene (18, 19). The absence of a significant correlation between RRS scores and serum retinol confirms that the field RRS should not be considered when serum retinol levels are of interest, as retinol detection occurs at a completely different wavelength, and lycopene, lutein and zeaxanthin do not have provitamin A activity (5).

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tive strong correlation when compared to the primary reference, i.e. serum carotenoids, whereas the correlation with fruit and vegetable intake scores, our secondary reference, was weakly positive.

The field RRS demonstrated reliability as evidenced by high intra-class correlations for intra- and inter-device repeatability. The device is simple to operate and the reliability appears not to be negatively affected when it is operated by lay people. This study has certain limitations: First, the selection, size and composition of the sample limits the external validity. The exclusion criteria and the high reported intake of fruit and vegetables make the results at this stage applicable to health-conscious people. We accepted the conventional serum β-carotene cut-off values as “gold standard,” while realising that, overall, such reference intervals have been questioned in their ability to demonstrate excess or deficiency (20).

In a country where fruit and vegetable intake is known to be low (21), this study provides a basis for follow-up research, particularly among those at risk of low intakes. Where the aim is a tool that is primarily sensitive, the field RRS holds promise as an easy-to-use, rapid, non-invasive instrument for the nutrition care professional screening for poor carotenoid status and low fruit and vegetable consumption.

Acknowledgments

This study was funded in its entirety through a grant from the Medical Research Council of South Africa (Ref: AS 677).

REFERENCES