

Effect of Fruit and Vegetable Intake on Skin Carotenoid Detected by Non-Invasive Raman Spectroscopy

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Background: Epidemiologic studies found the inverse correlation between fruit and vegetable intake and the risk of cardiovascular disease, various cancers, insulin resistance, and other chronic conditions. Skin carotenoid levels are highly correlated with serum levels; however, the direct measurement of skin carotenoids is difficult to perform. Raman spectroscopy has been described as a highly sensitive, specific and accurate method of skin carotenoid detection.

Objective: The authors assessed the relation between fruit and vegetable intake and skin carotenoid levels measured by Raman spectroscopy.

Material and Method: Twenty-nine healthy volunteers were enrolled in the present study. Demographic data and fruit and vegetable intake were recorded. Skin carotenoid levels were measured by Raman spectroscopy and were reported as Skin Carotenoid Score (SCS). The data were compared and were reported as 3 groups based on the amounts of fruit and vegetable intake.

Results: There were no significant differences of age, body weight, height and body mass index among the groups. Mean skin carotenoid score of low fruit and vegetable intake ($25,733 \pm 2,956$) was significantly lower than SCS of moderate intake ($31,333 \pm 4,792$, $p = 0.03$) and high fruit and vegetable intake ($35,125 \pm 6,081$, $p < 0.01$). Mean SCS of underweight participants ($29,250 \pm 4,621$) was not significantly different from normal ($33,384 \pm 6,614$) and overweight participants ($27,575 \pm 3,811$), $p = 0.06$.

Conclusion: Using Raman spectroscopy, the authors found that skin carotenoid levels were directly correlated with the degree of fruit and vegetable intakes. We suggest that Raman spectroscopy should be possible to replace the invasive chemical technique for the dermatologic carotenoid measurement.

Keywords: Carotenoid, Fruit and vegetable, Raman spectroscopy, Skin

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Carotenoids, a prominent class of phytochemicals, are compounds with vitamin A-like structure that play important roles of powerful antioxidants in humans. In epidemiologic studies, they have showed the inverse correlation between dietary carotenoid intake or circulating concentrations and the risk of cardiovascular disease, various cancers, insulin resis-

tance, and other chronic conditions⁽¹⁻⁷⁾. In human skin, carotenoids play an important role in the skin's antioxidant defense mechanism by acting as scavengers for free radicals, singlet oxygen, and other harmful reactive oxygen molecules⁽⁸⁻¹⁰⁾. The direct detection of carotenoids in skin usually requires invasive procedures, such as biopsies from multiple sites and processing of relatively large tissue volumes⁽¹¹⁾. Recently, the Raman spectroscopy, a novel non-invasive technique for dermatologic carotenoids detection, has been described⁽¹¹⁻¹⁴⁾. This technique is precise, sensitive, accurate and reproducible and provides a direct correlation to carotenoid detection in human skin⁽¹¹⁻¹³⁾.

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As fruits and vegetables are the main sources of carotenoids⁽¹⁵⁾ the Raman spectroscopy is being used to determine the association between human dermatologic carotenoids and fruit and vegetable intake. While the correlation between fruit and vegetable intake and serum carotenoid levels has been described, the data from the living human tissue are not well established⁽¹⁶⁾.

In the present study, the authors report the usefulness of Raman spectroscopy for carotenoid and show the association between human dermatologic carotenoids and fruit and vegetable intake.

Material and Method

Twenty nine healthy volunteers, health professionals at HRH Maha Chakri Sirindhorn Medical Center, were enrolled in the present study. Participants who reported having had an illness in the past 2 weeks or smoked more than 3 cigarettes per day were excluded. Demographic data (included age, sex, weight, height and body mass index), and amount of fruit and vegetable intake were recorded. The questionnaires of fruit and vegetable intake including the example size of amounts of fruit and vegetable per 1 serving were employed. Body Mass Index (BMI) (in kg/m²), calculated from measured height and weight, was graded into 3 groups as follows: underweight (BMI < 18.5 kg/m²), normal (BMI = 18.5-22.9 kg/m²) and overweight (BMI ≥ 23.0 kg/m²)⁽¹⁷⁾. Fruit and vegetable intakes were grouped in to 3 categories by using food exchange lists⁽¹⁸⁾ as follows: low intake (< 2 servings per day), moderate intake (2 to 4 servings per day), and high intake (> 4 servings per day). Raman spectroscopy (Biophotopic scanner, Pharmanex, USA) were performed in all subjects at the palm of the right hand and reported as Skin Carotenoid Score (SCS) by

one of the researchers without knowing the individual fruit and vegetable intake. Informed consent was obtained, and the study was approved by the Ethics committee of the Faculty of Medicine, Srinakharinwirot University.

Statistical analysis

Age, height, weight, BMI and SCS were reported as the means and standard deviations separately for each group regarding to fruit and vegetable intake. Differences in skin carotenoid score among the groups of fruit and vegetable intakes and BMI were tested with analysis of variance. To examine the relations between skin carotenoid score and fruit/vegetable intakes and BMI, SCSs were logged before running the linear regression model due to the skewness of data. Analysis of variance and Turkey HSD analysis was employed for multiple comparisons to find the differences between means. Statistical analyses were performed using a SPSS 11.0 software package, and the differences were considered as significant if p value < 0.05.

Results

Among 29 participants, 27 of them (93.1%) were females and 28 of them (96.6%) were non-cigarette smokers. The means age, height, weight and BMI of the participants were 31.9 ± 8.3 years, 158.2 ± 7.0 cm, 53.4 ± 10.4 kg and 21.2 ± 3.2 kg/m², respectively. There were no statistical differences in age, height, weight and BMI among the different groups of fruit and vegetable intake. The data of skin carotenoid score of each individual has been shown (Fig. 1). SCS and log-transformed SCS had statistically significant differences among the groups of low, moderate and high fruit/vegetable intake, p < 0.01 (Table 1). Mean

Table 1. Demographic data and skin carotenoid score of participant (n = 29)

Characteristics	Fruit and vegetable intake			p value*
	Low n = 9	Moderate n = 12	High n = 8	
Age, yr (SD)	30.3 (7.7)	33.9 (10.9)	30.8 (2.8)	0.57
Weight, kg (SD)	59.9 (13.9)	51.7 (7.0)	48.6 (7.2)	0.06
Height, cm (SD)	160.4 (9.3)	156.9 (4.4)	157.6 (7.8)	0.53
BMI, kg/m ² (SD)	23.0 (3.7)	21.0 (3.0)	19.5 (1.6)	0.06
SCS (SD)	25,733 (2,956)	31,333 (4,792)	35,125 (6,081)	<0.01
Log SCS	4.408 (0.05)	4.492 (0.065)	4.540 (0.075)	<0.01

BMI: body mass index; SCS: skin carotenoid score; Log SCS: log-transformed skin carotenoid score

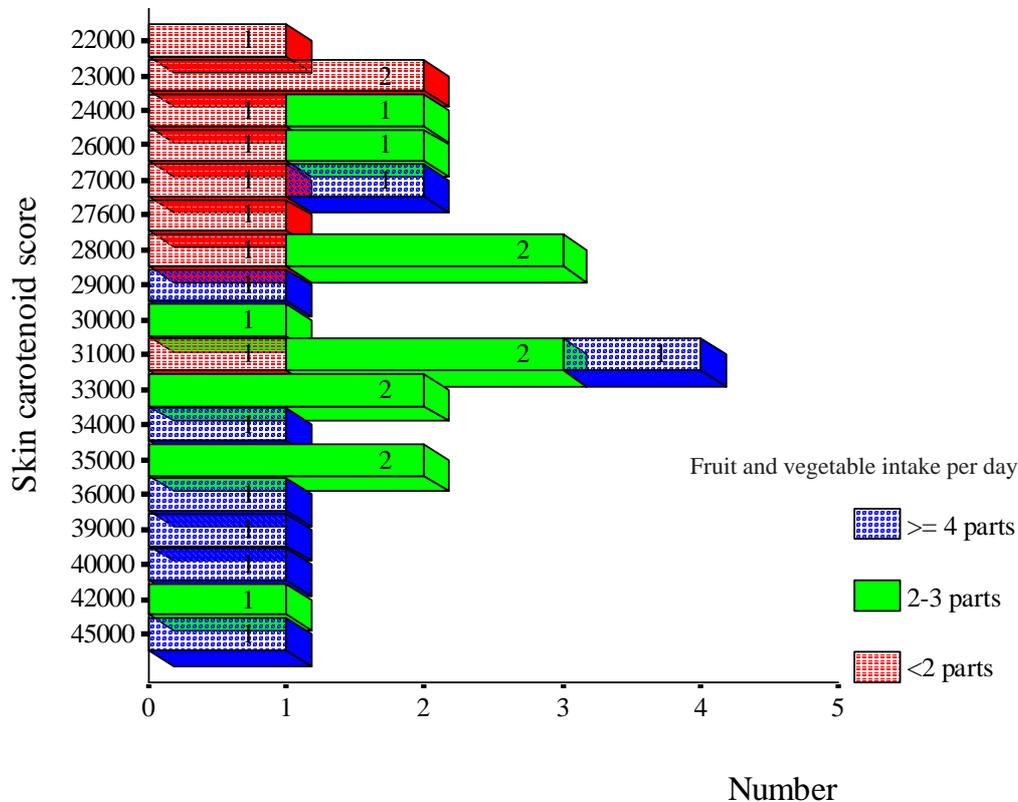


Fig. 1 Skin carotenoid score of each individual by amounts of fruit and vegetable intake per day (n = 29)

SCS of low fruit/vegetable intake ($25,733 \pm 2,956$) was significantly lower than SCS of moderate intake ($31,333 \pm 4,792$, $p = 0.03$) and high fruit/vegetable intake ($35,125 \pm 6,081$, $p < 0.01$). However, SCS of moderate intake did not reach statistically significant difference from high fruit/vegetable intake (Fig. 2). Mean SCS of underweight participants ($29,250 \pm 4,621$) was not significantly different from normal ($33,384 \pm 6,614$) and overweight participants ($27,575 \pm 3,811$), $p = 0.06$ (Fig. 3). Participants who consumed large amounts of fruits and vegetables tended to have lower BMI than those who consumed small amounts of fruits and vegetables, however, these findings did not reach statistical significance (Table 1). In univariate linear regression analyses of log-transformed SCS entered as a continuous variable, the authors found significant positive association between fruit and vegetable intake and SCS ($p = 0.01$) but not between BMI and SCS ($p = 0.60$).

Discussion

Antioxidant defense system in the human body is the network of intrinsic and extrinsic antioxidants

that work together to protect the body tissue from free radical, singlet oxygen and other harmful molecules. Intrinsic antioxidants such as L-glutathione, coenzyme Q10 and antioxidant enzymes are made in the body often from nutrients in food. Extrinsic antioxidants, such as vitamin E, vitamin C, alpha-lipoic acid, carotenoids and flavonoids, cannot be made by the human body and must be obtained from diet.

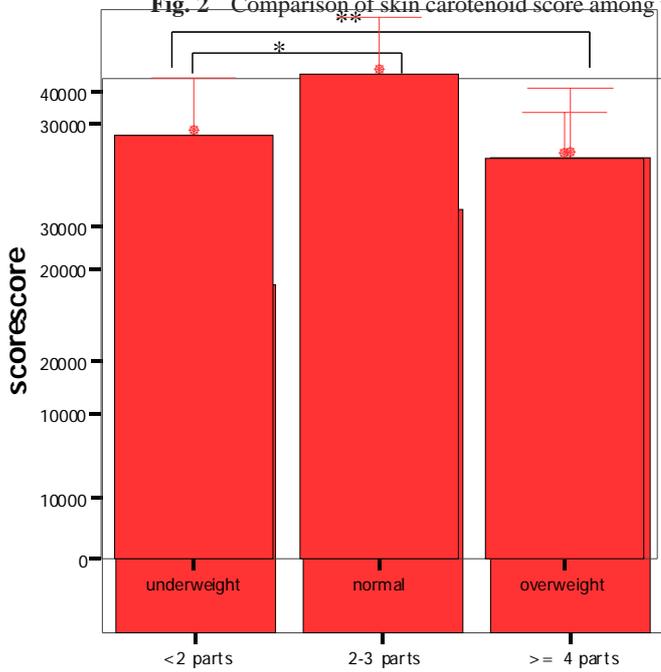
Carotenoids, the red, yellow and orange pigments distributed through nature particularly in fruits and vegetables, are an important part of antioxidant network of the human body. Many carotenoids have been described and the five most concentrated carotenoids found in humans skin are α -carotene, β -carotene, phytoene and phytofluene, lutein and lycopene⁽¹¹⁾. Carotenoids act sacrificially to protect other members of the antioxidant network from having to sustain free radical hits. A typical carotenoid molecule like lycopene or B carotene is able to sustain more than 20 free radical hits before it becomes completely destroyed^(19,20). In epidemiological studies, there were evidences of the role of dietary carotenoids in

T mean + SD

Fruit and vegetable intake per day

* p value = 0.03, ** p value < 0.01

Fig. 2 Comparison of skin carotenoid score among the groups of different fruit and vegetable intake



T mean + SD

Body mass index

p value = 0.06

Underweight (BMI < 18.5 kg/m²); Normal (BMI = 18.5-22.9 kg/m²); Overweight (BMI ≥ 23.0 kg/m²)

Fig. 3 Skin carotenoid score among the groups of different body mass index (n = 29)

preventing chronic diseases such as cancer, cardiovascular disease and age-related macular degeneration. In the skin, the unbalanced condition between carotenoids and reactive oxygen species can lead to premature skin aging, oxidative cell damage, and even the formation of skin cancer⁽¹¹⁾. In order to predict total antioxidant defense system in the body, Svilaas et al⁽²¹⁾ reported the reliability and accuracy of serum carotenoids to predict levels of other antioxidants. Skin carotenoid measured by High-Performance Liquid Chromatography (HPLC) has been shown to correlate significantly to serum carotenoid levels⁽²²⁾. However, the direct measurements of serum or skin carotenoids by HPLC are not feasible and inconvenient for routine screening in field studies.

Various spectroscopic methods have been applied to determine biologically relevant molecules in living organisms⁽²³⁾. With regards to the optical absorption properties of carotenoids, Raman spectroscopy, the novel technique, is employed to measure the tissue carotenoid levels. The optical concept is that each species of molecules can generate a different set of colors of light when stimulated with laser beam. Therefore, the color spectrum is a unique optical fingerprint of a particular molecule species. Raman spectroscopy uses a blue laser with wavelength of 473 nm. When this laser hits a carotenoid molecule, a unique spectrum with a prominent peak at 510 nm is generated by the carotenoid molecules. The green light is emitted out of the skin and is captured by a highly sensitive light detector. A computer analyzes the amount of green light and produces a numeric reading called the skin carotenoid score. Stahl et al⁽¹³⁾ reported that serum carotenoid levels were significantly correlated with skin carotenoid levels measured by spectroscopy, especially at the palm of the hand with the highest correlation coefficients at 0.94.

Skin at the inner palm is the most appropriate for spectroscopic measurement because of the convenience for accessibility and the highest carotenoid concentration compared with other regions⁽¹²⁾. Carotenoids are the lipophilic substances, thus, they are found predominately in the fatty areas of the body especially at palm skin that has a high lipid/protein ratio⁽¹¹⁾. Moreover, a minimal difference in skin pigmentation in this area and the prominent stratum corneum thickness, which helps to confine the laser in this layer, make the inner palm to be the most suitable site for examination⁽¹¹⁾.

In the present study, skin carotenoid levels directly correlated with the amounts of fruit and

vegetable intake. This finding provides further evidence in support of the hypothesis that fruit and vegetable intake is responsible for the skin carotenoid levels. This study is comparable with the study by Ford et al⁽¹⁶⁾ that reported the direct correlation between serum carotenoids, measured by HPLC, and fruit and vegetable intakes. The present results indicate the usefulness of Raman spectroscopy in evaluating the tissue carotenoid levels and may replace the invasive techniques as the standard screening method of dermatologic carotenoid detection. Compared to the existing study⁽¹⁶⁾, the present results did not show a significant association between BMI and carotenoids levels. This may be explained by the small sample size in this study, as there was a trend toward low carotenoid levels in participants who were overweight. Further studies with more participants are needed to determine this fact. Overweight participants had a trend toward consuming small amounts of fruits and vegetables compared with underweight and normal BMI participants that may cause lower carotenoid levels. Because most of the presented voluntary participants were females, the presented data needed to be cautiously interpreted within the limitation of the gender variation. The existing evidence from Europe⁽²⁴⁾ and US⁽²⁵⁾ showed that there were significant differences in serum carotenoid concentration between gender identities. These findings may be explained by the variation of food consumption among the sexes. Moreover, smoking status and alcohol intake also affected the carotenoid levels⁽²⁴⁾. Clearly, further studies are required to determine whether the actual impact of gender identity on skin carotenoid levels.

In conclusion, there is a significant association between skin carotenoid levels measured by Raman spectroscopy and the amount of fruit and vegetable intake, with the supported evidence of correlation between serum and skin carotenoid levels. The authors suggest that Raman spectroscopy could replace the invasive chemical technique for the screening method of carotenoid measurement.

As the benefits of carotenoid have been described, adequate amounts of fruit and vegetable intake, up to the recommended 5-9 servings per day⁽²⁶⁾, should be made.

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ผลของการรับประทานผักและผลไม้ต่อระดับแคโรทีนอยด์ที่ผิวหนังด้วยการตรวจโดยสเปกโตรสโกปี ชนิดรามัน

สงวนศักดิ์ ฤกษ์สุภผล, ลัคนา ฤกษ์สุภผล

ที่มา: การศึกษาทางระบาดวิทยา พบว่าการรับประทานผักและผลไม้จะมีความสัมพันธ์เชิงผกผันต่ออัตราเสี่ยงของโรคหัวใจและหลอดเลือด มะเร็ง เบาหวาน และโรคเรื้อรังบางชนิด การตรวจวัดระดับแคโรทีนอยด์ในกระแสโลหิตจะสัมพันธ์เป็นอย่างดีกับระดับแคโรทีนอยด์ที่ผิวหนัง อย่างไรก็ตามการตรวจวัดระดับแคโรทีนอยด์โดยตรงโดยเฉพาะอย่างยิ่งที่ผิวหนังทำได้ลำบากและซับซ้อน การตรวจวิธีการใหม่ที่ไม่ยุ่งยากและไม่เจ็บโดยสเปกโตรสโกปีชนิดรามัน พบว่ามีความไว ความจำเพาะ และความเที่ยงตรงสูงต่อการตรวจวัดระดับแคโรทีนอยด์ที่ผิวหนัง

วัตถุประสงค์: เพื่อที่จะประเมินความสัมพันธ์ของการรับประทานผักและผลไม้ต่อระดับแคโรทีนอยด์ที่ผิวหนังด้วยการตรวจโดยสเปกโตรสโกปีชนิดรามัน

วัสดุและวิธีการ: อาสาสมัครที่มีสุขภาพดีจำนวน 29 คนที่เข้าร่วมโครงการจะได้รับการสอบถามอายุ น้ำหนัก ส่วนสูง ค่ามวลดัชนีมวลกายและสอบถามปริมาณผักและผลไม้ที่รับประทานเฉลี่ยต่อวัน ผู้ร่วมโครงการจะได้รับการตรวจวัดระดับแคโรทีนอยด์ที่ผิวหนังด้วยการตรวจโดยสเปกโตรสโกปีชนิดรามันที่ฝ่ามือ ข้อมูลจะถูกนำมาประเมินโดยแบ่งกลุ่มตามปริมาณผักและผลไม้ที่รับประทาน

ผลการศึกษา: จากการศึกษาไม่พบความแตกต่างอย่างมีนัยสำคัญทางสถิติของอายุ น้ำหนัก ส่วนสูงและดัชนีมวลกายระหว่าง 3 กลุ่มที่รับประทานผักและผลไม้มาก ปานกลาง และน้อย ค่าเฉลี่ยของแคโรทีนอยด์ที่ผิวหนังของผู้ที่รับประทานผักน้อย ($25,733 \pm 2,956$) จะน้อยกว่าผู้ที่รับประทานผักปานกลาง ($31,333 \pm 4,792$, $p = 0.03$) และผู้ที่รับประทานผักมาก ($35,125 \pm 6,081$, $p < 0.01$) อย่างมีนัยสำคัญทางสถิติ ค่าเฉลี่ยของแคโรทีนอยด์ที่ผิวหนังของผู้ที่มีดัชนีมวลกายน้อย ($29,250 \pm 4,621$) ไม่แตกต่างอย่างมีนัยสำคัญทางสถิติจากผู้ที่มีดัชนีมวลกายปกติ ($33,384 \pm 6,614$) และผู้ที่มีดัชนีมวลกายมาก ($27,575 \pm 3,811$)

สรุป: จากการตรวจด้วยสเปกโตรสโกปีชนิดรามันพบว่าระดับแคโรทีนอยด์ที่ผิวหนังมีความสัมพันธ์เชิงตรงกับปริมาณผักและผลไม้ที่รับประทาน จากผลที่ได้มีข้อเสนอแนะว่า การตรวจแคโรทีนอยด์ที่ผิวหนังด้วยสเปกโตรสโกปีชนิดรามันอาจจะใช้แทนการตรวจทางชีวเคมีเดิมที่ยุ่งยากและทำให้ผู้ที่รับการตรวจต้องเจ็บปวด
