Comparison measurements between plasma carotenoids by mass spectrometry and palm carotenoids by skin carotenoid detector

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Abstract

Human body mainly acquires carotenoids, an antioxidant, from fruits and vegetables. The gold standard to assess human carotenoids is plasma or tissue carotenoid concentration measurement. Pharmanex Biophotonic Scanner S3 (S3 scanner) is a new innovation upgraded from Raman resonance spectroscopy to evaluate the levels of skin carotenoids. *Objective:* To study the reliability of skin carotenoid measurement by the skin carotenoid detector S3 scanner and its validity compared to plasma carotenoids by liquid chromatography-tandem mass spectrometry (LC-MS/MS). *Methods:* Subjects were allocated equally to three subgroups (low-, medium-, and high-level) depending on their mean skin carotenoid scores as screened by S3 scanner. Plasma carotenoid concentration was measured by LC-MS/MS within an hour after all three skin carotenoid assessments were done. *Results:* The study included 60 subjects (9 males and 51 females). Seven subjects of low-level subgroup had plasma carotenoid concentration below the measurable levels by LC-MS/MS method, therefore their data were not included in the correlation analysis between skin and plasma levels. Mean skin carotenoid scores were 25,385 ± 3,014.92 (n = 13), 39,083 ± 5,256.99 (n = 20), and 59,667 ± 5,949.10 (n = 20) for low-, medium-, and high-level subgroups. The mean plasma concentration (± SD) of each subgroup was 1.7108 ± 0.8181 ng/ml (n = 13), 2.5798 ± 1.4523 (n = 20), and 4.6987 ± 1.6326 ng/ml, respectively. Intraclass correlation coefficient among three skin carotenoid measurements were 0.9867. Estimate of the line of best fit from analysis of covariance, using 53 samples, yielded a Pearson correlation coefficient of 0.7471 ($P < 0.001$). *Conclusion:* Skin carotenoids measured by S3 scanner yielded a high reliability and a potential estimation of plasma carotenoid concentrations.

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Introduction

Carotenoids, compounds with vitamin A-like structures, are antioxidant phytonutrients existing in high concentrations in fruits and vegetables, and are usually taken up by human body through the diet.[1-3] Carotenoids are composed of a polyisoprenoid structure containing 40 carbon atoms and a system of conjugated double bonds.[4-6] Carotenoids have been suggested to play roles in the prevention of many cancers, heart disease, and age-related eye disease.[7,8] Moreover, studies showed that all-cause mortality was associated with their antioxidant property.[9,10]

The most widely used analytical method for assessing human carotenoids is high performance liquid chromatography (HPLC), which employs various absorbance detectors to measure plasma carotenoids.[11,12] Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is a developed technique that is more accurate and sensitive than classical HPLC to distinguish plasma carotenoids.[11] The LC-MS/MS was shown to have less overall analysis time and more efficiency for quantifying specific carotenoid substances.[11,13] These methods require expensive equipment, skilled chemists, and also necessitate the collection of biological samples. Consequently, accurate assessment of carotenoid status is not easily attained, particularly in large, population-based studies, and has therefore been limited to research and medical institutions.[4,5]

In humans, skin carotenoid levels have been shown to strongly and significantly correlated with plasma levels.[14,15] Direct measurement of skin carotenoids has been performed using HPLC; however, this involves extremely invasive skin biopsies.[16] Consequently, optical methods for assessing skin carotenoid levels could prospectively overcome these limitations.

Resonance Raman spectroscopy (RRS) is a highly noninvasive optical approach for quantitative measurement of carotenoids in human skin.[4,17] RRS employs a small probe with a laser at blue wavelength ($\lambda = 488$ nm) to measure total carotenoid concentration in the skin. The Raman scattered light produces a spectral fingerprint of the carotenoid molecules based

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on their unique molecular structure (alternating carbon double and single bonds) and vibrational energy levels.[1,6,18,19] Many studies have shown that RRS is a reliable, reproducible, and valid method for estimating skin carotenoid status, when compared with HPLC analyses of biopsied skin in healthy adults.[4,6,18,19] In addition, RRS skin carotenoid methodology had 0.9% less variance than plasma carotenoids assessed by the HPLC method ($P < 0.03$).[4]

Recently, an innovative portable skin carotenoid detector, Pharmanex Biophotonic Scanner S3 (S3 scanner), has been developed based on RRS technique, which could give results within 30 seconds and has a capability for iOS wireless data integration. Thus, this study was designed to investigate the correlation between plasma carotenoids measured by LC-MS/MS and skin carotenoids measured by the S3 scanner in healthy male and female Thai volunteers.

Materials and Methods

The study protocol and related materials were approved by Siriraj Institutional Review Board (IRB) of the Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand (approval number SI136/2016), in accordance with the current revision of the Declaration of Helsinki.

Volunteers

Inclusion criteria were Thai healthy male or female, age 18-55 years, who had a body mass index (BMI) between 18.0-30.0 kg/m^2. This study excluded pregnant women (positive pregnancy test at screening) or women in breastfeeding period. All participants were voluntary and gave written informed consent. Enrolled subjects were required only one visit at the Siriraj Clinical Research Center, for screening, three skin carotenoid measurements, and a blood sample collection. Subject screening procedures included demographic data, physical examination, body measurements (height and weight), vital signs, and skin carotenoid measurement. After the screening, each eligible subject was assigned the study number according to the sequence of arrival. Subjects then consecutively received three skin carotenoid measurements and a blood sample collection, respectively.

Skin carotenoid determination

Three measurements were performed over 15 minutes on the same palm of each subject, using the Pharmanex Biophotonic Scanner S3 (Pharmanex Electronic-Optical Technology, Shanghai, China; serial number CS75339). Carotenoid levels were determined by calculating the average height of the peak Raman absorbance signal obtained and quantified from excitation of skin carotenoids using a low-intensity blue ($\lambda = 473$ nm) LED light with green light (510 nm) detection. Skin carotenoids were reported as Raman intensity counts. The higher the count, the higher the concentration of carotenoid molecules detected at the site of measurement.

Eligible participants were divided into three subgroups (20 subjects per subgroup) according to the average score of three skin carotenoid measurements: low-level ($\leq$ 29,999), medium-level (30,000-49,999) and high-level ($\geq$ 50,000).

Liquid chromatography with tandem mass spectrometry

Liquid chromatography with tandem mass spectrometry (LC-MS/MS) was developed and validated for the determination of carotenoids in human plasma by Siriraj Bioequivalence Center, Department of Pharmacology, Faculty of Medicine Siriraj Hospital, as described in Method Validation Report. The lower limit of quantification (LLOQ) of the LC-MS/MS method in this study was 1.0000 ng/ml therefore the plasma carotenoid concentration less than 1.0000 ng/ml, called below limit of quantification (BLQ), could not be measured. One blood sample (6 ml) from a volunteer who was already allocated to a subgroup was collected within 1 hour after three skin carotenoid measurements. The blood sample was obtained from a forearm vein (or other appropriate area) via direct venepuncture and then processed for determining plasma carotenoid concentration using LC-MS/MS. Each volunteer provided matched blood sample and three skin determinations.

Data analysis

Demographics were characterized using descriptive statistics, *t*- and *F*-tests for differences in mean across subgroups and associations between categorical variables. Regression models were used to describe the association between demographics and levels of carotenoids. Intraclass correlation coefficients (ICCs) were used to assess the consistency of three skin carotenoid scores. Pearson correlation coefficients (r) were generated to verify the relationship between plasma carotenoids measured by LC-MS/MS and the mean skin carotenoids. Statistical analysis for each parameter was performed at the significant level of 0.05, using STATA12.1 (StataCorp, Texas, USA). Results are shown as mean ± SD.

Results

A total of 60 subjects, 9 males and 51 females, were included in this study. Subjects aged between 20 and 55 years, with a mean of 33.9 years, and had a mean BMI of 22.0 kg/m^2. The demographics of all subjects and each subgroup are summarized in Table 1. There were 7 males and 13 females in low-level subgroup. While there were only females in high-level subgroup, the medium-level subgroup had 2 males and 18 females. There was no statistically significant difference in BMI and weight among subgroups. The average age of low-level subgroup is significantly younger compared to medium-level and high-level subgroups.

Table 1 Baseline characteristics of the study population for each subgroup according to the skin carotenoid detector S3 scanner measurement. Data are mean (SD).

	Low [n = 20]	Medium [n = 20]	High [n = 20]	Overall [n = 60]
Female (%)	65%	90%	100%	85%
Age (year)	29.7	35.6	36.5	33.9 (7.9)
Weight (kg)	59.5	55.4	53	55.9 (9.6)
Height (cm)	162.8	158.5	156.5	159.3 (7.7)
BMI (kg/m^2)	22.5	21.9	21.6	22 (3.2)

Table 2 Mean scores (± SD) of three skin carotenoids measurements by the S3 scanner and plasma carotenoids measured by the LC-MS/MS.

	Low [n = 20]	Medium [n = 20]	High [n = 20]	Overall [n = 60]
Skin carotenoids	24100 (±4346)	39083 (±5257)	59667 (±5949)	40950 (±15574)
Plasma carotenoids	1.1120 (±1.0600)	2.5798 (±1.4523)	4.6987 (±1.6326)	2.7968 (±1.3816)

In this study, females had higher mean skin carotenoid score than males (43,614.38 *vs* 25851.85). For the mean plasma levels, females had 3.09 ng/ml compared to males 1.14 ng/ml. In low-level subgroup, linear regression analysis showed that sex did not significantly relate to both skin and plasma carotenoids (P = 0.463 and P = 0.707, respectively). There was a significant association between age and skin carotenoid scores at r = 0.5301 from linear regression (P = 0.037). BMI significantly related to plasma carotenoid levels at r = -0.185 (P = 0.025) but did not significantly relate to scores from the skin carotenoid detector (r = -0.775, P = 0.231).

The average skin carotenoid scores of three measurements using the skin carotenoid detector over 15 minutes among subgroups are presented in Table 2. ICC between individual measurements made on the same palm of each subject is 0.9867 (95% confidence interval, 0.9797-0.9916).

The average plasma carotenoid concentrations (± SD) was 1.1120 ± 1.0600 ng/ml in the low-level subgroup (n = 20). For the medium-level and high-level subgroups, mean plasma carotenoid concentrations were 2.5798 ± 1.4523 and 4.6987 ± 1.6326 ng/ml, respectively (n = 20 for each subgroup). In the low-level subgroup, seven subjects (no. 15, 31, 35, 53, 54, 55, and 57) who had BLQ data were not included in the analysis. Subsequently, the plasma carotenoid concentration in low-level subgroup was 1.7108 ± 0.8181 ng/ml (n = 13). After data of these seven subjects were excluded, the mean score of low-level subgroup was 25,385 ± 3,014.92 (n = 13).

Pearson correlation between skin carotenoids obtained via S3 scanner and plasma carotenoids measured by LC-MS/MS among 53 subjects yielded a coefficient r = 0.7471 (P < 0.001). Overall estimate of the line of best fit from analysis of covariance was shown in Figure 1.

Discussion

Carotenoid levels was recommended as a good marker for antioxidative protection from the damage caused by free radicals in human body. Unlike the invasiveness and complexity of plasma or tissue carotenoid assessment, the skin carotenoid measurement offers a more convenient and economical method. This study examined the reliability of skin carotenoid measurement using S3 scanner and the correlation between skin carotenoid scores from S3 scanner and plasma carotenoid levels determined by LC-MS/MS.

Our healthy volunteers were 85% female and 20 to 55 years old as shown in Table 1. The female predominance in higher-level subgroup was similar to other studies, suggesting that females might have higher intake or more absorption of carotenoids (retinol and beta-carotene) than males.[20,21] Linear regression analysis showed that age was associated with carotenoid levels at coefficient r = 0.5301 (P = 0.037) and 0.0520 (P = 0.121) for skin score and plasma concentration, respectively. The positive correlation between age and carotenoid levels is comparable to other findings among healthy volunteers.[20,22] The BMI inversely related to skin carotenoid score at a coefficient of -0.775 (P = 0.231) and plasma carotenoid concentration at -0.185 (P = 0.025), similar to those observed in Japanese population.[23,24] There are many other factors associated with carotenoid concentration, including lifestyle, illness, smoking, alcohol, and blood lipids;[25] however, different locations have specific skin physiologic properties (e.g. lipid composition). There are relative decreases in error on superficial part of skin as a result of less scattering and lower background noise.[26] Previous studies demonstrated that palm has the highest carotenoid concentration and highest ICCs. Therefore the S3 scanner was designed to measure the skin carotenoid concentration via either side of palm.[27,28]

This study allowed less than 15-minute intervals among three measurements because we tried to avoid

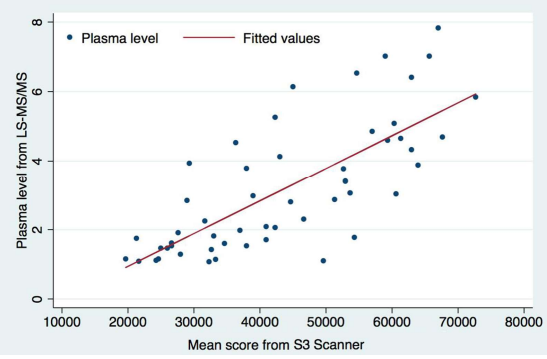

Figure 1 Mean skin carotenoids as measured by the S3 scanner compared with plasma carotenoids as measured by LC-MS/MS (validity study; n = 53). Pearson's correlation coefficient r = 0.7471 (P < 0.001).

the changes of sweat and sebaceous glands. These glands were expected to transport carotenoids on the skin surface.[27] Our results present a very strong consistency among three carotenoid measurements made on the same subject with ICC = 0.9867, similar to a previous study of RRS with ICC = 0.97.[28] Thus, using S3 scanner is a highly reliable technique for quantitatively assessing skin carotenoid levels.

Among 60 enrolled participants, seven subjects had plasma carotenoid concentration less than 1.00 ng/ml (BLQ), which could not be detected by LC-MS/MS in this study. Their mean skin carotenoid scores were categorized in low-level subgroup and ranged between 12,000 and 27,000. Some subjects with BLQ data had skin carotenoid scores more than those whose plasma carotenoid concentration was higher than 1.00 ng/ml, owing to the possibility that plasma carotenoid concentrations can be fluctuated by variability in daily carotenoid intake, with relatively short half-life (< 12 days) compared to skin.[29,30]

The mean plasma carotenoid concentrations of low-, medium-, and high-level subgroups, according to skin carotenoid scores by S3 scanner, are 1.7108 ± 0.8181 (n = 13), 2.5798 ± 1.4523, and 4.6987 ± 1.6326 ng/ml, respectively.

Mean skin carotenoids assessed by S3 scanner was significantly correlated with plasma carotenoid concentrations assessed by LC-MS/MS ($r = 0.7471$, $P < 0.001$) indicating that participants who had high skin scores were similarly high in plasma carotenoid concentration. Our results suggest that S3 scanner determination of skin carotenoids are valid and can effectively represent the plasma carotenoid concentration.

There was some limitation in subjects whose plasma carotenoid levels, as measured by LC-MS/MS, were less than 1.00 ng/ml (BLQ data). Further study should be designed to determine plasma carotenoid levels below 1.00 ng/ml. In addition, our study population was predominantly female, especially in the high-level subgroup, probably due to limited screening period; consequently, it may less represent the general population. Future studies should ensure the balance between males and females as well as expand over a greater age range.

Conclusion

Skin carotenoid scores measured by S3 scanner present a reproducible and highly correlated estimation of plasma carotenoid concentrations. S3 scanner is a valid technology to measure human carotenoid levels with convenience, non-invasiveness, and cost-effectiveness. It would be an appreciable device for monitoring carotenoid levels of large groups of people and leading to raise awareness of fruit and vegetable intake status.

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Conflict of Interest

The present project was funded by Nu Skin Enterprises, Inc., Thailand.

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