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Validating the Effects of Multivitamins on Nutrient Status Using Non-Invasive Spectroscopy

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Abstract

Background: The main objective of this randomized, double-blind, placebo-controlled study was to measure the impact of a multivitamin supplement on nutrient status and quality of life (QOL) and compare two non-invasive methods of detecting skin carotenoids. **Methods:** Subjects (46 healthy adults) were given either a supplement containing minerals, vitamins, carotenoids, and phytonutrients (n = 29) (SUP) or a placebo (n = 17) (PL) daily for 12 weeks. Skin carotenoid levels were measured at baseline, 1, 4, 8, and 12 weeks by Resonance Raman Spectroscopy (RRS) using a BioPhotonic Scanner S3 and Hyperspectral Absorption (HA) device. Subjects had blood drawn to assess safety and nutrient status for vitamin C and selenium at baseline and week 12. Subjects also filled out questionnaires related to QOL and skin attributes at baseline and week 12. **Results:** At week 12, the SUP group had a 44% increase in vitamin C levels ($p < 0.01$ vs baseline and PL) and a 26% increase in selenium levels ($p < 0.01$ vs baseline and PL) with no significant change in the PL group. RRS and HA both showed similar patterns in tracking changes in skin carotenoid levels over time. The SUP group reported an increase in skin carotenoid status as indicated by a 42% increase via RRS and 41% increase via HA at week 12 ($p < 0.05$ vs baseline and PL). At week 12, a high percentage of subjects in the SUP group reported QOL improvements in energy level, overall health, a stronger immune system, and quicker recovery after getting sick. Subjects receiving SUP compared to PL also reported more positive responses for skin attributes at the end of the study. **Conclusion:** Supplementation with a multivitamin significantly increased serum vitamin C, serum selenium, and skin carotenoid levels while also improving QOL and skin health attributes. These findings support the use of SUP to improve nutrient status and use of non-invasive methods to measure.

Keywords

Multivitamin, Carotenoids, Hyperspectral Absorption, Raman Spectroscopy

1. Introduction

Adequate nutrition is an important factor in staying healthy throughout one's lifespan. Healthspan is the years lived in good health, and unfortunately, the healthspan-lifespan gap has widened over the past two decades. The increase in the healthspan-lifespan gap implies that there is an increased burden of chronic disease at the end of one's lifespan [1].

Nutritional health is important for optimal health and wellness throughout one's entire life. Unfortunately, inadequate micronutrient intakes and related deficiencies are prevalent globally. On the basis of estimates of nutrient intake from food, not including fortification and supplementation, a high percentage of the global population does not consume enough vitamin E, calcium, iron, riboflavin, folate, and vitamin C [2]. Nutrient deficiencies contribute to a large burden of morbidity and mortality and impact aging processes. Healthy dietary patterns have been identified to promote healthy aging. Specifically, dietary patterns rich in plant-based foods, with moderate amounts of healthy animal-based foods, are associated with overall healthy aging [3]. Unfortunately, adhering to a healthy dietary pattern to obtain the needed nutrients is challenging, which is why supplementation can be useful.

A healthy, balanced diet paired with supplementation can fill nutrient gaps to ensure recommended daily intake levels of essential micronutrients are reached [4]. Additionally, nutrients delivering antioxidant benefits have been shown to have many mechanisms of activity in the body, including redox balance, support of immune cells, and mitochondrial function, and offer protection from many health conditions. Reactive oxygen species (ROS) are generated *in vivo*, and ROS are counterbalanced to prevent damage by an antioxidant defense network. Antioxidants can be synthesized *in vivo* or derived from the human diet [5].

One specific class of antioxidants is carotenoids. There are more than 1100 carotenoids found in nature, but only a few play a role in the human diet. The major carotenoids that are most abundant in the diet are α-carotene, β-carotene, β-cryptoxanthin, lycopene, lutein, and zeaxanthin [6]. It has been suggested that dietary carotenoids can even improve a healthy lifespan. A cross-sectional study in 27,338 adults from NHANES 1999-2018 found that increased dietary intakes of carotenoids was associated with lower biological aging indications [7]. Carotenoids are dietary pigments found in fruits and vegetables. However, only about 1 in 10 adults meet recommended intakes of fruits and vegetables [8]. Because fruits and vegetables, along with carotenoids, are known for numerous health benefits [9], measurement of these compounds is important to assess biomarkers of health and well-

ness.

Previously, human carotenoid levels have been assessed in serum and tissue using high-performance liquid chromatography (HPLC), considered the gold standard method of measurement. However, measuring carotenoids in the blood is invasive, inconvenient, time-intensive, and expensive. Carotenoids accumulate in the skin, and there are alternative technologies available that allow the levels of skin carotenoids to be detected non-invasively. Resonance Raman Spectroscopy is a method used to measure the concentration of carotenoids in the stratum corneum of the skin. A laser with a wavelength of 488 nm is radiated on the skin, and the spectrum of reflected light contains a carotenoid-related peak on a fluorescence background. The skin carotenoid levels measured by the Resonance Raman Spectroscopy device are highly correlated to blood carotenoid levels (R = 0.81) [10]. Unfortunately, Resonance Raman Spectroscopy devices are complicated, large, expensive, and require internal calibration. Therefore, a simpler alternative technology, Reflection Spectroscopy, is also being explored. Research is needed to compare devices measuring carotenoids using Resonance Raman Spectroscopy to Reflection Spectroscopy.

The main objective of this randomized, double-blind, placebo-controlled study was to measure the impact of a multivitamin supplement on nutrient status and quality of life (QOL) and compare two non-invasive methods of detecting skin carotenoids.

2. Materials and Methods

2.1. Study Design

A 12-week, parallel-group, two-arm, single-center, randomized, double-blind, placebo-controlled trial was completed. The trial protocol was approved by the Allendale Institutional Review Board, and this study conformed to the standards set by the latest revision of the Declaration of Helsinki. This study was retrospectively registered at clinicaltrials.gov (ClinicalTrials.gov trial registration NCT06749808) in December 2024. The study was conducted between July and October 2024.

Forty-eight healthy males and females between the ages of 20 - 65, BMI ≥ 18.5 and ≤29.9 kg/m^2, with a score of ≤30,000 Raman intensity counts (RIUs) using Raman spectroscopy (BioPhotonic Scanner S3, Pharmanex) were recruited for this study. Subjects were randomly assigned to either the supplement group (SUP) (n = 29) or the placebo group (PL) (n = 17). Unequal group allocation was planned a priori. For the study endpoints, the precision of the treatment effect estimate depended more heavily on the size of the active group than the placebo group. The small loss of power was adjusted by increasing the total sample size. Additionally, as biomarker analysis was a key part of the study objectives, a larger active group was planned to increase the informative value of these analyses. The placebo served as a comparator, not the main object of the study. Each group was instructed to take

the supplement or placebo twice daily (morning and evening with meals) for 12 weeks. They were also instructed not to change their eating or exercise habits for the duration of the study.

Subjects completed in-person visits to the research facility at baseline and weeks 1, 2, 4, 8, and 12. At each time point, subjects were given questionnaires on quality of life (QOL), skin attribute assessment, and completed non-invasive carotenoid skin measurements. Resonance Raman Spectroscopy (RRS) using a BioPhotonic Scanner S3 (Pharmanex, UT) was compared to a custom-built hyperspectral absorption device (HA) being developed (Rhyz Analytics, UT) that uses Reflection Spectroscopy. Hyperspectral absorption spectroscopy was utilized in the custom-built device. The device sequentially illuminated the fingertip with four discrete color bands: cyan, red, yellow, and green. A total of 798,720 optical measurements were acquired during each 15-second measurement. Incident and diffusely reflected light were recorded for each illumination cycle using a dual-photodiode configuration. One photodiode continuously monitors LED radiant output for real-time source normalization, while a second photodiode measures reflected light intensity following photon-tissue interaction and re-entry into the device. Wavelength-dependent absorption was quantified as a decrease in reflected intensity relative to the monitored incident illumination. Subjects also filled out a diet and lifestyle questionnaire at baseline and week 12. Additionally, at baseline and week 12, all subjects had blood draws completed to analyze for Vitamin C and Selenium levels. All subjects also had blood draws at baseline and week 12 for a comprehensive metabolic panel.

2.2. Recruitment and Randomization

Study volunteers were recruited through email databases in April 2024. Eligible participants were randomly assigned to one of two groups (SUP or PL). A participant identification number was allocated to each participant based on the order of enrollment. Identification of test articles was stored by the study sponsor and revealed after all data were analyzed. All test articles were packaged in identical packaging. Study investigators were blind to the treatment assignment until all study outcomes were collected.

2.3. Participants

Forty-eight (48) subjects were enrolled in the study. Forty-six (46) subjects completed all aspects of the study. Subjects satisfied the inclusion and exclusion criteria, accepted the prohibition and restrictions, and gave informed consent. The suitability of each subject to participate was confirmed prior to their acceptance into the study by completion and review of a pre-study questionnaire to determine eligibility. The inclusion criteria comprised healthy males and females between ages 20 - 65 years, BMI $\geq$ 18.5 and $\leq$29.9 kg/m^2, with a score of $\leq$30,000 Raman intensity counts (RIUs) using Raman spectroscopy (BioPhotonic Scanner, Pharmanex), no plans to start new treatments during the study, able to understand the

study procedures, and willing and able to adhere to all study procedures. The exclusion criteria comprised pregnant women, women who were nursing, or women planning to become pregnant; subjects who were currently taking vitamin C, selenium and carotenoid-containing supplements; known allergies to ingredients in the study dietary supplement; subjects with history of any gastrointestinal surgery and/or illness which in the opinion of the investigator would affect the digestive and absorption process of the body; use an indoor tanning booth; cigarette smokers, alcohol or narcotic addicts; concurrent therapy with any medication that might interfere with the study.

2.4. Intervention

The supplement was provided as four capsules in a sachet taken twice daily (8 capsules daily) containing 3 mg boron, 255 mg calcium, 100 mcg chromium, 0.9 mg copper, 150 mcg iodine, 180 mg magnesium, 75 mcg molybdenum, 2.3 mg manganese, 140 mcg selenium, 20 mcg vanadium, 15 mg zinc, 55 mg choline, 7.5 mg beta-carotene, 150 mcg biotin, 30 mg pantothenic acid, 122 mcg methyltetrahydrofolic acid, 118 mcg folic acid, 32 mg niacinamide, 10 mg pyridoxine, 8.5 mg riboflavin, 7.5 mg thiamine, 375 mcg vitamin A, 16 mcg methylcobalamin, 14 mcg vitamin cyanocobalamin, 1000 IU vitamin D3, 30 mg vitamin E, 40 mcg vitamin K2, 400 mg vitamin C, 150 mg grape seed extract, 40 mg olive leaf extract, 100 mg alpha lipoic acid, 2 mg lutein, 5 mg lycopene, 30 mg quercetin, and 5 mg resveratrol.

The placebo was provided as four capsules in a sachet taken twice daily (8 capsules daily) containing maltodextrin.

Each group was instructed to take the supplement or placebo twice daily with a meal (morning and evening) for the 12-week trial period. Subjects were also instructed not to change their eating or exercise habits for the duration of the study. Daily diaries were administered to assess product compliance.

2.5. Outcome Measures

In-person assessments were conducted at Weeks 0 (baseline), 1, 4, 8, and 12. Self-report diaries were also completed daily during the 12-week study duration. Primary outcome measures included at baseline, weeks 1, 4, 8, and 12 were skin carotenoid concentration measured on the finger via Hyperspectral Absorption (HA) with a custom device being developed, and on the palm using Resonance Raman Spectroscopy (RRS) using the BioPhotonic Scanner S3. Secondary outcome measures included at baseline and end of study (week 12) were changes in feelings of wellness as measured by the Quality of Life (QOL) questionnaire, skin appearance attribute changes via self-assessment, serum vitamin C levels, and serum selenium levels. Secondary outcome measures for safety included a comprehensive metabolic panel via blood draw at baseline and week 12, and self-reported adverse events (AEs) were assessed at each study visit to examine the safety and tolerability of the test articles.

2.6. Statistical Analysis

Descriptive statistics (mean and standard deviation) and change from baseline (T-Test, percent of subjects improving) were conducted for all study outcome evaluations. A Welch's T-Test was used, assuming unequal variances, and to account for differences in sample size for each group. For the Self-Perception questionnaires, frequency of responses was summarized for each question. Within-treatment analyses were done using a Binomial test with a null hypothesis of a 50/50 split of top box/not top box responses. A Mann-Whitney U Test was conducted for the categorical data responses. All statistical tests of hypotheses employed a level of significance of 0.05.

3. Results

3.1. Study Population

Forty-eight (48) subjects were enrolled in the study. Twenty-nine (29) subjects enrolled in the dietary supplement group completed the study, while seventeen (17) subjects enrolled in the placebo group completed the study. Two subjects enrolled in the dietary supplement group were lost due to scheduling conflicts. Baseline demographic characteristics are detailed in Table 1.

Table 1. Subject demographic characteristics.

SUP Group		
	Count	29
Age (Years)	Mean ± SD	37.8 ± 11.2
Sex	Female	18
	Male	11
Ethnicity Percentage (%)	Caucasian	71.4
	Asian	3.6
	Hispanic	21.4
	Other	3.6
PL Group		
	Count	17
Age (Years)	Mean ± SD	43.7 ± 9.0
Sex	Female	12
	Male	5
Ethnicity Percentage (%)	Caucasian	55
	Asian	15
	Hispanic	15
	Other	15

3.2. Outcome Measures

Skin Carotenoid Concentration: As detailed in Table 2, subjects in the SUP group had statistically significant improvement in BioPhotonic Scanner (RRS) scores over baseline and compared to placebo at weeks 4, 8, and 12. The PL group did not have a significant change from baseline throughout the study. By week 12, the SUP group had a 42% increase from baseline. As detailed in Table 3, subjects in the SUP group had statistically significant improvement in hyperspectral absorption device (HA) scores at weeks 8 and 12. The PL group did not have a significant change from baseline throughout the study. By week 12, the SUP group had a 41% increase from baseline score.

Table 2. BioPhotonic S3 scanner results (RRS).

Timepoint	BioPhotonic S3 Scanner Results			
	PL (RIUs) Mean ± SD	PL % Change from Baseline	SUP (RIUs) Mean ± SD	SUP % Change from Baseline
0	24,111 ± 5674	_	23,149 ± 5766	_
1 week	25,000 ± 5914	1	23,914 ± 6366	3
4 weeks	24,882 ± 6986	0	27,287 ± 8051[a]	18
8 weeks	24,463 ± 6481	1	31,011 ± 9740[a]	34
12 weeks	25,778 ± 5650	7	32,977 ± 9286[a]	42

a. $p < 0.05$.

Table 3. Hyperspectral absorption device results (HA).

Timepoint	Hyperspectral Absorption Device Results			
	PL (RIUs) Mean ± SD	PL % Change from Baseline	SUP (RIUs) Mean ± SD	SUP % Change from Baseline
0	30,073 ± 7977	_	27,722 ± 7555	_
1 week	30,881 ± 11,519	3	27,514 ± 9613	0
4 weeks	29,565 ± 9146	−2	29,720 ± 9835	7
8 weeks	30,273 ± 9500	0	35,859 ± 11,363[a]	29
12 weeks	32,330 ± 8673	8	39,045 ± 11,165[a]	41

a. $p < 0.05$.

Nutrient Status: Serum vitamin C levels significantly increased from baseline to the end of the study at 12 weeks in the SUP group compared to the placebo ($p = 0.0004$) (Table 4). The SUP group had a 44% increase in vitamin C levels from baseline. Serum selenium levels also significantly increased from baseline to the end of the study at 12 weeks in the SUP group compared to placebo ($p = 0.000001$) (Table 5). The SUP group had a 26% increase in selenium levels from baseline. No changes were seen for vitamin C and selenium levels in the PL group.

Table 4. Serum vitamin C levels.

Timepoint	Vitamin C Levels (mg/dL)			
	PL Mean ± SD	PL % Change from Baseline	SUP Mean ± SD	SUP % Change from Baseline
0	0.631 ± 0.326	_	0.663 ± 0.318	_
1 week	0.606 ± 0.293	3	0.956 ± 0.395[a]	44

a. $p < 0.05$.

Table 5. Serum selenium levels.

Timepoint	Selenium Levels (mcg/L)			
	PL Mean ± SD	PL % Change from Baseline	SUP Mean ± SD	SUP % Change from Baseline
0	191.31 ± 23.40	_	200.63 ± 31.22	_
1 week	190.85 ± 21.10	0	252.25 ± 45.97[a]	26

a. $p < 0.05$.

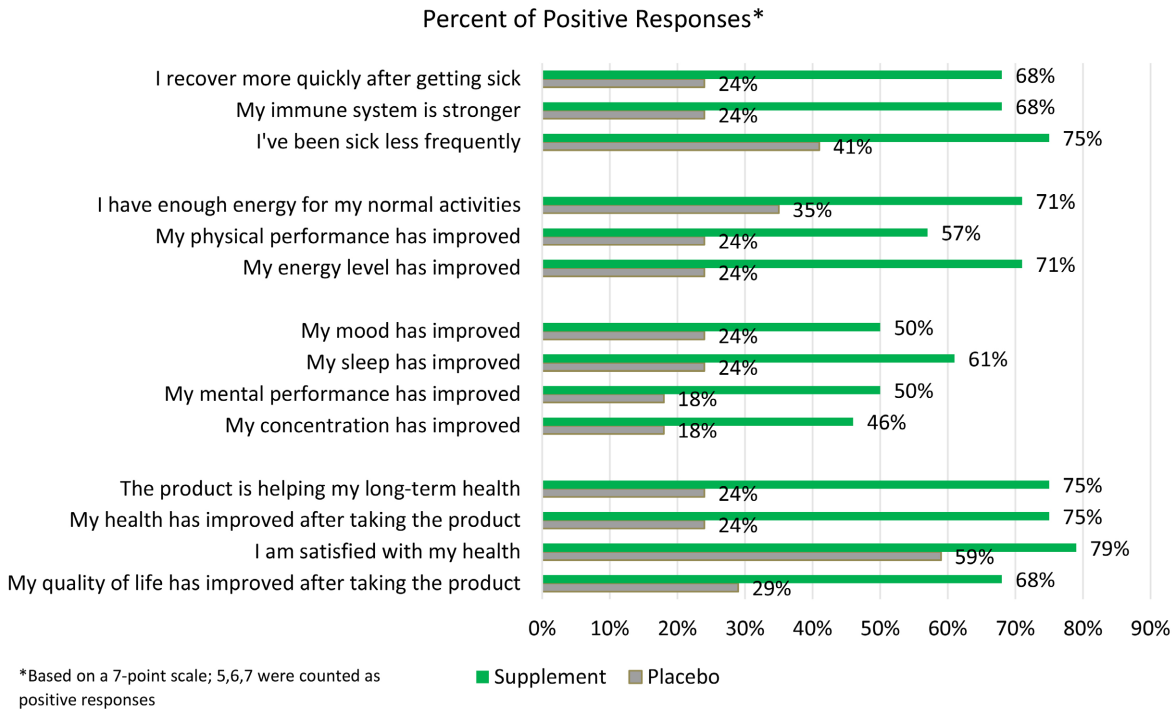

Figure 1. Quality of life results.

Quality of Life (QOL): At Week 12, participants were given a questionnaire that asked questions about quality-of-life parameters. The participants were asked to rate their experience on a 7-point scale, where 1 is strongly disagree, and 7 is strongly agree. A positive response was defined as any level of agreement to the question (including ratings of 5, 6, or 7). Results from the QOL questionnaire in-

dicate subjects in the SUP group perceived significantly greater benefit across all QOL attributes assessed, when compared to the PL group (Figure 1).

Skin Appearance Attributes: The subjects were asked to assess questions about skin attributes using the same 7-point scale. Results indicate subjects in the SUP group perceived significantly greater benefit across all skin attributes assessed, when compared to PL. Summary of results presented in Figure 2.

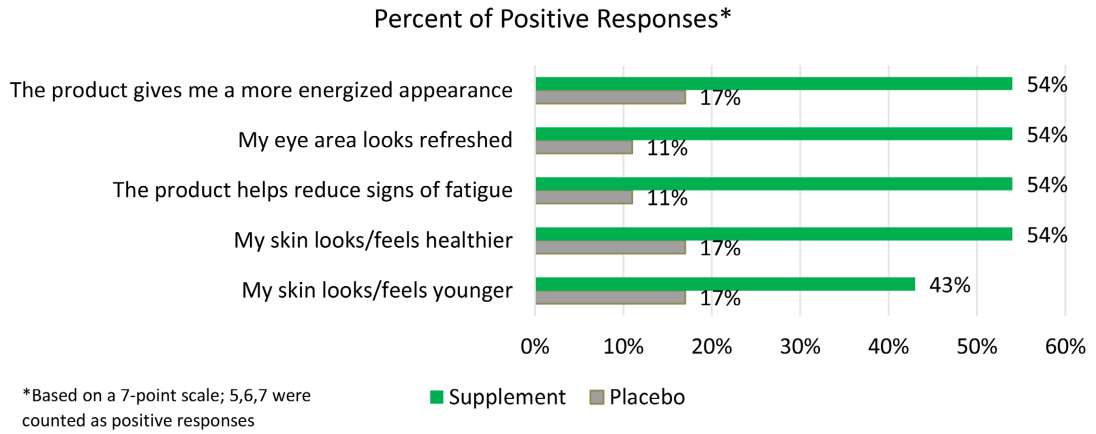

Figure 2. Skin appearance attributes.

Safety and Tolerability: No adverse events were reported during the study, indicating the dietary supplement was well tolerated. Additionally, no meaningful changes were seen with SUP compared to PL or baseline in the comprehensive metabolic panel (CMP) used for safety.

4. Discussion

Adequate nutrition is an important factor in staying healthy throughout one's lifespan and optimizing healthspan. Being able to support nutritional health and measure nutritional health non-invasively is essential. A healthy, balanced diet is important in receiving adequate vitamins, minerals, and phytonutrients. A healthy diet, along with supplementation, can help fill nutrient gaps to ensure recommended daily intake levels of essential micronutrients are reached [4]. This current study demonstrated that taking a supplement delivering vitamins, minerals, carotenoids, and phytonutrients resulted in an improvement in nutrient status, as shown by increased serum levels of vitamin C and selenium and skin carotenoid levels.

Nutrients delivering antioxidant benefits have been shown to have many mechanisms of activity in the body, including redox balance, support of immune cells, and mitochondrial function, and they offer protection from many health conditions. Many different nutrients can be considered antioxidants or promote antioxidant capabilities *in vivo* [5]. Vitamin C and selenium levels were measured in this study, in addition to skin carotenoids. Vitamin C is an important antioxidant, and selenium is an important cofactor for antioxidant enzymes. Significant in-

creases in vitamin C and selenium were seen after 12 weeks of SUP. Together, these results, along with an increase in skin carotenoids, show that overall antioxidant status was improved with SUP treatment.

Previously, human carotenoid levels have been assessed in serum and tissue using high-performance liquid chromatography (HPLC). However, technologies were used in the current study that allowed the levels of skin carotenoids to be detected non-invasively. Raman spectroscopy was the method used in the RRS device, and reflection spectroscopy (hyperspectral absorption) was used in the HA device. Both RRS and HA scanners showed similar patterns in tracking changes in skin carotenoid levels over time. The SUP group reported a statistically significant improvement in skin carotenoid status as indicated by a 42% increase via RRS and 41% increase on the HA device at 12 weeks ($p < 0.05$ vs Baseline and PL). At week 4, the RRS device was able to detect a significant increase from BL and PL with SUP, while the HA device could not detect a significant increase from BL and PL with SUP until week 8. The results from this study are similar to what has been published previously on comparing Raman spectroscopy to reflection spectroscopy for measuring carotenoid levels. Hwang *et al.* compared a Raman spectroscopy device to a reflection spectroscopy device to look at skin carotenoid levels. The study found that the rate at which skin carotenoid measurements increased using a Raman spectroscopy device was similar to that of a reflection spectroscopy device when subjects were consuming a high carotenoid diet [11]. In this study, different skin sites were used to measure skin carotenoids. For the RRS device, the palm skin was used, and for the HA device, the finger skin was used. Each of these scanners was designed to use these specific skin sites, but study results are showing there is a strong correlation between different skin sites on the same scanner and between scanners. A direct correlation analysis of the RRS and HA data collected in this study helps to better support the validation of the new device.

Quality of life measurements were shown to be improved in the current study. Other nutritional studies have also demonstrated improvements in QOL measurements. A supplement was given to healthy young adults that delivered vitamin C daily for 4 weeks. Compared to placebo, the vitamin C supplementation significantly increased attention and trended towards improving fatigue and work engagement [12]. Another study looking at the effects of a multivitamin mineral supplement in elderly adults found that it had a positive effect on improving psychological perspectives of quality of life [13]. This aligns with increased positive responses in subjects stating they have improved concentration, mental performance, and energy level in the current study. One limitation of the current study was that subjects only rated QOL outcomes; the study did not include quantitative measurements of cognitive ability.

The current study showed that subjects receiving SUP reported an improvement in skin appearance. Skin attributes were measured in the study because of the potential for certain nutrients in SUP to impact skin health and beauty. Other studies have demonstrated that improved attractiveness is linked to increased skin

yellowness, which suggests a role for higher skin carotenoid levels [14] [15]. The current study showed that the SUP group had improved skin carotenoid levels along with better-reported skin appearance. There are many mechanisms by which carotenoids specifically can impact skin health in addition to impacting skin color [16]. Additionally, having adequate nutrition and antioxidant protection in the skin is important to protect against oxidative stressors. Nutrients included in the SUP that may impact skin are not limited to carotenoids, and nutrients such as vitamin C, vitamin E, and vitamin D may also impact skin health. There may be a synergistic effect of the ingredients delivered in SUP. A synergy is supported by other studies that have found that carotenoid supplementation (25 mg total carotenoids) plus vitamin E (335 mg RRR-α-tocopherol) was more effective than the same level of carotenoids alone in reducing skin erythema following 8 weeks of supplementation [17]. One limitation of the current study was that it only measured skin attributes via subject self-perception and did not include dermatologist grading.

5. Conclusion

The randomized, double-blind, placebo-controlled study demonstrates that the supplement was well-absorbed and had a meaningful impact on both nutrient status (carotenoids, vitamin C, and selenium), QOL, and skin attributes. Both Raman Spectroscopy (RRS) and Hyperspectral Absorption (HA) devices were able to detect a significant change in skin carotenoid level with SUP compared to PL. Results from this study indicate that the HA device shows promise as a more accessible alternative to the RRS device. No adverse events related to the SUP were reported in this study, and there were no significant differences in safety parameters assessed, indicating good safety and tolerability.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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