Spectroscopic biofeedback on cutaneous carotenoids as part of a prevention program could be effective to raise health awareness in adolescents

Ruo-Xi Yu*,1, Wolfgang Köcher2, Maxim E. Darvin1, Monika Büttner3, Sora Jung1, Bich Na Lee1, Christoph Klotter4, Klaus Hurrelmann5, Martina C. Meinke1, and Jürgen Lademann1

1 Charité – Universitätsmedizin Berlin, Department of Dermatology, Venerology and Allergology, Center of Experimental and Cutaneous Physiology (CCP), Charitéplatz 1, 10117 Berlin, Germany
2 Opsolution Spectroscopic Systems GmbH, Goethestr. 25–27, 34119 Kassel, Germany
3 Elisabeth-Knipping-Schule, Mombachstraße 14, 34127 Kassel, Germany
4 Fulda University of Applied Science, Fulda, Postfach 22 54, 36012 Fulda, Germany
5 Hertie School of Governance, Friedrichstraße 180, 10117 Berlin, Germany

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1. Introduction

Reactive oxygen species (ROS) and other free radicals are well known to be a general metabolic byproduct in all aerobic organisms. In human beings, they are mainly produced in the mitochondrial respiratory chain [1]. At low concentrations they play an important role in physiological signaling pathways [2] and disinfection [3]. Various internal and external stress factors, e.g., inflammation [4], UV-irradiation [5, 6], extensive exercise [7–9] or alcohol consumption [10] can result in an excessive production of high amounts of free radicals and consequently cause oxidative stress which impairs the antioxidant defense system and damages all cell components including lipids, proteins and DNA [11–13].

Antioxidants are enzymes and non-enzymatic substances including several vitamins, carotenoids and flavonoids [14–16]. They form a defense system against the development of oxidative stress by neutralizing free radicals before those can damage cell structures [11, 17–19]. In the human skin, carotenoids are the biggest group of antioxidant substances [20]. Lycopene and beta-carotene represent the highest percentage of cutaneous carotenoids and it has been shown that lycopene has the highest antioxidant activity among all carotenoids [21].

It has been demonstrated that carotenoids can serve as marker substances for the general antioxidant status of human skin [22]. The various antioxidant substances form protection chains in the tissue, i.e., they are protecting each other against the destructive action of free radicals [23, 24]. The cutaneous carotenoid concentration also correlates with its concentration in the serum [25] and therefore can be seen as a biomarker for the general antioxidant capacity of the organism [22, 26]. It may be considered as a looking glass for the health-related lifestyle and nutrition of a subject [27].

Since humans cannot produce carotenoids and other important antioxidants themselves, an antioxidant-rich and balanced nutrition plays an important role in their supply [28]. Positive effects on health and beauty have been shown for various foodstuffs rich in antioxidants [29, 30].

At the same time, stress increases the ROS generation and, subsequently, reduces the carotenoid concentration in the skin. Thus, a person’s cutaneous carotenoid concentration can be seen as marker for his nutritional habits.

Recent studies suggest that a high concentration of cutaneous carotenoids correlates with better skin appearance [31–33] and that decreased carotenoid concentration may predispose to the development of skin cancer [34, 35]. One study investigating volunteers between 40 and 50 years found out that those subjects whose skin ageing values were highest exhibited low carotenoid concentrations [31]. If subjects at the age of 50 decide to improve their lifestyle, this will influence their appearance over the next decade, but even the healthiest diet will not rejuvenate their skin. Therefore, adolescents, who have the potential to influence their future health by today’s behavior, are to be convinced by biofeedback measurements to improve their nutritional behavior and reduce their stressors.

Teenagers show a weaker orientation to the future than adults and are more focused on instant rewards [36]. They often show a high-risk behavior towards risk factors for well-known diseases affecting their future health and economic welfare [37, 38].

Biofeedback in many forms has been approved in studies and also in clinical routine as a useful instrument to bring invisible physiological parameters to view and, consequently, influence a subject’s behavior [39]. In a recent study on the antioxidant status of adults, a non-invasive biofeedback on cutaneous carotenoid concentrations led to an enhancement of the volunteers’ health-related lifestyle and nutrition resulting in higher cutaneous carotenoid concentrations in many of them [40]. These measurements became only possible by the use of non-invasive spectroscopic methods like resonance Raman spectroscopy (RRS) and reflectance spectroscopy.

The question therefore arises, whether this biofeedback effect on cutaneous carotenoids can be found in adolescents and whether the instant feedback on their antioxidant status can serve as a means for behavioral health prevention in this age group.

2. Material and methods

2.1 Volunteers

The study was performed on 50 subjects (24 males and 26 females) aged between 17 and 20 years. They were all students of the 11th and 12th grade of the Elisabeth-Knipping School, a vocational grammar school in Kassel, Germany. They were students from five different classes, of which four had dietetics and one bioengineering as a special field. All volunteers had a healthy skin type II according to Fitzpatrick classification [41].

2.2 Study design

Prior to the measurements, the subjects were informed about the aims and procedure of the study and they were asked to complete questionnaires about their lifestyle and nutritional habits. Prior to
the second phase, specific information about the importance of antioxidants and the influence of diet and stressful lifestyle factors on their level was given to the students in the form of a lecture by Prof. Lademann (Dept. of Dermatology) and Prof. Klotter (Dept. of Nutritional Science).

The actual study took place in three phases. In a first phase from February 14th to March 18th, 2011, the current state on the antioxidant status and the students’ lifestyle was ascertained. Prior to the second phase from March 21st to April 15th, 2011, the students were informed once more about the influence of protective and stress factors on health in general and especially on the antioxidant status. The third phase was performed in September 2011, where the students were instructed to work on a healthy lifestyle and live on healthy food during the intervention phase. Moreover, they were asked to abstain from smoking, alcohol consumption and to avoid stress as far as possible. In addition, the students received a free lunch meal containing antioxidant-rich fruit and vegetables on every school day during the intervention phase.

The measurements were carried out during the students’ lunch breaks twice a week. Before each measurement, the volunteers filled in a questionnaire about their diets, lifestyle habits and possible stress factors such as smoking, sunbathing, illness or psychological stress over the last two days. After washing their hands, the volunteers measured the carotenoid concentration with the biozoom/C190 spectroscopic device [42] on the thenar eminence of both hands. The palm was chosen as measuring location because of its accessibility, the high concentration of carotenoid antioxidants, the absence of strong differences in the skin color of the ethnic groups [34, 43, 44] and the pre-existence of reference concentrations based on former measurements on the palm both by our group [42, 45] and by other groups using the RRS [27]. The measuring area was restricted to the thenar eminence in order to improve the reproducibility of the measured results. Measurements were carried out on both hands in order to avoid a bias caused by concentration differences between left and right, which had occurred in earlier measurements. The measured values of both hands were arithmetically averaged, rounded in accordance with standard commercial practice and then displayed in a scale from 1 to 12.

Ideally, there were eight measurements per person and phase. In 10 subjects, an additional ninth measurement took place at the end of phase 2 (Day 2.Z), because their first measurements of the second phase accidently started even before the first lunch meal was offered. Unexpected for the students, there was a third measurement phase, the follow-up, in September 2011 in order to review the sustainability of the effects achieved in phase 2. Measurements had been carried out by the students themselves as the device was easy to handle.

The subjects were informed about their own results not before the beginning of the intervention phase in order to prevent a premature biofeedback-effect. In phases 2 and 3, the volunteers were immediately informed about their values after each measurement.

Written consent was obtained from the students and their parents prior to the study. All procedures were in accordance with the Helsinki Declaration of 1975 as revised in 2008. A detailed description of the study progress can be seen in Figure 1.

### 2.3 Measuring method

Major problems with previous measuring methods for the determination of the antioxidant concentration in humans are the invasiveness of these methods, e.g., the high performance liquid chromatography for which blood or skin samples need to be taken [46, 47], the immobility of spectroscopic de-
sives like the RRS and the low sensitivity of other measurement methods [48]. Moreover, the classical analytical methods are cost-intensive and time-consuming. For a study under in vivo conditions in a school environment with adolescents, a portable, non-invasive and fast device for determining the carotenoid concentration in human skin was needed.

In the present study, we used the newly developed biozoom® spectroscopic system (Opsolution GmbH, Kassel, Germany) [42]. The working principle of the biozoom® device is based on reflectance spectroscopy using a light emitting diode (LED) as a source of irradiation. The LED emits a blue light spectrum ranging between 440 and 490 nm, which corresponds to the absorption maximum of carotenoids. The value of the small dip in the diffusely reflected spectrum measured in the range between 458 and 472 nm is associated with the concentration of carotenoids in the skin.

The measured values were presented in an ordinal scale ranging from 1 to 12, with 12 corresponding to 1.2 nmol g⁻¹ skin, which is the highest concentration measured with the RRS device, which had been developed by the Center of Experimental & Applied Cutaneous Physiology of the Charité – Universitätsmedizin Berlin, Germany for the quantitative determination of the carotenoid concentration in human skin [49]. With that system, more than 10,000 volunteers and patients, respectively, had been investigated over a period of ten years. The biozoom® system was calibrated using the RRS, and the comparison of the results measured with the biozoom® device and the RRS on the same volunteers showed a good linear correlation (Pearson’s $R = 0.88$) in earlier measurements as previously described in detail by our group [42].

The measurement with the prototype used in the study takes about 1.5 minutes for both hands. The final product is planned to require only a few seconds. In order to display the measured result, an additional output device with Bluetooth® technology (smartphone, tablet or PC) is necessary. In the present study, a laptop with a prototype output program was used. The final retail price of the measuring device is not yet decided, but it is designed to be affordable for private consumers. For schools, the developer plans special rates or sponsorships. In Figure 2, the use of the measuring system is demonstrated.

### 2.4 Statistical analysis

IBM SPSS® Statistics 19.0.0 (SPSS Inc., Chicago, IL) and Microsoft Excel 2008 for Macintosh were used for statistical analysis and generation of diagrams. The Mann-Whitney test was used for statistical analysis of independent samples and the Wilcoxon test for paired samples. For correlation analysis, Pearson’s $R$ and $R$-squared were calculated. A score ranging from 0 to 16 was conceived in order to evaluate the influence of multiple lifestyle habits such as nutritional or smoking habits and illness on the measured values. Higher scores signify better lifestyle habits and health (Table 1).

<table>
<thead>
<tr>
<th>Score:</th>
<th>0</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breakfast before school²</td>
<td>≤1 2–3</td>
<td>&gt;4</td>
<td></td>
</tr>
<tr>
<td>Cooking at home²</td>
<td>≤1 2–4</td>
<td>&gt;5</td>
<td></td>
</tr>
<tr>
<td>Fruit consumption³</td>
<td>≤1 2–4</td>
<td>&gt;5</td>
<td></td>
</tr>
<tr>
<td>Vegetables consumption³</td>
<td>≤1 2–4</td>
<td>&gt;5</td>
<td></td>
</tr>
<tr>
<td>Soft drink consumption³</td>
<td>≥5 2–4</td>
<td>≤1</td>
<td></td>
</tr>
<tr>
<td>Sweets consumption³</td>
<td>≥5 2–4</td>
<td>≤1</td>
<td></td>
</tr>
<tr>
<td>Smoking habits</td>
<td>daily occasional never</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days of illness⁴</td>
<td>&gt;50% 15–50% &lt;15%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹ Calculated through answers given to questions on lifestyle and nutritional habits. The score ranges from 0 to 16.
² In days per week. Students were asked how many days per week they eat breakfast before school or cook at home.
³ In portions per day. Students were asked how many regular portions of different foods they consume on a normal day.
⁴ The percentage of attended measuring days on which the volunteers have indicated illness on the measuring day or up to 3 days before.
3 Results

3.1 Compliance and attendance

In general, the volunteers were highly motivated and all of the participating students took part on all measurements when they were at school. If students were absent from school on a measuring day due to illness or other reasons, values were not ascertained for that day. In phase 1, an average attendance of 88% was reached. Due to illness of many students, attendance was lower in the second half of phase 2 and phase 3. The questionnaires accompanying the measurements were mostly duly completed. Questions about alcohol consumption, however, were often not answered completely.

In the follow up five months after the end of the intervention, two students had already left school and did not take part in these measurements.

During the first phase, the students were instructed to continue with their regular lifestyle habits. However, 14 of them reported that they lived healthier than normal during phase 1, as the study setting had raised their health consciousness. 36 of the volunteers claimed to have actively changed their lifestyle habits in phase 2 beyond the lunch meal at school. All volunteers took part at the school lunch offered in phase 2, nine of them daily, the rest missed it at least once.

3.2 Measured results in the steady phase

In the first phase, the median carotenoid concentration of each subject ranged between 3 and 9. The average of the median values of the volunteers was $5.73 \pm 1.37$, which is significantly higher than the average value of $5.36 \pm 1.81$ determined by Meinke et al. with the RRS in 151 healthy volunteers aged between 18 and 75 years in Berlin, Germany [45].

The measured values were steady over the whole phase and for every measuring day the median value of all subjects together was 6. There were no significant differences based on age or gender, nor between students with dietetics and those with bioengineering as special field.

23 of the volunteers smoked at least occasionally during phase 1, ten of them on a daily basis. 35 of them drank alcohol on one or more days during phase 1. The distribution of the measured values is shown in Figure 3a, b.

3.3 Influencing factors on the carotenoid concentration

The measured carotenoid concentrations correlated with the volunteers’ lifestyles. Several well-known health-related lifestyle factors were assessed through questionnaires; and it became apparent that high carotenoid concentrations are associated with better nutrition, non-smoking and good health. However, the individual influence factors on their own, e.g., the incidence of illness (Pearson’s $R = -0.178$; $p = 0.23$) or consumption of fruits (Pearson’s $R = 0.273$; $p = 0.06$) do not correlate significantly with the measured values, even though a clear tendency was found.

However, we assumed that different factors have a synergetic effect on the antioxidant status as they have on health in general and therefore created a score that takes multiple influencing factors into ac-
count (Table 1). The median of the measured values correlates significantly with this health-related lifestyle score (Pearson’s $R = 0.303; P = 0.03$; a.u.: arbitrary units).

Figure 4 Correlation of the health-related lifestyle score with the measured values in phase 1. The score takes into account smoking, nutritional habits and the incidence of illness. Pearson’s $R = 0.303; P = 0.03$; a.u.: arbitrary units.

* This subject claimed that she already improved her nutrition and lifestyle habits during the first phase of the study as she ate less meat than usual and took food supplements in the period of the first two measurements. These changes were not taken into account in the health-related lifestyle score since the score is calculated on the basis of the information given before the measurements took place.

Moreover, an association of high carotenoid concentration and good psychosocial status, which was ascertained through questionnaires, could be found. Students with high carotenoid concentrations showed higher satisfaction and better performance at school, claiming more often that they had enough time for themselves and to spend with hobbies and friends. The subjective view on the current overall quality of life correlates significantly with the measured antioxidant concentration. On a scale from 1 to 10, 1 being the worst imaginable and 10 the best imaginable life, subjects indicated where they see themselves at the moment (Figure 5). A higher score is significantly associated with a higher carotenoid concentration (Pearson’s $R = 0.328; P = 0.02$; a.u.: arbitrary units).

There was no correlation between BMI and measured concentrations. According to findings of Meinke et al. [45], a BMI of over 30 is correlated with a significantly lower carotenoid concentration. However, only one subject in the study at hand had a BMI of over 30.

3.3 Measured results in the intervention phase

In the second phase, seven subjects had a lower median measured value than in phase 1 and twelve subjects kept their median value. 30 volunteers increased their median value in phase 2, eight of them by two or more points. The average of the median values in phase 2 was $6.45 \pm 1.12$ thus exceeding the median measured in phase 1 by 0.71. The measured values increased during the second phase (days 2.1–2.Z in Figure 8 below). The average of the median values for the second half of phase 2, i.e. day 2.5 to 2.Z amounts to $6.67 \pm 1.03$, which is even 0.94 points higher than in phase 1 (Days 1.1–1.8). The development of the measured values of all subjects together throughout phases 1 and 2 can be
seen in Figure 6. The approximately bell-shaped distribution of measured values in phases 1 and 2 is shown in Figures 3a, b.

In general, subjects with a low baseline in phase 1 achieved the most significant increase in their values in phase 2 (Figure 7). However, the ranking in relation to the median value was not significantly changed. The extent to which the median measured concentration changed was not dependent on age or gender.

3.4 Changes in behavior and circumstances in the intervention phase

Apart from higher carotenoid concentrations, there are more changes in the second phase towards the baseline. More than half of the subjects reported in free text questionnaires that they had felt healthier in the second phase. One female volunteer reported that with the healthier lifestyle in phase 2, her skin appearance improved.

According to their answers in the questionnaires, 36 of the volunteers tried actively to enhance their lifestyle in the intervention phase beyond the free lunch meal at school. In general, the subjects consumed more fruit and vegetables in the second phase compared to the first.

5 of the 23 smokers from phase 1 did not smoke in phase 2, which means that 22% of the smokers from phase 1 quit smoking at least for the period of the intervention phase. Also the number of cigarettes smoked was lower in phase 2. Prior to the intervention, 10 of the students smoked on a daily basis. Two of them reduced their cigarette consumption radically in phase 2, their median measured values increased by 1.5 and 2 points, respectively, which is significantly higher than the average increase of 0.71. The volunteers also reduced their alcohol consumption. 27 subjects drank alcohol on one or more days in phase 2 compared to 35 subjects in phase 1, also the amount of alcohol was lower in phase 2.

In phase 2, the volunteers wrote more school exams. 24 subjects stated that they had a significantly higher level of stress due to exam preparations in phase 2. On average, the subjects wrote two exams per week in the first and five exams per week in the second phase. Another uncontrollable change in phase 2 was the higher incidence of illness in the students. Teachers reported that many subjects suffered from a flu-like infection in phase 2.
3.5 Follow-up

In a third measuring phase five months after the intervention, the sustainability of the achieved effects on the carotenoid concentration and the lifestyle enhancements was reviewed. 47 volunteers took part in the two-week follow-up measurements; two had already dropped out of school.

Only 13 students smoked during the follow-up, which is not only fewer than at the beginning of the study but also than in the intervention phase. One former smoker and one non-smoker had dropped out of school. Significant changes in the occurrence of illness and diet were not observed in comparison to the second phase.

A consolidation of the measured values can be found. The average of the median of all subjects participating in phase 3 is higher by 0.42 ± 1.10 points than in phase 2 (p = 0.002). Excluding the two dropped out subjects, it is higher by 0.40 points. Compared to phase 1, it is 1.14 points higher. The change of the measured values from phase 2 to phase 3 is not as significant as from the first to the second phase (change in phase 2: +0.71 points, p < 0.001).

9 in 47 students kept the median measured value from phase 2; another 9 of them had a lower and 29 a higher median value than in the intervention phase. Compared to baseline, 5 of the 47 remaining subjects had lower levels, 7 maintained their previous value, and 35 subjects increased their value, 16 of them by two or more points.

3.6 Individual changes in the volunteers

While in the previous paragraphs the results of the groups were explained, the individual kinetics of the carotenoid concentrations in selected volunteers are discussed in this paragraph.

Some volunteers experienced significant lifestyle changes or very unusual situations in the course of the study. The question therefore arises whether significant changes can also be seen in the course of their carotenoid concentration. In this section, those volunteers are looked at more closely.

Subject A is the subject with the lowest measured values in both phases (Figure 8). She does not smoke, rarely drinks alcohol and usually eats a lot of fruit and vegetables. In the first phase, where her values varied greatly, she was sick on five of eight measurement days. Also her fruit and vegetable consumption was very irregular in phase 1, she ate more of both in phase 2. She had a lot of private stress in both phases and said that especially the days around measuring day 2.4 involved “a lot of stress, anxiety and worry” due to conflicts with friends and family.

Subject B had a relatively low level in phase 1 and experienced the highest increase of all the subjects in the course of the second phase (Figure 8). She ate more fruit, vegetables, drank more fruit juices, ate fewer sweets and took food supplements on three days in phase 2. In the second phase, she experienced a little more stress at school due to exam but fewer conflicts at home, which had been very burdening in phase 1. Overall, she was in a better mood in phase 2 and experienced it as less stressful than phase 1.

Figure 8 Measured carotenoid concentration over the course of phases 1 and 2. Volunteer A: Phase 1 median: 3; phase 2 median: 4. Volunteer B: Phase 1 median: 4.5; phase 2 median: 6. Volunteer C: Phase 1 median: 9; phase 2 median: 9. *Volunteer C stated that she ate less fruits and vegetables than normally around these days.
Subject C had the highest values in both phase 1 and phase 2 (Figure 8). Especially in phase 1, she ate significantly more vegetables and fruit than the average subject, did not smoke or drink in both phases. She never experienced any private stress in both phases and describes herself as a contented person. In both phases, she was preoccupied with exam preparations for three days, which is less than most of the subjects. The volunteer claimed that she already had changed her diet in the first phase in so far as she ate less meat than usual and took food supplements in the period of the first two measurements. Her consumption of fruit and vegetables decreased in phase 2 but was still above average. Especially on the days prior to measuring day 2.3 in phase 2, when the measured value was lower than on the other measuring days, she ate little fruit and vegetables.

After the second phase, the students were asked to write down their thoughts on the study anonymously. The following quotes are taken from those protocols:

“You are really surprised when you see: ‘Oh God, my value is now at two or three!’ But then you have the motivation to eat better from now and thereby achieve a better value.”

“Especially, I have reduced stress, now I start earlier to study when we are going to write an exam and try to be more relaxed in general.”

“By the better diet I got a better skin complexion.”

“It’s not something, where you need years to see wrinkles or diseases. You have immediate results, the better physical condition is also there immediately.”

“I started eating fruits and vegetables every day and tried to reduced fat in my diet.”

“We have developed a real ambition to improve our [carotenoid] values.”

4. Discussion

The pilot study at issue was carried out in order to investigate the influence of lifestyle habits on carotenoid concentrations in adolescents. In addition it explored to what extent biofeedback measurements influence those lifestyle habits in terms of healthy nutrition and stress reduction in adolescents.

It provides the findings that carotenoid concentrations in this cohort are variable but in average stable over the period of four weeks and higher than in the average adult population.

The results also indicate that lifestyle habits and stressful events are reflected in cutaneous carotenoid concentrations. Most of the results on factors influencing the cutaneous carotenoid concentration are consistent with former findings [40, 45], in particular that higher carotenoid concentrations can be found in non-smokers and subjects with healthy nutrition and little stress. Contrary to Meinke et al. [45], no differences between male and female subjects were found. A possible explanation is that adolescents of both genders have more similar lifestyle habits than adults since they are co-educated and their parents and school mainly determine their diet. Moreover, in this age group, the distribution of smokers is more balanced between the sexes whereas there are significantly more male than female smokers within older adults.

A possible explanation for the high baseline concentrations in our volunteers might be that the subjects were within a relatively homogeneous group of young and in general healthy students with a comparatively high level of education. Moreover, the volunteers were students from a school, where nutritional science is one of the main focuses, which may have influenced their nutritional habits. Also, 14 subjects claimed they had lived healthier than usual during the first phase and it is not unlikely that more volunteers unwillingly did so. Moreover, only 10 in 49 (20.4%) volunteers smoked on a daily basis at baseline. The smoking prevalence in the general German population is 32% for men and 22% for women [51]. In the study of Meinke et al. [45], 28 in 108 (25.9%) volunteers, who had given statements about their smoking habits, were smokers and those had a significantly lower carotenoid concentration (4.52 ± 1.77) than the non-smokers (5.69 ± 1.78). It is therefore likely that the low smoking prevalence also contributed to higher values in the present investigations.

The study at hand indicates that a biofeedback system on cutaneous carotenoids as part of an integrated health prevention program is able to help increasing the motivation to adopt a healthy lifestyle in high school students. Moreover, its use is correlated with an increase in the cutaneous carotenoid concentration and therefore the general antioxidant status. Furthermore, a consolidation of these two major results can still be seen after five months. Those findings correspond to former findings on adults [40].

To our knowledge, the study at hand is the first one investigating the antioxidant status of high school students and also the first study, in which the subjects carried out the measurements themselves. There has only been one study investigating antioxidant concentrations in US-American preschool children, which was the first of its kind being performed in a school environment [52]. This pilot project has proven that the spectroscopic device is suitable for use at school and by students themselves and has paved the way for the development and implementation of further field studies.
5. Limitations

The results cannot be fully generalized since the investigations involved a rather small number of volunteers, all within a very homogeneous group with regard to age, education and habitat. Another limitation is the subjectivity of the answers stated in the questionnaires, especially when it comes to the evaluation of the health state or the influence of stressing events. Also, the students filled in the questionnaires about the previous two or three days by memory, which limits the accuracy of the answers.

Another limitation of the impact of the study might be the limited practicability of a multi-modal prevention program including changes in the catering, especially in countries, were school lunches are provided by a well-established industry.

Although a significant increase in the carotenoid concentration can be seen in the volunteers, there is only limited evidence for each individual influencing factor on this result. Also, the influence of the biofeedback on specific aspects of lifestyle was not acquired in particular. However, the results indicate several possible influencing factors on the increase of the carotenoid concentration in phases 2 and 3. In phase 1, non-smokers, volunteers with a high consumption of fruit or vegetables, and volunteers who often cook at home had higher measured values. Since students smoked less and ate more vegetables and fruit in the second phase, there is reason to presume that those facts contributed to the increase in their values. Further investigations with larger populations in a controlled environment and with a more sensitive acquisition of individual changes are needed in order to make clear statements on those and other specific influencing factors.

Even though volunteers had significantly higher measured values after intervention, the effect of biofeedback and consequent lifestyle modification might be underestimated. On the one hand, the volunteers were exposed to a higher prevalence of illness and a higher stress level due to significantly more exams in phase 2. On the other hand, many of the volunteers had changed their lifestyle already in the first phase as mentioned above, knowing that they would be measured. This might have contributed both to the high baseline values and a smaller increase than possible in the intervention phase.

As mentioned above, an increase of the carotenoid concentration was particularly noticeable in the second half of phase 2. On the one hand, this can be explained by the simultaneous start of the intervention and the measurements of the second phase, so that the intervention-related effects were not reflected in the early measurements of phase 2. Earlier investigations on the kinetics of carotenoids have shown that it takes up to three days until the positive effects are reflected in the skin [40]. On the other hand, the increase in the course of phase 2 can be interpreted as the effect of the gradual change in the subjects’ lifestyles. Some volunteers stated that they felt like being in a competition and compared their results with fellow students, one volunteer said in an interview that they therefore “developed a genuine ambition to improve [their] values???” in the course of the intervention phase [53].

The consolidation of both the healthy lifestyle and the increased carotenoid levels is particularly astonishing since no measurements were taken within the five months before the follow-up measurements. A continuous biofeedback might have presumably contributed to an even greater increase within that time. However, the increase towards the follow up might not be only due to the sustainability of the biofeedback effect since former findings have shown significant seasonal variations of cutaneous carotenoid concentrations with higher concentrations in the summer and autumn months presumably due to the better availability of fresh fruit and vegetables in temperate latitudes [27, 40]. Nevertheless, the “seasonal increase” was not pronounced during the spring months [40], when measurement phases 1 and 2 were performed. However, in September, the “seasonal increase” is maximal, which could have influenced the results of phase 3. Thus, it cannot be said whether seasonal influences or biofeedback and previous health education events had more influence on the carotenoid increase in the skin for phase 3.

The examples of volunteers as shown in Section 3.6 are individual cases only. In some subjects, no reasonable explanation could be found for the course of antioxidant concentrations. However, measurements took place only every three to four days; and the course of antioxidants on the days between the measurements is unknown. At the same time, the questionnaires cover questions about habits and events on the days before each measurement. A recent study investigating the kinetics of cutaneous carotenoids showed that the decrease of the concentration can be as fast as within two hours after a stressful event, but the increase after the supplementation of carotenoid-rich products takes one to three days [40]. Therefore, investigations with more frequent measurements are needed in order to get further into the question of the influence of certain events.

The study at hand only covered a small number of volunteers and only shows a few significant results. Nonetheless, tendencies can clearly be seen. Further investigations with more cases are needed in this field.
6. Conclusion

In conclusion, the present results provide evidence that health-promoting lifestyle habits and a good state of physical and mental health are associated with a high concentration of cutaneous carotenoids. Positive factors such as good psychological status and consumption of antioxidant-rich food, and negative factors such as sleep deprivation, cigarette smoking and alcohol consumption, multifactorially influence the carotenoid concentration. Therefore, the concentration of cutaneous carotenoids can be seen as a useful indicator for general health.

At the end of the study, the students were asked to summarize their experience gained from the application of the biozoom® device. Nearly all students revealed that they are reluctant to follow good advice from their parents or teachers, whereas they are very serious about the information gained from their own body. They reported also that group measurements result in a competition for the best values.

The biozoom® system was appreciated by the volunteers and asserted itself to be a quick and non-invasive option to measure carotenoid concentrations. It provides the potential to serve as a readily accessible “looking glass” for general health and could be used within prevention and training programs.

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Author biographies Please see Supporting Information online.

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