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Research paper

Cutaneous lycopene and β-carotene levels measured by resonance Raman spectroscopy: High reliability and sensitivity to oral lactolycopene deprivation and supplementation

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ABSTRACT

Carotenoids, naturally occurring lipophilic micronutrients, possess an antioxidant activity associated with protection from damage induced by free radicals.

The present study investigated an innovative non-invasive method to measure cutaneous levels of lycopene and β-carotene and to monitor the distribution of orally administered lactolycopene in human skin and plasma.

A double-blind placebo-controlled randomized study was performed in 25 volunteers, who were under a lycopene-deprived diet (4 weeks prior to study until end of the study) and orally received either lactolycopene or placebo for 12 weeks. Skin and plasma levels of lycopene and β-carotene were monitored monthly using Raman spectroscopy and HPLC, respectively.

Cutaneous levels of lycopene and β-carotene monitored by resonance Raman spectroscopy showed high reliability. Irrespective of the investigated area, cutaneous levels were sensitive to lycopene deprivation and to oral supplementation; the forehead showed the closest correlation to lycopene variation in plasma.

Plasma and skin levels of lycopene were both sensitive to oral intake of lactolycopene and, interestingly, also skin levels of β-carotene. Thus, oral supplementation with lycopene led to an enrichment of β-carotene in human skin, possibly due to the fact that carotenoids act in the skin as protection chains, with a natural protection against free radicals.

1. Introduction

Plant constituents, such as carotenoids include a class of natural fat-soluble pigments which are found in numerous types of fruits and vegetables. These lipophilic micronutrients have powerful antioxidant properties and are widely distributed in the human body. The two predominant carotenoids in human tissues, β-carotene and lycopene, represent about 70% of the carotenoids in the skin and are found in the liver, adrenal glands, fat, pancreas, lungs, testes and kidney [1–3].

Carotenoids are involved in the light-protective system in plants and contribute to the prevention of external damage in humans, such as from UV-irradiation, smoke exposure and contact with environmental hazards. The consumption of a carotenoid-rich diet has been epidemiologically correlated with a lower risk for several diseases [4–7]. Antioxidants in the skin form protection chains, which mean that they protect each other from damage caused by free radicals [8–12].

β-Carotene is an endogenous photoprotector, and its efficacy in preventing UV-induced damage has been widely demonstrated.

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Dermal accumulation of carotenoids may protect human skin from light-induced lesions [13–15]. When comparing the skin surface structure of volunteers in a similar age group, it has been recently demonstrated that a higher amount of lycopene in human skin was associated with a reduced number of furrows and wrinkles [16], indirectly suggesting a photoprotective and subsequently an anti-ageing effect. Recent studies have shown that supplementation and topical application of lutein and zeaxanthin show photoprotective activity [17] as well as the supplementation of an antioxidant complex, vitamins (lycopene, β-carotene and α-tocopherol), selenium [18] or vitamins E and C in combination with carotenoids, selenium and proanthocyanidins [19].

Lycopene is the major carotenoid in tomatoes; thus, ingesting lycopene or tomato-derived products leads to a photoprotective effect on the skin [20–22]. Dietary carotenoids are derived exclusively from exogenous sources, originating from the intake of fruit, vegetables and dairy products. Lycopene is more efficiently absorbed by the body from processed foods, such as tomato paste and tomato juice treated with oil rather than from fresh tomatoes or tomato juice [23]. This can be ascribed to the destruction of all membranes during food processing with subsequent release of carotenoids from the cells [24–26].

The purpose of this double-blind placebo-controlled randomized study was designing a new method for measuring skin levels of lycopene and β-carotene after 4, 8 and 12 weeks of daily oral supplementation with 25 mg lactolycopene in different body areas of volunteers under a lycopene-deprived diet and to monitor the distribution of lycopene taken orally in skin and plasma.

2. Materials and methods

2.1. Study group

The study protocol was submitted and approved by the local ethics committee of the Charité Universitätsmedizin – Berlin. Twenty-five healthy volunteers aged between 18 and 45 years were included in the study protocol (19 female and 6 male) after having given their written informed consent. The median age was 25.7 years (range: 21.8–39.4) in the verum group and 27.3 years (range: 22.3–45.8) in the placebo group. All volunteers had a skin phototype between I and III, a body mass index (BMI) between 18 and 22 kg/m^2, a plasma triglyceride level $\leqslant$180 mg/dL and a total cholesterol level of $\leqslant$200 mg/dL. They were non-smokers and followed a lycopene-deprived diet controlled by a dietary protocol. The volunteers received a nutritional information sheet and were clearly instructed not to eat tomatoes or any tomato-derived foods, watermelon, pink grapefruit, guava, papaya and apricot. Volunteers neither used any cosmetic products containing antioxidant ingredients nor did they take vitamin supplements. Volunteers were excluded if pregnant, lactating or taking inadequate contraceptive measures or if they were vegetarian or had an intolerance to whey proteins or lycopene. The intake of β-carotene, vitamin(s) or mineral(s) and the use of antacid drugs, gastrointestinal dressings, alkali therapy and dietary fibre supplementation were prohibited during the whole trial period. Twenty-three volunteers finished the complete study protocol with excellent compliance; 1 volunteer withdrew for personal reasons unrelated to the investigational study products, 1 was withdrawn for intake of vitamin pills.

2.2. Study products

Two oral products (verum and placebo) both produced as capsules containing granules of whey proteins as carriers were used. The volunteers ingested, according to their randomisation group daily three capsules during breakfast with half a glass of water either of the verum (2% lactolycopene = 25 mg lycopene from tomato oleoresin embedded in a whey protein matrix) or of the placebo (granules of whey protein without lycopene) and continued the same intake over the 12-week treatment period.

2.3. Study protocol

At the screening visit (TS), 4 weeks prior to starting oral intake of the study product, inclusion and exclusion criteria were checked, in particular cholesterol and triglycerides levels. Plasma and skin levels of lycopene and β-carotene were measured on four body areas: the palm of the hand (left for right-handed people and vice versa), forehead, lower back and forearm (arm opposite to the chosen palm). After satisfying screening visit, all volunteers started a lycopene-deprived diet that lasted up to the final study visit.

Four weeks after TS, all volunteers were randomized either to the verum group (25 mg lycopene) or to the placebo group at visit T0, and each volunteer was given capsules for daily supplementation (3 per day). At monthly intervals, plasma and skin measurements of β-carotene and lycopene were performed. After a 12-week oral intake, the study included a 4-week follow-up period under lycopene deprivation but without supplementation. At T16, the same parameters as at screening visit were measured and recorded.

The cutaneous measurements of carotenoids using Raman spectroscopy as described thereafter were always performed on four selected body areas. For optimal reliability, a template of the corresponding surface skin area (1 cm^2) to be measured was provided for each single participant in advance so that the same area was investigated at every time point.

2.4. Techniques for evaluation

2.4.1. Skin tests

Laser-based resonance Raman spectroscopy was used as a fast and non-invasive optical method for measuring the absolute levels of β-carotene and lycopene in living human skin [27,28]. β-Carotene and lycopene have different absorption values at 488 and 514.5 nm and, as a result, different Raman scattering efficiencies at 488 and 514.5 nm excitations, respectively. These differences were used for assessing β-carotene and lycopene levels in living human skin. Both components were detected consecutively and the measurements lasted approximately 10 s. The analytical method for the separate determination of carotenoids β-carotene and lycopene in the skin applied in the present study, as well as measuring setup, has been previously described in detail by Darvin et al. [28].

Fig. 1 shows a typical Raman spectrum of carotenoids, obtained *in vivo* from the human skin under resonance excitation conditions. Three prominent Raman peaks are well recognized in face of the high fluorescence background, which is produced by collagen, lipids, porphyrins, elastin, etc. The Raman peaks at 1005, 1156 and 1523 cm^{-1} originate from the rocking motions of the methyl groups, from the carbon–carbon single bond and carbon–carbon double-bond stretch vibrations of the conjugated backbone, respectively.

The intensity of the strong Raman peak corresponding to the C=C vibration of the conjugated backbone of a carotenoid molecule at 1523 cm^{-1} was used for the measurement of carotenoid concentration [27].

2.4.2. Skin thickness

At visits T0 and T12, skin thickness was measured at the four skin measurement areas using ultrasound of a 12 MHz probe with a maximal axial, respectively, lateral resolution of 0.1118 and

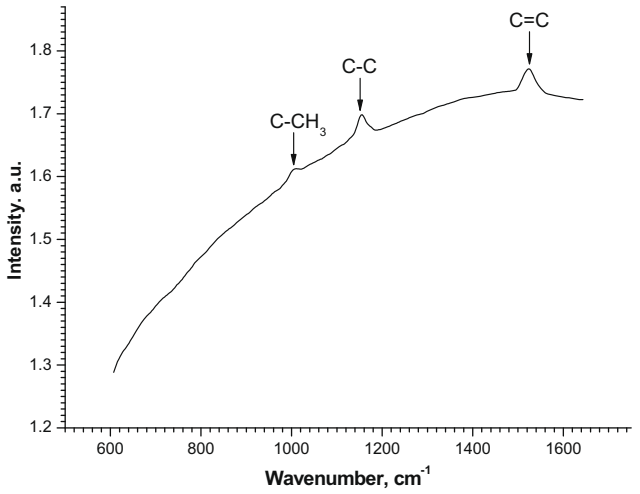

Fig. 1. Typical Raman spectrum obtained from the human skin measured *in vivo* under an excitation at 514.5 nm.

0.164 mm. Before measurement, volunteers had to rest for 15 min in a room with a constant temperature (20–23 °C). The device calibration had to remain the same throughout the entire study course. The ultrasound technique applied was the high-end TECHNOS device (ESAOTE, Italy) with a range of applicable frequencies between 3.5 and 14 MHz. Based on the applied frequency, this ultrasound device allows for an ever increasing detailed insight into changes of thickness of the dermis but similarly at the same time for visualization of the subcutaneous tissue. The 12 MHz probe was used to measure thickness of the dermis. Repeated measurements (at least 3) within one single B (Brightness)-scan were taken as well as measurements in two cross sections on each of the selected areas on each visit. The ultrasound method was performed to monitor whether there were any variations in skin thickness possibly influencing the outcome of cutaneous lycopene and β-carotene measurements.

Raman spectroscopy as well as ultrasound examinations were always performed by the same investigator to avoid inter-investigator bias and assure quality control.

2.4.3. Blood sample analysis

Plasma carotenoids were extracted for measuring out lycopene and β-carotene levels using high performance liquid chromatography (HPLC) as described by [29,30]. Until final processing, plasma was stored in darkness at −70 °C for a maximum of 2 weeks.

Two-hundred microlitre of plasma was diluted with 200 μL of pure water (for HPLC, Roth, Karlsruhe, Germany). Twenty microlitre of internal standard echinenone (DHI Water and Environment, Hoersholm, Denmark) was added and protein was precipitated by adding 200 μL ethanol (for HPLC, Roth, Karlsruhe, Germany, containing 0.01% ascorbic acid). For carotenoid extraction, 800 μL hexane (for HPLC, Roth, Karlsruhe, Germany) was used and the sample was vortex mixed for 20 min, centrifuged at 1000 g, and subsequently 400 μL of the hexane layer supernatant was carefully removed and evaporated under a stream of nitrogen. The residue was reconstituted in 30 μL tetrahydrofuran (THF) (for HPLC, Roth, Karlsruhe, Germany) and 70 μL mobile phase (90% methanol for HPLC, Roth, Karlsruhe, Germany, 10% THF). The samples were prepared avoiding sun-light exposure and were stored in dark and cooled at 4 °C before analysing with HPLC.

The HPLC system consisted of a Perkin Elmer System 200 equipped with an auto sampler and a pump, and a UV–Vis-detector from GAT (LCD 500, PerkinElmer Life and Analytical Sciences, Shelton, USA). Lycopene and β-carotene were detected at 450 nm. The separation took place using a C18 column (YMC-Gel ODS-A, 150 mm × 4 mm i.D., Omnichrom, Berlin, Germany) and the mobile phase running at a flow rate of 1.0 mL/min within 18 min. The signal output was recorded by a computerised data management system (Tubochrom 4, PerkinElmer) and data have been compiled in a special dataset. Each sample was analysed in triplicate in minimum, and mean values were generated.

2.5. Statistics

All parameters of the two groups were compared using the Student's *t*-test for each visit. The correlation between skin and plasma levels and between skin thickness and skin levels of lycopene and β-carotene were calculated using the Pearson correlation coefficient.

Despite the small number of subjects, analyses (efficacy and safety) were performed on the full analysis set ($n = 23$) defined as the randomized set, excluding the two withdrawn subjects.

3. Results

Twenty-five healthy female ($n = 19$) and male ($n = 6$) volunteers enrolled in the study were randomised into either the verum group ($n = 11$, taking lycopene capsules) or the placebo group ($n = 12$, taking placebo capsules). Twenty-three of them completed the 12-week treatment and 4-week post-treatment period. Two female volunteers withdrew for personal reasons or violation of the study protocol. The median ages (years) for the two groups were 25.7 (range: 21.8–39.4) for the verum group and 27.3 (range: 22.3–45.8) for the placebo group. The 23 subjects were all within phototype range II ($n = 18$) or phototype III ($n = 3$).

At baseline, in all subjects, mean (±SEM) total cholesterol and triglycerides were 171.3 (±3.9) mg/dL and 73.0 (±5.1) mg/dL, respectively, which corresponds to a normal lipid profile. The mean body mass index (BMI ± SEM) was 20.11 (±0.48) kg/m^2 in the verum group and 20.29 (±0.38) kg/m^2 in the placebo group. These values were close to the lower limit of normal BMI range (Table 1).

Protocol deviations included a total of 17 volunteers, who reported intake of at least one lycopene-containing food during the 20-week lycopene-deprived period and 8 volunteers, who did not take the stipulated number of capsules. Compliance within the study course was calculated for the 23 volunteers through the percentage of exposure to treatment, and calculated compliance for the whole treatment period was in the median at 98.9% (median T0–T12). During the clinical trial, 12 subjects experienced non-cutaneous adverse events with no causal relationship to the study products.

Table 1
Patient characteristics at baseline.

Parameters		N	Median	Minimum	Maximum
Age at T0 (years)	Verum	11	25.7	21.8	39.4
	Placebo	12	27.3	22.3	45.8
Weight (kg)	Verum	11	56.0	53.0	79.0
	Placebo	12	59.8	48.0	75.0
Height (cm)	Verum	11	171.0	161.0	190.0
	Placebo	12	171.5	159.0	185.0
BMI (kg/m^2)	Verum	11	19.9	18.0	21.9
	Placebo	12	20.7	18.2	22.0
Cholesterol (mg/dl)	Verum	11	164.0	133.0	198.0
	Placebo	12	186.0	145.0	200.0
Triglycerides (mg/dl)	Verum	11	95.0	37.0	115.0
	Placebo	12	65.0	22.0	87.0

3.1. Lycopene level in plasma

At screening visit (TS), all volunteers started a lycopene-deprived diet resulting in a significant decrease in plasma lycopene level in both groups, i.e., from 0.535 ± 0.070 μmol/L to 0.199 ± 0.018 μmol/L in verum and from 0.474 ± 0.040 μmol/L to 0.248 ± 0.036 μmol/L in placebo group (Fig. 1).

3.1.1. Course of plasma lycopene levels compared to screening visit (TS)

In the verum group, the mean plasma lycopene level progressively and significantly increased to reach 0.730 ± 0.063 μmol/L ($p = 0.005$) at T4 (after a 4-week supplementation), 0.748 ± 0.052 μmol/L ($p = 0.004$) at T8 (after 8 weeks) and 0.824 ± 0.068 μmol/L ($p < 0.0001$) at T12 (after 12 weeks).

In the placebo group, the mean plasma lycopene level significantly decreased to 0.253 ± 0.032 μmol/L ($p < 0.0001$) at T4, 0.251 ± 0.037 μmol/L ($p < 0.0001$) at T8 and 0.279 ± 0.052 μmol/L ($p = 0.0015$) at T12 (Fig. 1).

3.1.2. Course of plasma lycopene levels compared to start of supplementation (T0)

In the verum group, the mean plasma lycopene level steadily increased to reach a statistically significant difference ($p < 0.0001$) at T4, T8 and T12. In the placebo group plasma levels of lycopene remained stable, the lowest level being already reached and not altered by the placebo treatment. The differences in plasma lycopene level between the verum and placebo groups were statistically significant at T4: 0.478 ± 0.069 ($p < 0.0001$), at T8: 0.497 ± 0.063 ($p < 0.0001$), and at T12: 0.544 ± 0.085 ($p < 0.0001$).

After stopping the 12-week intake of the verum or placebo but continuing lycopene-deprived diet for a further 4 weeks, the mean plasma levels in the verum group significantly decreased to 0.356 ± 0.047 μmol/L at the follow-up visit (T16). This level was significantly lower than the plasma level at the screening visit (−0.178 ± 0.054 ($p = 0.0078$)) but significantly higher than the level at T0 after a 4-week lycopene-deprived diet (+0.157 ± 0.038, $p = 0.0019$). In the placebo group, plasma lycopene level did not differ from that at week 0 (T0, $p = 0.869$) but was lower than the level at TS ($p = 0.0002$) (Fig. 2). At TS and at T0, the difference between the two groups was not statistically dissimilar (Table 2).

3.2. Skin lycopene level

Table 3 summarizes the different skin lycopene levels (nmol/g) on four different skin areas at the screening visit (TS). The baseline skin lycopene levels differed from one area to the other. Within each skin area, the baseline lycopene level was not different between the two groups. At TS, lycopene levels could be ranked as follows: palm = forehead > forearm > back.

As a result of dietary lycopene deprivation, skin level decreased significantly in all areas of the verum group. In the placebo group, the statistical significance was limited to the back and forehead. Within each skin area, there were no statistically significant differences in lycopene levels between the two groups.

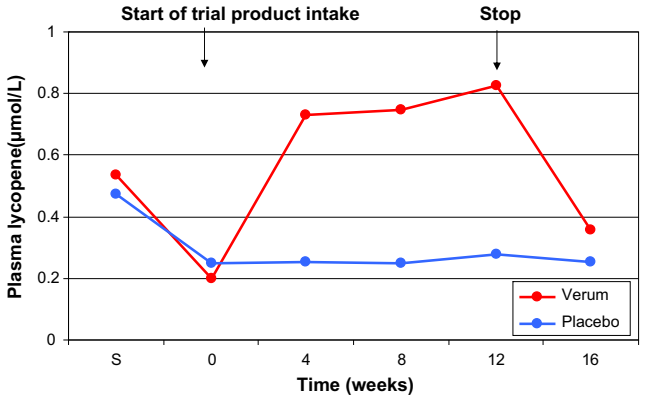

Fig. 2. Course of mean lycopene plasma levels in the verum and placebo groups at TS (4 weeks prior to starting of supplementation), T0 (start of supplementation), T4, T8, and T12 (supplementation course) and T16 (after 4 week follow-up).

Table 2
Plasma lycopene change (statistical significance).

Plasma lycopene	Screening	T0	T4	T8	T12	T16
Verum: level compared to TS		↓S	↑S	↑S	↑S	↓S
Placebo: level compared to TS		↓S	↓S	↓S	↓S	↓S
Verum: level compared to T0			↑S	↑S	↑S	↑S
Placebo: level compared to T0			NS	NS	NS	NS
Verum/placebo: difference at visit	NS	NS	S	S	S	NS

S: significant, when $p < 0.05$ ↓: reduction.
TS: trend to S, when $0.05 \leqslant p \leqslant 0.1$ ↑: increase.
NS: not significant, when $p > 0.1$.

Table 3
Skin lycopene level (nmol/g) at the screening visit.

	Forehead	Lower back	Inner arm	Palm
Verum group	0.297 ± 0.061	0.181 ± 0.041	0.194 ± 0.036	0.326 ± 0.089
Placebo group	0.283 ± 0.025	0.179 ± 0.024	0.222 ± 0.031	0.279 ± 0.043

3.2.1. Course of skin lycopene level compared to screening visit (TS)

In the verum group, skin lycopene levels on the forehead and back did not change over the 16-week study period. There was a reduction with a tendency to significance at T4 on the palm and at T12 on the arm. In all four areas, skin lycopene levels at the different clinical visits were similar to or lower than at TS and unlike in plasma, never exceeded these levels (Fig. 3).

As expected, skin lycopene level in the placebo group was significantly decreased in all skin areas at T4, T8 and T12 compared to TS (Fig. 3).

3.2.2. Course of skin lycopene level compared to start of supplementation (T0)

In the verum group, skin lycopene level was increased at T4 on the back ($p = 0.007$), forearm ($p = 0.037$) and forehead ($p = 0.077$) but was not statistically different on the palm at T4 or in any test areas at T8 and T12 (Fig. 3).

In the placebo group, skin lycopene levels did not statistically differ on the back neither at T4, T8 and T12 nor on the forehead and palm at T4. They decreased significantly on the forehead and palm at T8 and T12 and on the forearm at T4, T8 and T12 (Fig. 3). The minimum skin lycopene level was only reached after 8 weeks (on forearm and back) and after 12 weeks (on forehead and palm). This contrasts with the effects on plasma levels, where the minimal level was already reached as early as after a 4-week dietary deprivation.

In all investigated skin areas, the maximum lycopene level under the intake of verum or placebo was higher in the verum group compared to the placebo group. A statistical significance or a tendency to statistical significance was reached at least once between T4 and T12 (Fig. 3).

At the 4-week follow-up visit (T16), lycopene level in the skin decreased in the verum group with a tendency to significance in forearm and back compared to TS, but was not significantly different compared to placebo. Lycopene level remained at the lower level in the placebo group. A statistical difference between the two groups was only found on the forehead.

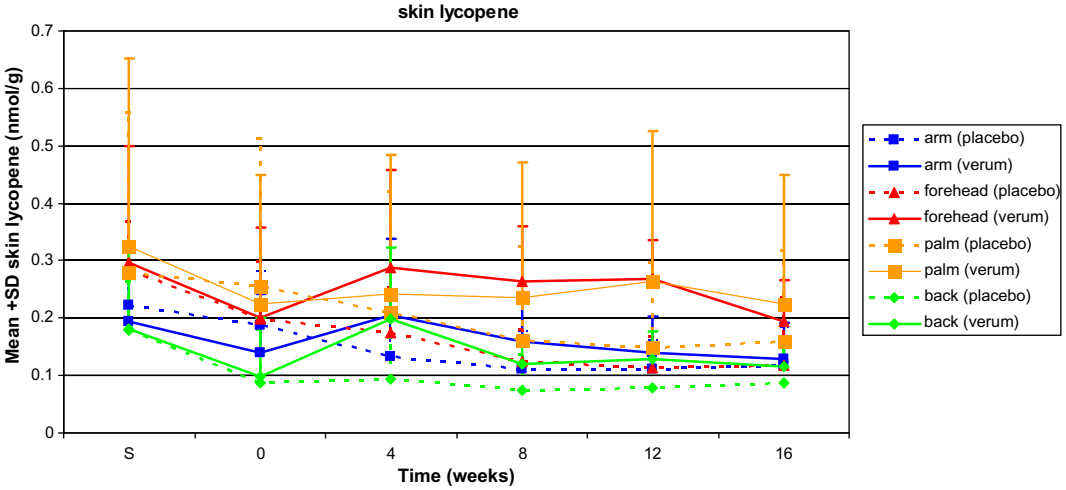

Fig. 3. Course of mean cutaneous lycopene levels measured in the four investigation areas in the verum group (2% lycopene supplementation) and in the placebo group.

3.3. Course of β-carotene level in plasma

At TS, mean plasma β-carotene level was similar between the two groups. The 4-week lycopene dietary deprivation had no impact on the plasma level of β-carotene, which remained stable during this period. No statistically significant inter- or intra-group differences were found (Fig. 4).

During the 12-week supplementation period, plasma β-carotene level did not show significant variations, neither in the verum nor in the placebo group.

During the follow-up period without treatment (T16), the plasma β-carotene level decreased in both groups, but with no statistical significance compared to TS and T0 in the verum group and, with statistical significance when compared to TS in the placebo group (Fig. 4).

3.4. Course of β-carotene level in the skin

At TS, β-carotene levels could be ranked as palm > forehead > arm = back (Table 4). In contrast to plasma, skin levels were sensitive both to lycopene deprivation and to oral supplementation. With supplementation, skin β-carotene level increased significantly ($p < 0.05$) above TS level in the four areas, whereas it tended to remain stable in the placebo group.

During the 12-week treatment period in the verum group, β-carotene levels in the skin increased in all four areas compared to TS and T0 (with statistical significance at T8 and/or T12 depending on the investigated area). In contrast to skin levels of lycopene, which never reached baseline levels during lycopene supplementation, skin β-carotene levels in the four areas were always above TS level (Fig. 5).

In the placebo group, compared to T0, the skin level increased with statistical significance on the back at T4, T8 and T12, and on the arm at T12. Compared to T0, the skin levels significantly decreased at T4 and T8 on the palm. A difference in favour of the verum group was observed on the forehead (T8 and T12) and on the palm (T8) (Fig. 5).

At the follow-up visit (T16), β-carotene level in the skin decreased in the verum group but remained statistically different from the level on the back and on the palm at TS and T0. In the placebo group, β-carotene concentration in the skin also decreased and was statistically different from T0 on the back. No statistical differences between the two groups were found in any area.

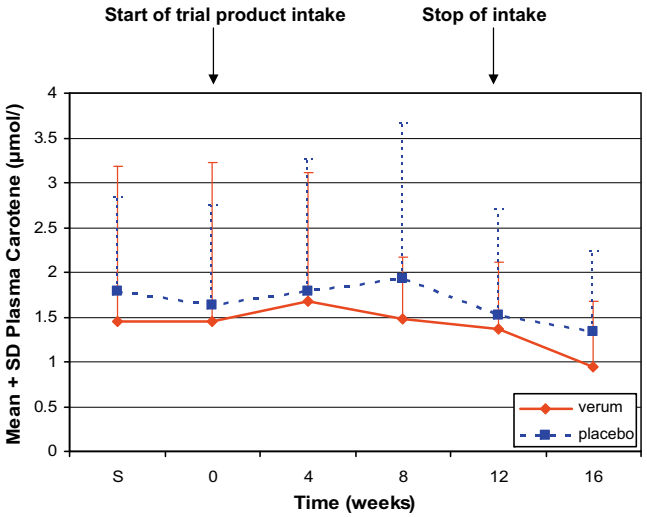

Fig. 4. Course of mean β-carotene plasma levels in the verum and placebo groups at TS (4 weeks prior to starting of supplementation), T0 (start of supplementation), T4, T8 and T12 (supplementation course) and T16 (after 4 week follow-up).

Table 4
Skin β-carotene level (nmol/g) at the screening visit.

	Forehead	Lower back	Inner arm	Palm
Verum group	0.377 ± 0.044	0.291 ± 0.035	0.353 ± 0.054	0.596 ± 0.047
Placebo group	0.402 ± 0.038	0.325 ± 0.049	0.385 ± 0.038	0.625 ± 0.052

3.5. Skin thickness

Evaluation of skin thickness in the four test areas was assessed at T0 and T12. Skin thickness varied non-significantly between the different areas and between volunteers (male thicker than female) during the whole study period and without any correlation between skin thickness and cutaneous lycopene level.

4. Discussion

In the present study, lycopene from tomato oleoresin embedded in a whey protein matrix, named lactolycopene was investigated for its ability to influence lycopene and β-carotene skin and plasma

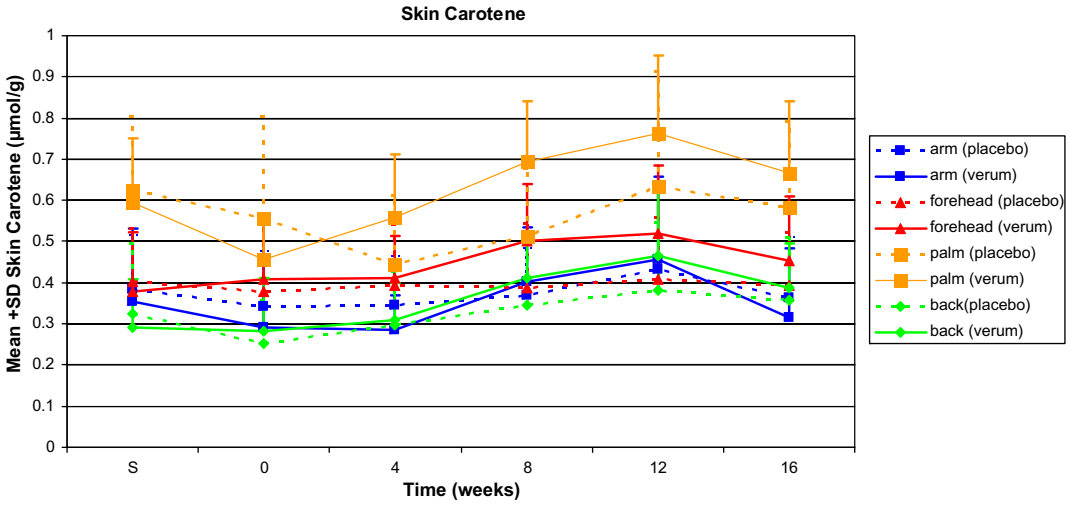

Fig. 5. Course of mean cutaneous β-carotene levels in the four investigational areas in the verum group (2% lycopene supplementation) and in the placebo group.

levels. It has recently been demonstrated that this lycopene formulation has the same bioavailability in plasma and buccal mucosa cells as that from tomato paste [31]. In the present study, resonance Raman spectroscopy was used for the first time to assess the concentration of lycopene in the skin using an innovative non-invasive method. Plasma levels of lycopene and β-carotene were analysed using classical high pressure liquid chromatography (HPLC) [30].

Plasma lycopene level has been reported to significantly increase after a 2-week daily supplementation with lactolycopene (lycopene 25 mg) with a similar time–concentration curve as with daily supplementation of tomato paste [24]. Daily oral intake of lactolycopene (lycopene 25 mg) was also shown to increase lycopene level in buccal mucosa cells and both lycopene and β-carotene levels in the skin [31].

In contrast to other studies where the appearance of antioxidants was measured indirectly by the variation of skin properties such as elasticity, skin lipids or photoprotection, the present investigation permitted to directly and non-invasively measure the amount of carotenoids in the skin using Raman spectroscopy. Nevertheless, Palombo et al. have shown that the photoprotective activity increases by supplementation of lutein and zeaxanthin, an effect which could even be enhanced by simultaneous topical application of antioxidants [17]. Similar results were obtained for hydration, superficial skin lipids but not for elasticity, which could be likewise improved by topical application of the antioxidans. The study pointed out that additional benefit was achieved through the simultaneous administration of these carotenoids by both routes.

This double-blind, placebo-controlled randomized clinical trial, investigating the effect of a 12-week lycopene supplementation on plasma and skin bioavailability of lycopene in 23 healthy volunteers under a lycopene-deprived diet showed a high sensitivity of plasma lycopene to oral uptake. Under lycopene-deprived diet conditions, plasma lycopene level rapidly decreased and reached a significantly low level remaining constant under placebo supplementation. Similarly, lycopene level rapidly increased with lycopene supplementation to reach a level significantly higher than at baseline. These changes in plasma level are in agreement with those previously reported by Richelle et al. [31] following lycopene supplementation with a similar formulation.

Lycopene is not homogeneously distributed in the skin; although well described, the reason remains unknown. Distribution of lycopene could possibly be affected by the exposure to light or the thickness of the skin of any given area. Therefore, skin thickness was measured in each volunteer to detect any possible correlation between skin thickness and lycopene enrichment in the skin. At baseline skin lycopene levels differed depending on the body area and skin thickness. The differences in skin thickness and vascularization at various sites, as well as differences in UV-light exposure, might play a role and possibly help to explain these differences of cutaneous lycopene distribution. Orally administered antioxidants have been reported to be transported to the skin surface and the SC by the sebaceous gland secretion route [32]. The forehead has a higher sebaceous gland density, whereas the palm a high sweat gland density compared to the back and forearm which let us assume that lycopene is transported via the sebaceous gland secretion route or possibly also via sweating.

Irrespective of the skin area, cutaneous lycopene levels were sensitive to oral uptake of lycopene with a delayed effect compared to the plasma level. Unlike plasma level, variations of skin level were rarely statistically significant in the verum group where lycopene level remained always below the baseline level. Even in the placebo group, a statistically significant lower level was reached compared to T0, suggesting a slow but steady release of stored lycopene from the skin when needed.

When analysing cutaneous lycopene and β-carotene levels in the four studied areas, the forehead seems to be the area where the change in lycopene level shows the closest profile to that monitored in plasma. Conceivably, skin structure, hair follicle density and sebum excretion rate are possible parameters which might influence the lycopene and β-carotene levels in the skin. Further studies are required to investigate the importance of hair follicle density and sebum secretion on cutaneous lycopene and β-carotene levels.

Interestingly, we could not observe any variations of β-carotene level in the plasma during the study. The statistically significant decrease in β-carotene in the skin after a 16-week lycopene-deprived diet may suggest that a longer period of lycopene-deprivation could unbalance the β-carotene plasma level.

As observed with skin lycopene levels, β-carotene skin levels differ according to the area. In contrast to plasma, skin level of β-carotene is sensitive to lycopene-deprived diet and to supplementation.

With lycopene supplementation, skin levels of β-carotene increased significantly above screening level in the four areas, whereas skin levels of lycopene tend to remain stable in the lycopene-deprived diet group. A difference in favour of the verum group is seen in forehead and in palm. The finding of increasing

concentrations of β-carotene in the skin as a result of systemic intake of lycopene is highly interesting [33]. This may possibly be caused by the fact that the antioxidant substances act as protection chains, protecting each other from the direct damage caused by free radicals. This means that the increase in lycopene in the skin may also stimulate the enrichment of β-carotene in the human skin which is not linked to free radical damage but rather to maintain the same skin lycopene to β-carotene ratio. The reason why this effect was not observed in plasma β-carotene levels, the latter is possibly due to the fact that in contrast to blood, skin represents a storage reservoir for antioxidants for several weeks [6].

In the present study, we could neither find any significant variation of skin thickness between T0 and T12 nor establish any correlation between the lycopene or β-carotene level depending on the investigated area and the time points. This may possibly be due to the device used for skin thickness evaluation in the present study protocol. Very recently, Heinrich [15] reported an increase in skin thickness as well as in skin density as a result of the combined application of lycopene, β-carotene, vitamin E and selenium at different doses vs. placebo. In this study the effects were revealed using a 20 MHz ultrasound device (Dermascan, Cortex Technologies, Hadsund, Denmark), which is more convenient for this purpose due to its ability to depict the slightest change. However, the observed changes in the above-mentioned study could also be ascribed to the combination effect of the different antioxidants compared to the uptake of lycopene alone. Further studies should examine lycopene effects by measuring skin thickness with a 20 or 50 MHz device.

In conclusion, this 20-week, double-blind placebo-controlled randomized trial using lycopene dietary supplement clearly demonstrated that lycopene plasma level was very sensitive to lycopene uptake (diet or supplementation), with rapid variation and within a high range. In contrast, lycopene skin levels are less sensitive or show a slower response to oral supplementation as compared to plasma. However, reduced lycopene uptake induces a slow but significant decrease, whereas a supplementation only tends to maintain baseline levels. In addition, β-carotene plasma level is poorly influenced by the lycopene intake, whereas β-carotene skin levels tend to be stable under lycopene-deprived diet and to increase to a higher level than baseline under lycopene supplementation. Finally, the test product was well tolerated by all volunteers as shown by no treatment-related adverse events.

This is the first study proving the ability to monitor skin carotenoid levels using resonance Raman spectroscopy as a fast non-invasive diagnostic tool. In addition, skin levels of lycopene showed clear variations which could be allocated either to lycopene deprivation or to supplementation. This innovative device enables the investigator to confirm that different body areas present different storage behaviour for lycopene and β-carotene antioxidants.

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