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Dietary lutein supplementation protects against ultraviolet-radiation-induced erythema: Results of a randomized double-blind placebo-controlled study

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ABSTRACT

Various studies showed promising photoprotective and anti-aging effects of lutein, but it was mostly investigated in combination with other antioxidants, and some study results are contradictory. The aim of this randomized, double-blind placebo-controlled intervention was to investigate the effects of dietary lutein supplementation on minimal erythema dose (MED) as a measure of skin's photoprotective potential, and other skin parameters. Thirty healthy women received supplementation of lutein (20 mg/day; liquid formulation) or placebo for 12 weeks. In the test group, MED was significantly increased, indicating greater individual's resistance to the production of erythema following UV radiation. The overall treatment effect was 0.114 J/cm^2 corresponding to a relative increase of photoprotective activity of 22%. On the other hand, we were unable to confirm supportive effects for skin regeneration. Study findings show that dietary supplementation with lutein improved skin photoprotective potential and could contribute to skin defense against UVR-mediated skin damage.

1. Introduction

Skin is the outermost organ and provides protection for the human body from external stress factors such as ultraviolet radiation (UVR). UV radiation causes different photochemical reactions in the skin, and secondary interactions including increased formation of reactive oxygen species (ROS), leading to a decrease of physiological homeostasis and resulting in damaging effects such as erythema, hyperplasia, hyperpigmentation, edema, immunosuppression, photoaging, and photocarcinogenesis (Afaq & Mukhtar, 2011; Farage, Miller, & Maibach, 2017). UVB, which represents around 5% of solar UVR, acts preferentially on the epidermis, and is therefore primarily responsible for sunburn and erythema. It is the primary activator of the inflammatory response through activation of inflammasomes, leading to the release of cytokines, chemokines, and ROS (Feldmeyer et al., 2007; Nasti & Timares, 2012). UVA radiation (which constitutes up to 95% of solar UVR) penetrates deeper and causes damage to both epidermis and dermis; it can damage the connective tissues and blood vessels along with other major skin components, including the extracellular matrix (ECM), leading to premature aging, characterized by wrinkling and loss of elasticity (Ahuja, Gupta, Mishra, & Rani, 2017). Newer findings suggest that blue light, part of sunlight's visible spectrum, also contributes to skin aging, similar to UVA (Nakashima, Ohta, & Wolf, 2017).

The skin possesses a variety of mechanisms for protection against environmental assault, including antioxidants, which help reduce potential damage from the ROS produced in skin due to sunlight exposure, but its defensive capability is rapidly depleted by even moderate UV light exposure (Thiele, Dreher, & Packer, 2002). Systemic protection against sunlight-induced skin damage by nutritional means has received attention from various groups in recent years (Balić & Mokos, 2019; Chen, Damian, & Halliday, 2014; Pérez-Sánchez, Barrajón-Catalán, Herranz-López, & Micol, 2018), with convincing evidence that several dietary antioxidants, including different carotenoids, can provide some protection against skin damage from sunlight and reduce sunburn-associated erythema following UVR exposure (Balić & Mokos, 2019; Chen, Damian, & Halliday, 2014; Evans & Johnson, 2010; Jansen,

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Wang, Burnett, Osterwalder, & Lim, 2013; Krutmann & Humbert, 2011; Nwanodi, 2018; Parrado, Philips, Gilaberte, Juarranz, & González, 2018; Pérez-Sánchez, Barrajón-Catalán, Herranz-López, & Micol, 2018; Petruk, Del Giudice, Rigano, & Monti, 2018; Zerres & Stahl, 2020; Stahl, 2011); ; .

Lutein is a member of the xanthophyll group, a subgroup of the carotenoid family and often coexists with its stereoisomer zeaxanthin (Granado, Olmedilla, & Blanco, 2003). As it cannot be synthesized in the human body, lutein is present in human skin as a result of dietary intake (Wingerath, Sies, & Stahl, 1998). Foods such as dark green vegetables, different fruits, grains, and eggs represent its major source (Abdel-Aal, Akhtar, Zaheer, & Ali, 2013; Yang et al., 2018; Zhao, Cheng, Jiang, Yao, & Han, 2014). It is also a common component of functional foods and food supplements, usually using commercial lutein extracted from marigold flower (*Tagetes erecta*) (Bone, Landrum, Guerra, & Ruiz, 2003; Ochoa Becerra, Mojica Contreras, Hsieh Lo, Mateos Díaz, & Castillo Herrera, 2020).

Although in the human body lutein is found in several tissues, its concentration is highest in the retinal tissue of the eye (Khachik, de Moura, Zhao, Aebischer, & Bernstein, 2002; Madaan et al., 2017). It is also a major carotenoid present in the skin (Wingerath et al., 1998). In the skin as well as in other tissues, lutein can act as blue light filter and antioxidant, potentially benefitting the immune system, and contributing to protection against the damaging effects of UVR as well as visible light that penetrates the skin (Roberts, Green, & Lewis, 2009; Shegokar & Mitri, 2012).

Although there are some *in vitro* and *in vivo* studies on photoprotective and anti-aging effects using a combination of antioxidants including lutein supplementation (Evans & Johnson, 2009; Heinrich et al., 2003; Heinrich, Tronnier, Stahl, Bejot, & Maurette, 2006; Meinke et al., 2013; Pérez-Sánchez et al., 2018; Shegokar & Mitri, 2012), only a few studies have specifically investigated the *in vivo* effects of lutein or lutein/zeaxanthin supplementation on human skin. According to Roberts et al. (Roberts et al., 2009), the earliest indication of the efficacy of lutein oral supplementation in human skin was reported in 2002 (original reference unavailable). After 8 weeks of oral supplementation with lutein/zeaxanthin (6 mg/0.18 mg), lipid peroxidation in the skin was reduced and skin hydration improved. The effects of lutein/zeaxanthin combination in soft gel capsules administered orally (10 mg lutein/0.6 mg zeaxanthin daily) and/or applied topically on human skin were later evaluated in a randomized, placebo-controlled, 12-week clinical trial on 40 healthy women (aged 25–50 years, mean age 35.1 years) with expressed signs of skin aging (Palombo et al., 2007). Although in all lutein groups an increase of superficial skin lipids, skin hydration, and skin elasticity, suppression of skin lipid peroxidation, and an increase of photoprotective activity were observed, oral lutein administration resulted in better photoprotective activity than topical application, but later was more efficient for improvement of skin elasticity. For all parameters, the best results were achieved using combined topical and oral administration, which was shown to provide the highest degree of antioxidant protection. In another randomized, placebo-controlled trial, dietary intake of lutein/zeaxanthin (10 mg/2 mg, soft gel capsules) over 12 weeks was tested on 48 healthy volunteers (18–45 years, mean age 36.1 ± 5.3 years, 43 female, 7 male) with mild-to-moderate dry skin (Juturu, Bowman, & Deshpande, 2016). Results showed improvement of skin tone and luminance (CIE L*) in the test group.

The aim of this randomized, double-blind placebo-controlled study was to investigate the effects of supplementation with lutein (20 mg daily) in the form of a liquid food supplement over 12 weeks on minimal erythema dose (MED), as a measure of photoprotective potential. Considering indications that lutein might support repair of radiation-damaged skin, the secondary objectives of the study were to investigate effects of supplementation on skin viscoelasticity as well as dermal density.

2. Materials and methods

2.1. Study design

The study employed a single-center, randomized, placebo-controlled, parallel design. The study was in full compliance with the principles laid out in the Declaration of Helsinki. The study protocol was approved by the Slovenian National Medical Ethics Committee (Ministry of Health, Republic of Slovenia), identification number KME 0120-63/2018 (approval letter ID 0120-63/2018/4, date of approval: 20 February 2018) and was registered at ClinicalTrials.gov (ID: NCT03811977). The study was performed in compliance with the requirements of local authorities. Before participation in the study, all subjects signed a written informed consent form (ICF).

2.2. Study population

Invitation to participate in the study was published on the institutional website of the Higher School of Applied Sciences (Slovenia) and social media. The study population included healthy Caucasian female subjects, aged between 25 and 55 years with Fitzpatrick skin phototypes (FT) II and III from the Ljubljana area (Slovenia).

Exclusion criteria were pregnancy or breastfeeding, known or suspected allergy to any ingredient of the tested products, photosensitivity, changes in dietary habits and dietary supplementation in last month prior to inclusion, veganism, changes in cosmetic facial and body-care routine in last month prior to inclusion, diagnosed and uncontrolled/untreated/unregulated disease, including acute skin diseases, any skin conditions in the group of photodermatoses, connective tissue diseases, prior or existing melanoma or non-melanoma skin cancer, any clinically significant history of serious metabolic disease, digestive tract disease, liver disease, kidney disease, hematological disease, acute skin diseases, regular consumption of food supplements containing carotenoids or other antioxidants in last month before inclusion in the study, invasive rejuvenation treatments (e.g., needle rollers, needle mesotherapy, deep/medium-deep chemical peels) in last 6 months prior to study entry, non-invasive rejuvenation treatments (e.g., radiofrequency, electrotherapy, ultrasound therapy) in last 2 months prior to study entry, skin pigmentation disorders, skin abnormalities in the test areas, gluteal hyperpigmentation, and mental incapacity that precludes adequate understanding or cooperation. Subjects were asked not to change their dietary habits and routinely used skin-care regime on the test sides during the entire study period. Consumption of any additional food supplements containing carotenoids or other antioxidants as well as sunbathing and use of tanning beds or tanning products was not allowed during the study.

Subjects' compliance with the inclusion and exclusion criteria was checked before their inclusion in the study. A total of 38 subjects were assessed for eligibility and 8 of these did not meet the inclusion criteria, therefore 30 subjects were enrolled onto the study. The subjects were randomly assigned to either (a) a placebo group, receiving placebo syrup or (b) a test group receiving investigational product, lutein syrup, with 15 subjects per group. Randomization was performed using a simple randomization procedure (computerized random numbers).

2.3. Study products and intervention

All subjects consumed 10 mL of a syrup daily for 12 weeks with a meal. The test group received the investigational product, the test syrup (2 mg lutein/mL; daily lutein dose 20 mg), and the placebo group received 10 mL of flavored and colored placebo syrup without lutein. Ingredients of the test syrup: water, xylitol, lecithin, fruit concentrate, lutein, sodium benzoate, flavor. Placebo syrup did not contain lutein, but colouring was added instead (allura red, quinoline yellow). Study products were formulated within the Food4Future (F4F) programme and produced by Valens Int. d.o.o. (Šenčur, Slovenia) following good

manufacturing practice guidelines.

2.4. Assessments

Regular checks of the subjects were carried out three times during the study: at the baseline (T0), after 6 weeks (T6), and after 12 weeks of supplementation (T12). To follow their compliance with the protocol, subjects kept a diary of test product intake for the whole 12-week intervention period, and it was checked after 6 and 12 weeks of intervention (T6 and T12; concomitant interview with the subjects). To further check participant's compliance, also empty and unused packages of products from subjects were collected and assessed after 6 and 12 weeks of supplementation. MED determination and other assessments of skin parameters were performed at baseline and after 12 weeks of intervention (T0 and T12). The results were obtained during March 2019 and July 2019. All measurements were carried out on subjects lying in a room with a temperature of 20–25 °C and relative humidity 40–60%. Assessments started after a 30-min acclimatization period in the same atmospheric conditions.

No application of skin-care products 12 h before and 24 h after UV application was allowed on the gluteal area. Subjects were instructed to clean their face at least 2 h before the time of measurement and to not apply any cosmetic products on their face 2 h or less before the measurement.

2.5. Minimal erythema dose determination

Irradiation for determination of MED (J/cm^2), was performed with automated erythema tester Dermalight® 80 MED Tester (Dr Hoenle Medizintechnik GmbH, Germany; UVB 311 nm). Increasing UV doses (exact dosages depending on the individual's skin phototype following the Fitzpatrick classification; 100% dose for FT II: 0.800 J/cm^2, FT III: 1.127 J/cm^2) were applied on a gluteal area via 10 small square apertures within the MED tester, at T0 on the left half of the gluteal area, at T12 on the right half of the gluteal area. Testing was performed under standardized conditions. MED readings were done 24 h post irradiation. The UVB dose received in the first square with perceptible and unambiguous redness, with clearly defined outlines, interpreted 24 h after exposure to UVB, was determined as MED according to standard procedures (Agache & Humbert, 2004).

MED values before and after supplementation were used to calculate the photoprotective activity for each subject, as described elsewhere (Palombo et al., 2007) according to the following equation:

Photoprotective activity = MED for treated skin/MED for untreated skin.

MED for untreated skin corresponds to baseline MED values, and MED for treated skin corresponds to MED values after 12 weeks of supplementation.

2.6. Dermal density measurements

Dermal density (intensity score 0–100) was measured according to standard procedures (Agache & Humbert, 2004) using ultrasonography with DermaLab® Series, SkinLab Combo, 20 MHz ultrasound probe (Cortex Technology ApS, Denmark). Measurements were taken on a predetermined area on the right cheek, in the center between the alarofacial groove and earlobe under zygomatic bone on the outlined measurements area (approx. 4 cm^2). Measurements were repeated three times and average calculated. A constant gain curve was applied for each volunteer. Digital image analysis was done using the integrated software of DermaLab (SkinLab, Cortex Technology ApS, version 1.04.1, Hadsund, Denmark).

2.7. Viscoelasticity measurements

Viscoelasticity (VE; MPa) was measured using DermaLab® Combo SkinLab, elasticity probe (Cortex Technology ApS, Denmark) on the left cheek, in the center between the alarofacial groove and earlobe under zygomatic bone on the outlined measurements area (approx. 4 cm^2). The elasticity was measured with settings for normal skin condition and individual settings of skin thickness. Measurements were repeated three times and average calculated.

2.8. Sample size calculations

According to our unpublished pilot tests and results of previous studies with lutein supplementation (Juturu et al., 2016; Palombo et al., 2007), the percentage of variance in MED explained by the effect of treatment product was presupposed at 25% (partial $\eta^2 = 0.25$). Using power calculation based on this assumption, we determined that a total sample size of 26 participants is needed to achieve 80% power at 5% significance level. Assuming a 10% drop-out rate, a total number of 30 participants (15 per group) were included in the study.

2.9. Data and statistical analysis

Data were analyzed using STATA version 15.1 (StataCorp LLC, TX, USA). For descriptive statistics, Microsoft Excel version 16.0 (Microsoft Corporation, Redmond, WA, USA) was used. The data were tested for normality by a Shapiro–Wilk test. Differences in sociodemographic variables between groups were tested using *t*-test for independent variables or two sample z-test of proportions. The measured skin parameters were evaluated by descriptive analysis at T0 (baseline) and T12 (after 12 weeks of supplementation). Efficacy analyses for continuous variables (MED, VE, density) were performed using analysis of covariance (ANCOVA), with baseline measures as covariate. A nested two-factor ANCOVA tested for time and a time-by-treatment effect on the depended measures between groups. The nested ANCOVA, using the baseline score as a covariate, determined when significant effects occurred. Differences were considered significant at $p < 0.05$.

3. Results

Out of the 30 subjects enrolled on the study, 28 completed the entire 12-week trial (placebo group: 14 subjects, test group: 14 subjects). There was one drop-out in each of the groups, both due to personal reasons. In the placebo group one subject discontinued intervention during the trial and in the test group one subject was lost to follow-up at the last regular check. No side effects or adverse events of any kind were reported. The trial design and the flow of subjects through the trial are represented in the Consolidated Standards of Reporting Trials (CONSORT) flow diagram in Fig. 1.

The baseline characteristics of the subjects that finished the whole 12-week trial and were included in analysis are shown in Table 1. Only women of Caucasian ethnic origin were included. The mean age of subjects was 39.2 ± 10.8 years, with no significant difference in age between both groups ($p = 0.44$). The distribution of phototypes (FT) II and III was equal in both groups. There was also no significant difference in the distribution of smokers in both groups ($p = 0.36$).

We also confirmed that randomization concerning all investigational variables was successful, as both groups were the same at baseline regarding MED ($t(26) = 1.47$; $p = 0.16$), VE ($t(26) = 1.22$; $p = 0.23$), and density ($t(26) = 0.21$; $p = 0.84$).

Our first and second hypotheses concerned the degree to which the use of formulation might result in variance changed in MED, VE, and density. We hypothesized that the group receiving the experimental treatment would outperform the control group on the MED, VE, and density. In addition, we hypothesized that MED, VE, and density in the experimental group would be higher following 12 weeks' treatment. To

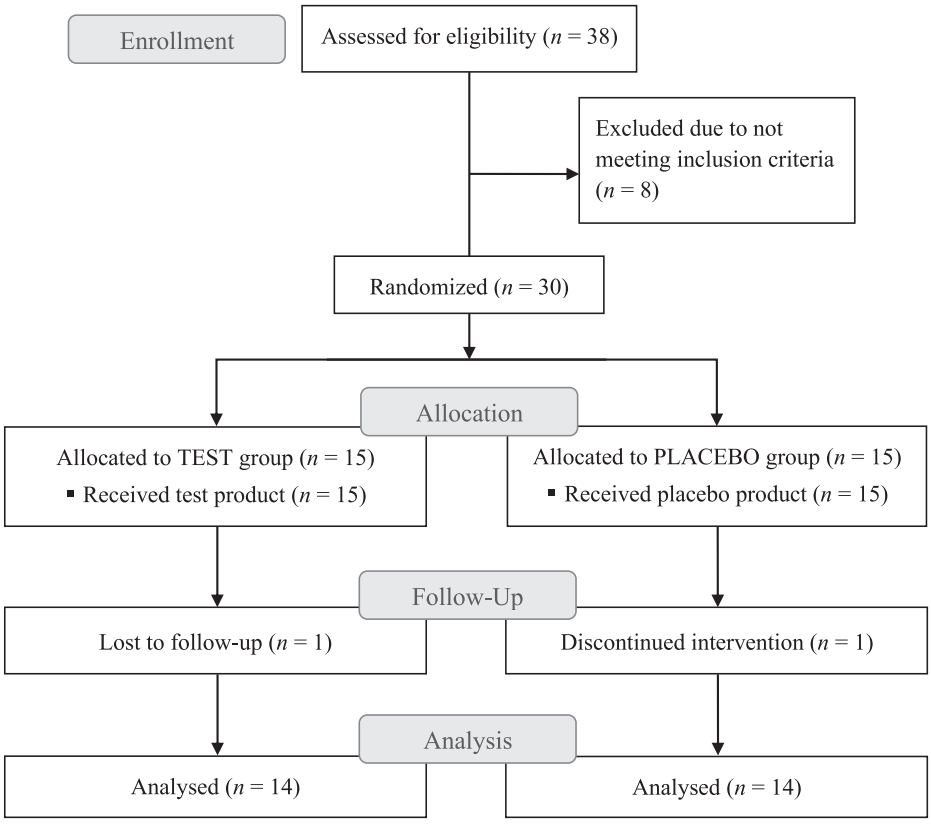

Fig. 1. Consolidated Standards of Reporting Trials (CONSORT) flow diagram showing trial design and subjects' assignment and progression through the trial.

Table 1
The baseline characteristics of the subjects that finished the trial and intervention period.

Details	Placebo Group (n = 14)	Test Group (n = 14)
Sex (F/M)	14/0	14/0
Age (years, mean ± SD)	40.8 ± 11.6	37.7 ± 10.8
Phototype		
– II (n)	7	7
– III (n)	7	7
Ethnic origin	Caucasian (n = 14)	Caucasian (n = 14)
Smokers		
– No	12	10
– Yes	2	4
Intervention		
– Start	March 2019	March 2019
– End	June 2019	June 2019

examine these two hypotheses, a nested two-factor ANCOVA was performed, using STATA GLM. The independent variables consisted of the group (treatment and control) and time (at baseline and 12 weeks' follow-up), factorially combined. To account for individual differences in the baseline due to randomization process, we used the baseline data as covariates in the analysis. To meaningfully interpret the univariate F tests for the different groups, we determined whether any statistical assumptions underlying the use of ANCOVA were violated in the dataset. An examination of Bartlett's test of sphericity demonstrated that the data are not intercorrelated (MED: $\chi2(1) = 0.351$, $p = 0.553$; VE: $\chi2(1) = 1.724$, $p = 0.189$; density: $\chi2(1) = 1.540$, $p = 0.215$). Moreover, the results of the tests of between-participants' effects demonstrated that the assumption of homogeneity of regression slopes were successfully met (MED: $F(1,48) = 0.42$, $p = 0.52$; VE: $F(1,48) = 1.03$, $p = 0.32$; density: $F(1,48) = 0.03$, $p = 0.87$). The analysis showed a significant effect of time and the interaction between group and time on the change of MED. The analysis reveals significant improvement in the MED from baseline to end point for the intervention group in magnitude of 0.092 J/cm^2 ($t = 5.09$, $p < 0.001$) and no change for the placebo group. The results are presented in Supplementary Table S1.

Results for MED measurements are shown in Table 2. While in the placebo group MED remained without significant change after 12 weeks of intervention, in the test group lutein supplementation resulted in a significant increase over baseline values. Comparison of the test and placebo group treatment using two-factor ANCOVA found a significant interaction effect (i.e. treatment effect) after 12 weeks of supplementation, measured as change of MED. The treatment effect of lutein supplementation was 0.114 J/cm^2 (95% CI: 0.061, 0.166; $p < 0.001$; Supplementary table S1). Another way of looking into those results is to calculate photoprotective activity; the results are shown in Fig. 2. Lutein supplementation over 12 weeks resulted in 22.2% (95% CI: 11.1, 33.2) significantly higher photoprotective activity in comparison to placebo.

The starting level of dermal density in the placebo group was 36.9 (95% CI: 34.7, 39.2) and 36.4 (95% CI: 34.1, 38.7) in the test group, and it remained without significant change in both groups until the end of the study (39.5 (95% CI: 37.2, 41.7) and 35.3 (95% CI:33.0, 37.6), respectively; $p > 0.05$ for both groups).

Table 2
Minimal erythema dose before and after supplementation.

Group	Baseline (J/cm^2)	Week 12 (J/cm^2)	Change from baseline (ΔMED; J/cm^2)	p-value for change from baseline	p-value for changes between groups
Test group	0.529 (0.503–0.555)	0.620 (0.594–0.647)	0.092	<0.001	<0.001
Placebo group	0.587 (0.561–0.613)	0.565 (0.539–0.592)	−0.022	ns	

Note: Numbers in parentheses are confidence intervals (CI) of adjusted means; ns - nonsignificant.

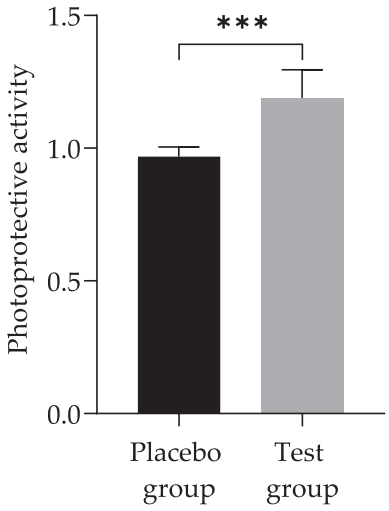

Fig. 2. Photoprotective activity of lutein (test group) in comparison to placebo group (after 12 weeks); $p < 0.001$.

The starting level of VE in the placebo group was 2.0 MPa (95% CI: 1.8, 2.2) and 1.6 MPa (95% CI: 1.4, 1.8) in the test group, and it remained without significant change in both groups until the end of the study (2.0 MPa (95% CI: 1.8, 2.2) and 1.7 MPa (95% CI: 1.5, 1.9), respectively; $p > 0.05$ for both groups).

4. Discussion

Our double-blind placebo-controlled study showed that daily dietary supplementation with lutein (20 mg/day, liquid formulation) improves photoprotective activity of the skin, as the MED that defines the amount of UVB irradiation needed to induce visible erythema on skin was significantly increased in the test group after the intervention. Higher MED indicates greater individual resistance to the production of erythema following UV irradiation. The overall treatment effect was 0.114 J/cm^2, corresponding to a relative increase of photoprotective activity of 22.2%. The observed mean increase of photoprotective activity is lower than in a previous study by Palombo et al. (2007), where supplementation with 10 mg/0.6 mg of lutein/zeaxanthin combination daily over 12 weeks led to an almost 150% increase of photoprotective activity. On the other hand, the observed effect of lutein supplementation in our study is far greater than in a study by Juturu et al. using 10 mg/2 mg of lutein/zeaxanthin daily (Juturu et al., 2016). Although they observed a small increase of MED of 0.006 J/cm^2 over baseline in actives group, it was not significant when compared to the placebo. Both mentioned studies included test groups that were similar to the one used in our study regarding age. While our study included women aged 25–55 years (average 39.2 years), a study by Palombo et al. included women aged 25–50 years (average age 35.1 years) with expressed signs of premature skin aging and Juturu et al. included both female and male subjects aged 18–45 years (average 36.1), but women were in the majority and they should have mild-to-moderate dry skin.

The main underlying mechanism for lutein's photoprotective effects and ability to reduce erythema after UVR exposure are likely due to its antioxidant action and anti-inflammatory effects. Those were exploited in several other studies. For example, lutein supplementation was shown to decrease ROS generation following UVR exposure, UVB-induced inflammation, immunosuppression, epidermal hyperplasia, and formation of apoptotic (sunburn) cells (González, Astner, An, Goukassian, & Pathak, 2003; Lee et al., 2004), and partially reduced photoaging and photocarcinogenesis (Astner et al., 2007) in the skin of hairless mice. It was also shown that lutein can protect the ECM by regulation of its remodelling in dermal fibroblasts, melanoma cells, and UVR-exposed fibroblasts (Philips et al., 2007). It was also reported that lutein supplementation reduces levels of lipid peroxidation after UV irradiation in human skin (Palombo et al., 2007; Roberts et al., 2009). Protective effects of oral supplementation with lutein (10 mg daily, 12 weeks) in human skin were also studied on a molecular level in a study focusing on the expression of genes involved in solar radiation-induced skin damage such as oxidative stress, photodermatoses, and photoaging (Grether-Beck, Marini, Jaenicke, Stahl, & Krutmann, 2017). It was shown that lutein supplementation can inhibit UVA/B- and UVA1-induced upregulation of gene expression including heme-oxygenase 1 (HO1), which is an indicator of oxidative stress and intercellular adhesion molecule 1 (ICAM1), which has a role in skin inflammation. There are also several indications that lutein can support repair of radiation-damaged skin. In some *in vitro* studies, it was shown that lutein can protect the ECM by regulation of its remodelling in dermal UVR-exposed fibroblasts (Philips et al., 2007), and to inhibit UVA/B- and UVA1-induced upregulation of expression of matrix metallopeptidase 1 (MMP1), which is involved in dermal collagen breakdown and consequently in skin photoaging (Grether-Beck et al., 2017). Those effects are expected to reflect in improvement of skin physiological parameters, connected to photoaging, such as dermal density and elasticity. However, although one of the previous studies showed improved skin elasticity due to lutein supplementation (Palombo et al., 2007), in our study there was no improvement of either skin viscoelasticity or dermal density in the test group receiving lutein supplementation. Therefore, while we showed lutein's photoprotective effects, we were unable to confirm its effect on skin regeneration.

Strengths of this study include the fact, that it was conducted with lutein – without other components, which could interfere with the results. This is particularly important because many previous studies investigated effects of combinations of different antioxidants on UV-radiation-induced erythema. Another strength of this intervention study was that effects were studied using different skin parameters, and that we had a comparison with a placebo. However, we also need to mention some limitations. While we were not able to observe significant effects of the intervention to skin viscoelasticity and dermal density, we should note that with consideration of cycle of the skin regeneration, longer observation period could improve detection of changes in skin structure and might therefore result in different magnitude of the effects. Another limitation is that we did not measure intake of lutein with foods. However, it should be noted that daily lutein dosage used in this study was for several factors higher than typical dietary lutein intake. The effect of dietary lutein intake was also minimised with study inclusion criteria, for example exclusion of vegans, which could have higher lutein intake due to higher vegetable intake. To better explain some inter-individual differences (i.e. those related with differences in absorption efficiency) it would be also useful to measure serum lutein levels during conduction of the study, but this would considerably increase invasiveness and could reduce compliance rates. Furthermore, due to complex metabolic processes measurement of plasma lutein metabolites is challenging. However, study compliance was checked by other means, particularly with a compliance diary of test product intake for the whole 12-week intervention period, and verification of returned used/unused test products.

5. Conclusion

In conclusion, findings of our double-blind placebo-controlled human intervention study with lutein supplementation confirmed its ability to improve skin photoprotective potential. Although the extent of protection achieved that way is not comparable to the use of high sun protection factor sunscreens, a systemic increase of basal protection could contribute to skin defense against UVR-mediated skin damage.

6. Ethics statement

The study was in full compliance with the principles laid out in the

Declaration of Helsinki. The study protocol was approved by the Slovenian National Medical Ethics Committee (Ministry of Health, Republic of Slovenia), identification number KME 0120-63/2018 (approval letter ID 0120-63/2018/4, date of approval: 20 February 2018) and was registered at ClinicalTrials.gov (ID: NCT03811977). The study was performed in compliance with the requirements of local authorities. Before participation in the study, all subjects signed a written informed consent form (ICF).

CRediT authorship contribution statement

Katja Žmitek: Conceptualization, Methodology, Formal analysis, Investigation, Project administration, Writing - original draft, Writing - review & editing. **Janko Žmitek:** Conceptualization, Funding acquisition, Project administration, Supervision. **Mirjam Rogl Butina:** Conceptualization, Writing - review & editing. **Hristo Hristov:** Formal analysis, Writing - review & editing. **Tina Pogačnik:** Conceptualization, Methodology, Investigation. **Igor Pravst:** Funding acquisition, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2020.104265.

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