Modulating effects of oral administration of Lycii Fructus extracts on UVB-induced skin erythema: A Randomized, placebo-controlled study

MAKOTO TSUNENAGA[1], WENSI XU[2,3], TAKUMI MYOJIN[2], TOSHIYUKI NAKAMURA[2], TATSUYA KON[1], YOSHIMASA NAKAMURA[2] and OSAMU UEDA[1]

[1]Shiseido Co., Ltd. MIRAI Technology Institute, Frontier Business Research and Development Center, Yokohama, Kanagawa 220-0011; [2]Graduate School of Environmental and Life Science, Okayama University, Okayama 700-8530 Japan; [3]College of Life and Environmental Sciences, Hunan University of Arts and Science, Changde, Hunan 415000, P.R. China

Received February 1, 2022; Accepted May 10, 2022

DOI: 10.3892/br.2022.1545

Abstract. Severe UV exposure induces skin inflammation, causing erythema. Lycii Fructus (*Lycium barbarum* and *Lycium chinense*) is a potential antioxidant agent with a high content of polyphenols, including rutin and chlorogenic acid. This study examined the effects of Lycii Fructus extract (LFE) on UVB-induced skin erythema in humans. Healthy volunteers were randomly assigned to one of two groups and received UVB irradiation at 1.5 minimal erythemal dose (MED) on day 0 at three designated sites on their backs, and the skin color was measured until day 7. After an 8-week treatment with LFE (900 mg/day) or placebo, UVB irradiation (1.5 MED) was applied again at different sites on day 63. Skin color was continuously measured in each group until day 69. LFE tablet administration for 8 weeks significantly inhibited UVB-induced erythema formation and increased the MED by 13%. Erythema formation peaked on the first day after UVB irradiation, but gradually dissipated over the next several days. LFE tended to accelerate erythema disappearance. To determine the polyphenol responsible for the protection against UVB-induced skin damage, the effects of LFE-derived polyphenols and their metabolites on UVB-induced cytotoxicity were examined *in vitro*. The major intestinal metabolite of rutin and LFE significantly attenuated phototoxicity and in human keratinocyte HaCaT cells. Quercetin enhanced intracellular glutathione levels in HaCaT cells, even though LFE did not increase it. Together, the results showed that LFE inhibited erythema formation and accelerated erythema dissipation, possibly through its direct antioxidative action.

Correspondence to: Dr Osamu Ueda, Shiseido Co., Ltd. MIRAI Technology Institute, Frontier Business Research and Development Center, 1-2-11 Takashima, Nishi-ku, Yokohama, Kanagawa 220-0011, Japan
E-mail: osamu.ueda@shiseido.com

Key words: UV-induced skin damage, Lycium Fructus extract, erythema, glutathione, polyphenols

Introduction

The skin is the largest organ, protecting the body from external stimuli and preventing foreign matter from entering the body. Sunlight is necessary for human life, but certain components of sunlight can damage the skin. According to Halliday *et al* (1) exposure to even low doses of sunlight during everyday activities can suppress immunity. UV irradiation is known to cause inflammation and thus increases the risk of skin cancer with an enhanced frequency of mutations (2-4). For example, according to Martincorena *et al* (5), a substantial number of somatic mutations (from 2-6 mutations per megabase per cell) were observed in the sun-exposed epidermis (5). This mutational frequency is similar to that seen in several types of cancer and reflects the characteristic damage caused by UV exposure (5). Studies of perceived age, a valid biomarker of systematic aging (6,7), suggest that exposure to UV can expedite the aging process (8,9). Sunscreens can protect the skin from UV-facilitated aging (10). Fatty acids are also promising for photoprotection according to a previous report. showing that the topical application of eicosapentaenoic acid inhibited UV-induced epidermal damage (11). Although sunscreens are effective in protecting the skin against UV-induced aging (11), they fail to protect against sub-erythemal UV exposure, resulting in severe DNA damage when applied unevenly or insufficiently around the eyes, where topical application is awkward (12).

UV exposure can cause DNA damage, either directly or via active oxygen, to induce an inflammatory reaction (sunburn) and prompt the release of inflammatory mediators (13). DNA damage also induces apoptosis - a process of regulated cell death (14). The UV-induced damage also leads to oxidative stress, but it complementarily activates nuclear factor erythroid 2-related factor 2 (Nrf2), thus inducing Phase-II drug-metabolizing enzymes (15). The expression of γ-glutamylcysteine synthetase, the rate-limiting enzyme in the production of

reduced glutathione, is mediated by Nrf2 expression (15). It has been highlighted that certain food components influence the inflammasome possibly through Nrf2-related enzymes (16).

Previous studies suggested that antioxidant supplementation (such as vitamins C and E) could help combat reactive oxygen species (17,18), and augment the protective effects of sunscreens (19). Other effective treatments against UV damage include green tea, coffee, fruit, and other products rich in antioxidants (20-22); some carotenoids have been reported to show similar effects (17,23,24). A systematic review of Lycii Fructus, namely goji berries (*Lycium barbarum* and *Lycium chinense*), found that these plants have been used as a folk medicine and traditional foods for centuries (25). The body of research demonstrated that Lycii Fructus is effective for enhancing kidney and liver function, protecting ocular health, and boosting immunity (25). Lycii Fructus is rich in polysaccharides, water-soluble vitamins, carotenoids, and polyphenols such as flavonoid glycosides and phenolic acids (26). As noted in a review by Ulbricht *et al* (25), some of these components exhibit antioxidative activity and are able to inhibit UVB-induced cell death (25). This view is supported by Amagase and Farnsworth (26), who examined the effects of goji berries on a man who had a pruriginous eruption on a sun-exposed area of skin (27). This study demonstrated that the man's minimal erythema dose for UVB was decreased following intake of the goji berries (27). A study using rodents also showed that orally consumed goji berry juice inhibited UV damage in mice (28). In a study on human participants, Kuwazuru *et al* (29) examined the effects of supplementation containing goji berries or their ethanol extracts (29). The results indicated that the supplementation was effective in inhibiting UV-induced erythema formation. However, the study was limited by its small size, and the trial was not randomized.

In the present randomized, double-blind, placebo-controlled clinical trial study, we examined the effects of LFE on UV-induced epidermal damage to confirm whether LFE is effective for inhibiting erythema formation. Considering previous reports, 44 participants were recruited and 22 participants were randomized to each treatment (17,21-24). We also investigated the possibility that LFE exerts antioxidative effects *in vivo* as LFE has been reported to facilitate cytoprotective gene expression and enhance the production of glutathione in hepatocytes (30). To elucidate which polyphenols participate in protection against UVB-induced skin damage, we examined the effects of the LFE components on antioxidative capacity after human keratinocytes were directly exposed to UV.

Patients and methods

Study design. The present study was a randomized, placebo-controlled, double-blinded study, with a parallel group test conducted by Derma Labo, Inc. This trial conformed to the Declaration of Helsinki (31) and was approved by the Ethics Committee of Tactics (Hokkaido Activation Center) on December 14, 2016 (approval no. 2016-100). Written informed consent was obtained from all potential participants prior to participant selection. Fig. 1 shows the test schedule. The test was conducted between January 16 and May 1, 2017.

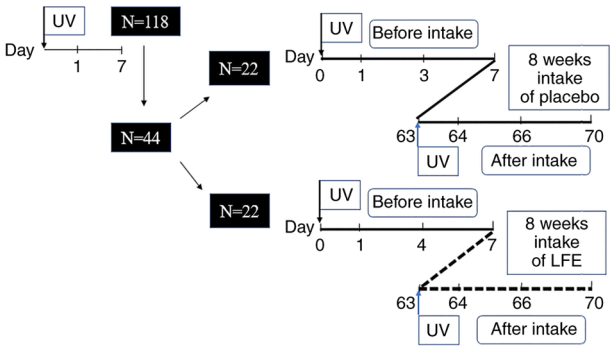

Figure 1. Test schedule. Subjects were randomly assigned to one of two groups. Both groups received 1.5 MED UV radiation on day 0 at three designated sites on their backs, and measurements were performed until day 7. The placebo group (upper) and the LFE group (lower) ingested placebo (solid line) or LFE (dotted line), respectively, for 8 weeks. Subsequently, UV irradiation at 1.5 MED was applied again at different sites on day 63 and measurements were performed until day 70 with continued placebo or LFE ingestion. Further, the subjects were irradiated with UV of a predetermined intensity, which was the same as irradiation at the time of screening on day 63 and the MED of each subject was determined again. MED, Minimal Erythema Dose; LFE, Lycii Fructus extract.

The individual in charge of test materials allocation performed random allocation, such that the primary background factors were not biased amongst each group. The allocation of the test materials was performed based on an allocation table (key code) prepared from a random number table in advance by the individual in charge of the test materials allocation. The allocation table was sealed by the person in charge of test materials allocation and kept secret until opening. After confirming that all data was fixed, the individual in charge of test materials' allocation opened the key after confirming the sealing status of the allocation table and emergency key code. The individual in charge of test materials allocation submitted the allocation table after opening the key and created a record.

Participants. The purpose of the study was to clarify the effects of LFE ingestion on UVB-induced skin erythema. After screening, participants were recruited from among 118 individuals in the Exam's volunteer bank, which was managed by Derma Labo. Inc, who agreed to participate. Participants were Japanese nationals aged between 20 and 60 years of age.

Individuals were excluded from the study if any of the following circumstances applied: i) Pigmentation, inflammation, or other significant reactions were present at the skin site; ii) the participant had a history of photo sensitivity or presented symptoms of such; iii) the participant was suspected to be allergic to the test materials (for example, the participant had experienced allergic reactions to the applied food in the past); iv) sunburn had occurred in the skin site during the 3 months preceding the study; v) they had chronic skin conditions (such as atopic dermatitis) present at the skin site; vi) the participant was pregnant (or planned to get pregnant during the study) or lactating; vii) the participant had asthma or a similar chronic condition and regularly took medication for the condition; viii) the participant was taking medication that may affect the test results, including medication for freckles, UV-induced pigmentation, or liver spots (e.g., drugs containing L-cysteine,

vitamin C, or tranexamic acid); ix) during the 3 months preceding the study, the participant was regularly taking (>5 days a week) skin whitening supplements or other nutritional supplement with claims of antioxidant properties (e.g., supplements rich in catechins, flavonoids, or polyphenols); x) the participant was currently undergoing, or had undergone during the past three months, a trial that involved the ingestion of food or drugs, the use of cosmetics, or something similar; xi) the participant was a regular smoker; xii) the participant had applied topical medication to the skin site during the past week; xiii) the participant intended to travel abroad or to swim in the sea during the study period (between screening and end of the study); xiv) the participant was undergoing hormone replacement therapy following the start of menopause or a menopausal disorder; and/or xv) the participant was otherwise deemed ineligible for the study by the physician-in-charge or principal investigator.

During the study, participants were required to adhere to the following rules and prohibitions: i) Participants were not allowed to apply topical medication to the skin site, take medication for freckles, UV-induced pigmentation, or liver spots (e.g., drugs containing L-cysteine, vitamin C, or tranexamic acid), or use non-medical drugs (officially known in Japan as 'quasi-drugs') or cosmetics. Participants were permitted to use personal hygiene products such as soap and shower gel, but they were not allowed to switch these products during the study period. If participants unavoidably needed to use the above medication, quasi-drugs, or cosmetics, they were instructed to report the product name, volume used, period used, and reason for use in a journal; ii) participants were not allowed to take medication (or apply topical medication) or take or apply newly designated 'quasi-drugs' or traditional Chinese medicines; iii) participants were not allowed to ingest newly designated health foods or foods carrying health or nutritional claims (officially known in Japan as 'food for specified health uses' or 'food with function claims'). If participants unavoidably needed to eat such foods, they were instructed to either report the product name, ingredients, and volume consumed in their journal or to affix the label (or a copy thereof) to their records. Participants were also instructed to report the name and amount consumed of any health food, 'food for specified health uses,' or 'food with function claims' that they were regularly consuming; iv) For a 2-week period preceding the study, participants had to refrain from any activity that could cause sunburn, such as participating in outdoor sports or using a tanning bed. Participants were asked to take steps to avoid UV exposure altogether (from both indoor and outdoor sources). They were instructed, for example, to wear sun-protective hats and clothing and to use sunscreen. If participants nonetheless suffered sunburn, they were instructed to report the time, duration of exposure, and severity (e.g., 'skin red, feels sore') in their journal; v) on the evening prior to a test day, participants were required to bathe or shower before going to bed. They were not allowed to bathe or shower before testing on the test day; vi) on a test day, participants were not allowed to engage in intense exercise until the test was over; vii) on a test day, participants were not allowed to consume any spicy food or drink such as curry, chilies, or hot sauce (e.g., Tabasco); vii) participants were not allowed to scrub the skin site with abrasive personal hygiene products; ix) for a 1-month period preceding the study, participants were not allowed to undergo beauty treatments such as a chemical peel or spa treatment; and x) participants were not allowed to start any regular supplement regimens.

Table I. Composition of LFE and placebo tablets.

Component	LFE tablets, mg/6 tablets[a]	Placebo tablets, mg/6 tablets[a]
LFE	900	-
Microcrystalline cellulose	966	933
Carmellose calcium	36	36
Calcium stearate	18	18
White potato	-	933
Total	1920	1920

[a]Each subject took 6 tablets per day. LFE, Lycii Fructus extract.

Test materials. The supplement used in this study was a tablet containing LFE, which was provided by Matsuura Yakugyo Co., Ltd., and consisted of microcrystalline cellulose, carmellose calcium, and calcium stearate. This supplement was comparable to a placebo containing the same components, except that the LFE was substituted with potato starch (Table I). A total of six LFE tablets (the daily dose) contained 900 mg LFE; the amount of rutin and chlorogenic acid contained in the extract of LFE in this human study was 926 and 876 μg/day, respectively, with 966 mg microcrystalline cellulose, 36 mg carmellose calcium, and 18 mg calcium stearate. A total of 6 placebo tablets contained 933 mg microcrystalline cellulose, 36 mg carmellose calcium, 18 mg calcium stearate, and 933 mg potato starch. The LFE and placebo tablets were supplied by Shiseido Pharmaceutical. The tablets were administered orally at a rate of six per day for 8 weeks.

Screening. The candidates reported their skin type with reference to the Fitzpatrick scale (32). Those with Type I (always burns, never tans) and Type II (usually burns, tans minimally) were selected for screening. Solar Light's Model 601 Multiport solar simulator was used to produce six stages of erythema on the selected candidates' backs at increments of ~20% (11.4, 15.0, 19.2, 21.9, 27.6, and 30.6 mJ/cm^2). Each irradiated surface area was 0.5 cm^2 (Φ=8 mm). UV irradiance was measured using a Solar Light's PMA2100 radiometer and PMA2108 biologically weighted UV-B detector. The following day, the irradiated areas were examined to determine each participant's MED. Minimal tanning dose (MTD) was determined on the 7th day following irradiation. Based on the MED and MTD results, 44 candidates were selected for participation in the study. These candidates were randomly assigned to be divided into two groups to avoid bias in major background factors among the groups. Allocation of test materials was carried out based on the allocation table prepared in advance from the random number table. The assignment table was sealed by the person in charge of the trial food assignment and was strictly stored until opening.

Other screening processes included a lifestyle survey [with items on medical history, present symptoms, lifestyle,

medication, use of cosmetics and supplements, and physical characteristics such as height (cm), weight (kg), BMI, systolic blood pressure (mmHg), diastolic blood pressure (mmHg), and pulse (beats/min)], a general biochemical blood test, a blood test for *in vitro* antioxidant potential test, and a questionnaire about diet (brief-type self-administered diet history questionnaire) (33).

Induction of erythema and measurement of skin color. Of the candidates judged eligible to participate by the physician-in-charge, 44 individuals were finally selected. All the candidates were highly UV-sensitive (8 men, 36 women; median age was 50 years old; age range 22 to 59 years).

During the study, MED was determined as follows. Before and after an 8-week course of the active agent or placebo (intervention), the skin sites were exposed to six stages of irradiation as in the screening. A total of 24 h after irradiation, the pigmentation in the testing sites was measured, and the pigmentation after intervention was compared with that before intervention.

Three skin sites on the back were irradiated. The irradiated UV intensity was 1.5x higher than that of the participant's MED. The participants were divided into two groups: An experimental (LFE) group and control (placebo) group. To control for MED differences, the participants were assigned to groups such that the groups had a similar average MED. Before intervention, pigmentation was measured four times: Before irradiation, and on days 1, 3, and 7 after irradiation. The participants then took their tablets for 8 weeks; those in the LFE group took the supplement containing LFE and those in the placebo group took the placebo. After the 8-week intervention, the participants underwent irradiation with the UV intensity at 1.5x their MED. As before, pigmentation was measured before irradiation, and on days 1, 3, and 7 after irradiation (Fig. 1).

The devices used to measure skin pigmentation were a Minolta CR-200 Chromameter (Konica Minolta), a Mexameter MX 16 (Courage + Khazaka Electric GmbH), and a C-Cube (Pixience SA) (34). The measurements were conducted in a room with a constant temperature (23±2˚C) and humidity (45±5%), and the participants were given 20 mins to acclimatize themselves to the room before measurement. Two skin hydration values were measured: Stratum corneum hydration (SCH) and transepidermal water loss (TEWL). The former was measured using a Corneometer CM825 (Courage + Khazaka Electric GmbH). Each irradiated site was measured five times and the average SCH was determined as arbitrary units (AU). Non-irradiated sites were measured as well to provide a control value.

The latter value, TEWL, was measured using a Tewameter TM300 (Courage + Khazaka Electric GmbH). The TM300 was placed on the target skin site for 15 secs and then activated for 20 secs. Five readings were taken after 10 secs following activation, and the average (where the deviation was least) was defined as the TEWL (g/hm^2).

Analyses of antioxidants and oxidative markers in serum. Blood samples were taken just before trial and at 8 weeks. In each case, the samples were taken before irradiation by a nurse under a physician's supervision. The blood sample was used to determine the *in vivo* antioxidative capacity. Each sample was collected using a vacuum blood collection tube with a separating agent. The blood was centrifugally separated for 15 min at 2,000 x g at room temperature. The separated blood was then stored at -30˚C before measurement. Antioxidant volume was used as a measure of antioxidative capacity. The blood samples were sent to Hoken Kagaku's Sapporo laboratory for biochemical and hematological analysis. Four markers of in-serum antioxidative capacity were used. The first was total glutathione disulfide (GSH+GSSG), measured using an OxiSelect total glutathione assay kit (cat. no. STA-312). The second was carbonylated protein, measured using an OxiSelect protein carbonyl ELSIA kit (cat. no. STA-310-T). The third was lipid peroxides, measured using an OxiSelect 8-iso-prostaglandin F2 α Elisa kit (cat. no. STA-337). These three OxiSelect devices were provided by Cell Biolabs. The fourth marker was 8-hydroxy-2-deoxyguanosine (8-OHdG), measured using a highly sensitive 8-OHdG check ELISA kit (cat. no. KOG-HS10E) available on the market by the Japan Institute for the Control of Aging (Nikken Seil Co. Ltd.).

Daily diet. The participants' daily diet was ascertained using the brief-type self-administered diet history questionnaire (BDHQ). The results were analyzed by the survey provider's support center (the 'DHQ Support Center').

Analysis of antioxidative effect on keratinocytes. An immortalized human keratinocyte cell line (HaCaT) was used to analyze the antioxidative effect. HaCaT cells were obtained from Deutsches Krebsforschungszentrum with a material transfer agreement. The HaCaT cells were cultured using DMEM (Thermo Fisher Scientific Inc.) supplemented with 10% FBS (Thermo Fisher Scientific Inc.) and antibiotics (50 units/ml penicillin and 50 μg/ml streptomycin, both provided on the market by Fujifilm Wako Pure Chemical Industries). Alongside LFE, two reagents were used on the HaCaT cells: Chlorogenic acid and quercetin, both of which were provided by Sigma-Aldrich; Merck KGaA. To obtain the LFE, hydrous ethanol was dehydrated, and the remnants were dissolved in DMSO (final concentration 0.1% (v/v). The protective effect of LFE was measured in terms of cell viability after exposure to UVB. The HaCaT cells were treated with the reagent for 24 h 1 day after pre-culture. Then, the culture medium was replaced with Hank's Balanced Salt Solution (Thermo Fisher Scientific Inc.) and exposed to 50 mJ/cm^2 UVB. The cells were incubated for an additional 24 h after which the cell viability was determined using an MTT (FUJIFILM Wako) assay.

Glutathione (GSH), a marker of intracellular antioxidative capacity, was measured using the DTNB method (Total Glutathione Quantification Kit, Dojindo Molecular Technologies, Inc.). A total of 24 h after inoculation, the cells were treated with LFE for 24 h. Cells were then collected, and the intracellular GSH was measured.

Statistical analysis. Statistical analysis was performed using Microsoft Excel 2013 (Microsoft Corporation). A paired Student's t-test was used for intragroup comparisons. Multivariate regression analysis was used to compare changes in MED between the two groups. Covariance analysis was used for erythema time series results, including for time point

comparisons. An unpaired Student's t-test was used for the corresponding time points. All values are presented as the mean ± SD. P<0.05 was considered to indicate a statistically significant difference.

UMIN registration. This study was registered at the University Hospital Medical Information Network (registration no. UMIN000025593).

Results

Participants' age, MED, and BMI. All 44 healthy volunteers selected in the screening process participated in this study. However, six participants were subsequently discontinued; four were voluntarily withdrawn, one was excluded after taking medication, and another was excluded after the MED results indicated a concerning reaction to UV exposure. The final trial consisted of 38 remaining participants, 20 of whom were in the placebo group and 18 of whom were in the experimental group. There were no significant inter-group differences in these items (the placebo group's average MED was 17.5±4.1 mJ/cm^2, and the LFE group's was 18.2±3.8 mJ/cm^2; the placebo group's average BMI was 22.5±3.1 kg/m^2, and the LFE group's was 22.6±3.5 kg/m^2). There were no harmful effects of test material based on general biochemical blood tests.

Change in MED following LFE administration. Table II shows the placebo and LFE groups' MED values at week 0 (pre-intervention) and week 8 (post-intervention), as well as the change between the two-time points. The LFE group's average MED was significantly higher post-intervention (P=0.002). The week 8 figure was 20.6±3.9 mJ/cm^2, which marks a 13% increase from the week 0 figure of 18.2±3.8 mJ/cm^2. There was no significant change in the placebo group's MED (P=0.38). The week 0 figure was 17.5±4.1 mJ/cm^2 and the week 8 figure was 18.0±5.1 mJ/cm^2. The degree of change significantly differed between the groups (P=0.02). In the LFE group, MED changed by 2.4±2.6 mJ/cm^2, while in the placebo group, it only changed by 0.5±3.5 mJ/cm^2.

Changes in erythema measurements. This section outlines the results for the erythemas that formed at the three sites on the participants after exposure to UV at 1.5x their MED. The data included 20 placebo participants and the 18 remaining participants in the LFE group. Table III shows the changes in pigmentation over the four time points before intervention (Day 0, Day 1, Day 3, and Day 7) and after intervention (Day 63, Day 64, Day 66, and Day 70). The a* values indicate CR-200 readings, while 'melanin index' indicates MX 16 readings. Table IV shows the changes in erythema index as measured by the C-Cube. Fig. 2 shows the changes before and after intervention. In Tables IV and V, values in parentheses indicate change relative to the pre-irradiation figure (Day 0/Day 63), which was scaled at 100.

As shown in Table III, the a* value peaked at the first day (Day 1/Day 64) and declined over the next two time points. Conversely, the melanin index rose across the four time points. The LFE group did not differ significantly from the placebo group in terms of the score differences for either of the two indices before and after intervention.

Table II. Change in MED by the intake of LFE.

	MED, mJ/cm^2 (%)[b]		
Group	0 week	8 week	Δ8 weeks
Placebo	17.5±4.1 (100)	18.0±5.1 (102.9)	0.5±3.5
LFE	18.2±3.8 (100)	18.2±3.8 (113.2)	2.4±2.6[a]

[a]P<0.01 (using a paired t-test). Data are presented as the mean ± SD. Values in parenthesis are percentage values relative to 0-week. LFE, Lycii Fructus extract; MED, Minimal Erythema Dose.

As shown in Table IV, the C-Cube-measured erythema index rose between Day 0/Day 63 and Day 1/Day 64 before declining. Before intervention, the LFE group's results for the C-Cube-measured erythema index did not differ significantly from that of the placebo group (P=0.72). After intervention however, the LFE group's results for the C-Cube-measured erythema index differed significantly from that of the placebo group (P=0.001). On Days 66 and 70 (the 4th and 7th days after irradiation), the placebo group's values were significantly higher than that of the LFE group. In addition, UV exposure also affected SCH and TEWL, but the LFE group's post-intervention results did not significantly differ from that of the placebo group after 8 weeks of LFE ingestion (data not shown).

Evaluation of antioxidative capacity in the serum. Table V shows the results for the four markers of in-serum antioxidative capacity. Total GSSG increased in both groups after intervention, but not significantly. In the placebo group, total GSH was 3.09±1.67 nM at pre-intervention and 3.27±1.44 nM at post intervention; in the LFE group, it was 3.04±1.68 nM at pre-intervention and 4.17±3.38 nM at post intervention. While 8-OHdH in serum were significantly suppressed after intervention in both the Placebo and LFE groups, there were no differences between the two groups. Carbonylated protein measurements showed no differences before or after intervention, or between groups. Carbonylated protein levels declined following intervention in both groups, but the decline was not significant. The remaining two markers, lipid peroxides and 8-iso-pro F2 alpha, were significantly lower after intervention, but there was no significant intergroup difference.

Protection against UV-induced oxidative stress in HaCaT cells. The HaCaT, a spontaneously transformed human keratinocyte cell line derived from the epidermis was used to determine the protective effects of LFE as well as chlorogenic acid (a major component of LFE) and quercetin (an intestinal metabolite of rutin, another major component of LFE). Pretreatment with LFE for 24 h exhibited a significant protective effect on the UVB-induced cytotoxicity at a concentration of 50 μg/ml, but this effect decreased at 1,000 μg/ml (Fig. 3A). Similarly, chlorogenic acid showed a significant effect at 5 μM, but this effect was absent at 100 μM (Fig. 3B). Quercetin also exhibited an inhibitory effect from 0.5-5 μM (Fig. 3C). The pretreatment of LFE, chlorogenic acid or quercetin significantly inhibited the hydrogen peroxide-induced cytotoxicity (data not shown).

Table III. Changes in the *a value and melanin index[a].

A, *a value

	Before ingestion				After ingestion			
Group	Day 0	Day 1	Day 4	Day 7	Day 63	Day 64	Day 66	Day 70
Placebo								
Value	5.24±1.22	7.01±1.27	5.85±1.11	5.32±1.13	5.37±1.29	7.59±1.39	6.75±1.39	6.44±1.38
Relative difference, %[b]	100	137.05±22.19	113.45±11.95	102.37±8.17	100	144.48±22.49	127.11±12.62	121.06±12.49
LFE								
Value	4.81±1.56	6.50±1.37	5.41±1.48	4.94±1.53	5.01±1.33	7.13±1.52	6.21±1.30	5.88±1.24
Relative difference, %[b]	100	143.39±37.88	115.39±17.91	104.58±14.46	100	144.93±19.90	126.23±17.35	119.78±14.35

B, Melanin index

	Before ingestion				After ingestion			
Group	Day 0	Day 1	Day 4	Day 7	Day 63	Day 64	Day 66	Day 70
Placebo								
Value	92.67±33.94	94.28±31.29	100.66±35.93	98.80±33.80	82.03±39.83	84.79±38.08	90.35±43.23	87.07±33.80
Relative difference, %[b]	100	103.76±12.29	110.14±10.23	108.00±14.43	100	102.26±16.18	111.33±10.65	112.67±18.73
LFE								
Value	82.96±41.79	83.82±41.06	86.76±41.21	88.33±37.64	65.83±36.68	69.29±36.09	74.14±35.26	72.12±34.59
Relative difference, %[b]	100	101.44±6.90	108.09±19.06	114.67±35.95	100	107.47±19.90	118.39±25.54	112.85±16.30

[a]Placebo, n=20; LFE, n=18; one patient withdrew from the LFE group. [b]From Day 0.

Table IV. Changes in Erythema index.[a]

	Before ingestion				After ingestion			
Erythema index	Day 0	Day 1	Day 4	Day 7	Day 63	Day 64	Day 66	Day 70
Placebo								
Value	45.69±4.10	50.22±4.47	48.75±4.48	47.51±4.24	45.16±5.25	54.83±4.90	49.56±5.21	47.83±5.23
Relative difference, %[b]	100	110.05±7.30	106.82±4.99	104.12±4.99	100	122.07±9.06	110.05±6.27	106.12±5.20
LFE								
Value	44.63±5.57	49.90±6.41	47.06±5.78	47.04±5.81	45.99±5.81	53.81±6.91	48.49±5.49	45.90±5.14
Relative difference, %[b]	100	112.12±8.90	105.62±5.32	105.58±5.65	100	117.27±8.90	105.78±6.52	100.16±6.56

[a]Placebo, n=20; LFE, n=18; one patient withdrew from the LFE group. [b]From Day 0.

Thus, chlorogenic acid and quercetin as well as LFE are effective for inhibiting cell damage resulting from UV exposure, possibly through their antioxidative effects.

Fig. 4 shows the effect of LFE upon intracellular total GSH, which was determined using the DTNB method. Quercetin increased total GSH at concentrations of 2.5 and 10 μM, LFE and chlorogenic acid showed no significant effect for increasing total GSH in HaCaT cells, even though LFE has been reported to enhance the cellular GSH level in hepatocytes (30).

In the BDHQ survey, we asked the participants how much tea and coffee they consumed. The responses indicated that participants, who consumed plenty of tea or coffee prior to the start of the test, tended to frequently have these beverages in the second half of the study (at 8 weeks). Based on this, we assumed that the increasing response in serum total GSH levels comes from the participants who consumed low amounts of tea or coffee per day. There were 10 participants in the LFE group who consumed <220 g of tea or coffee a day and 12 such participants in the placebo group (total GSH: from 2.7±1.6 to 4.7±3.2 nM). The increase in serum GSH in the 10 LFE participants (total GSH: from 2.7±1.7 to 3.2±1.4 nM) was not significantly greater than in the placebo group (P=0.11). It would be necessary to control tea/coffee consumption to confirm the cause-effect relationship.

Discussion

When an erythema forms on the human skin following exposure to UV, melanin production and deposition causes the skin to darken (35). The damaging effects of UV irradiation are significant not only in the epidermis, but also in the hypodermis, resulting in inhibition of an immune response and acceleration of skin aging (36). In addition to sunscreens, consumption of certain nutrients can protect the skin from UV irradiation. Green tea catechins, carotenoids in vegetables and fruits, lycopene in tomatoes and collagen peptides have been reported to inhibit erythema formation (20,37-39). Similarly, Kuwazuru *et al* (29) and Gomez-Bernal *et al* (27) suggested that LFE has the potential to inhibit UV-induced damage. However, neither study conclusively confirmed this effect.

The present study examined the effects of an 8-week supplementation of LFE in Japanese men and women aged 20-60. The protective effects were determined by measuring the participants' MED and the change-over-time in erythema formation after exposure to UV irradiation at 1.5x the MED. The study adopted a placebo-controlled, double-blind design, and participants took either the LFE supplement or a placebo at 900 mg a day for 8 weeks. As shown in Table II, the LFE group's average MED was increased significantly by 13%. MED increased in the placebo group too, although this increase was not significant. Moreover, the increased MED in the LFE group was significant in comparison to that of the placebo group. As shown in Table III, the LFE intervention did not significantly affect the formation or change-over-time of erythema as measured by a* values (CR-200) or melanin index (MX 16). However, the supplementation did significantly affect such according to the C-Cube-measured erythema index (Table IV). Erythema formation in the LFE group was significantly lower than that in the placebo group on the day after irradiation (day 64); moreover, on subsequent days, the

Table V. Composition of LFE tablets and placebo tablets.

	LFE, n=19		Placebo, n=20	
Marker	Before	After	Before	Before
Total GSH, nM	3.04±1.68	4.17±3.38	3.09±1.67	3.09±1.67
8-OHdG, ng/ml	2.75±1.83	2.25±1.67[a]	3.50±3.18	3.50±3.18
Carbonylated protein, ng/ml	0.316±0.07	0.297±0.04	0.302±0.07	0.302±0.07
8-iso-PRO F2α, nmol/ml	81.72±40.92	36.58±31.91[a]	104.51±62.75	104.51±62.75

[a]P<0.05 vs. Before. LFE, Lycii Fructus extract. Antioxidant measurements was performed on serum collected on days 0 and 63.

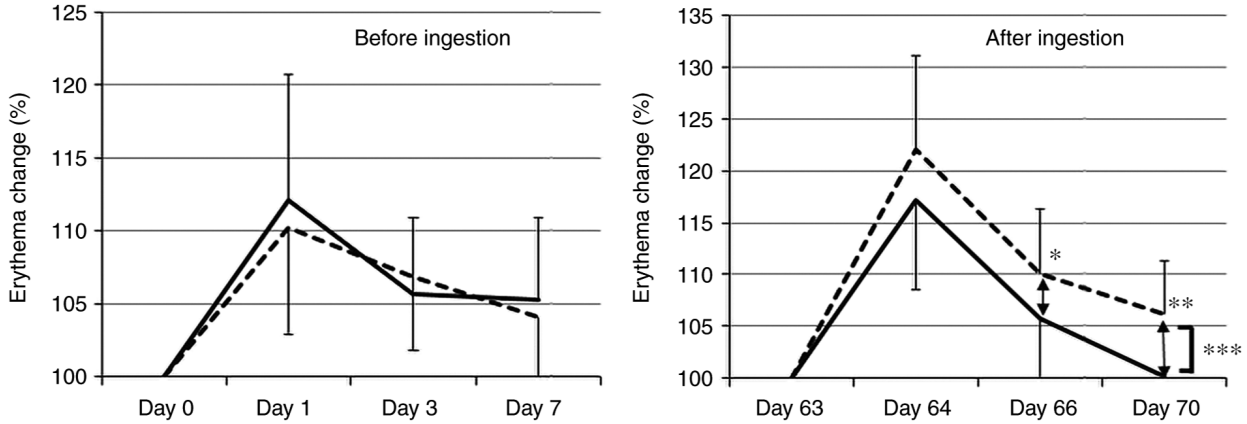

Figure 2. Erythema formation and disappearance following UV irradiation. The solid line indicates the LFE group and the dashed line indicates the placebo group. Before intervention, changes in erythema did not differ significantly between the LFE and placebo groups. According to the covariance analysis, after intervention, erythema disappeared earlier in the LFE group than they did in the placebo group, which was not the case before intervention. Based on the unpaired t-test, at day 66 and day 70, erythema in the LFE group was significantly lower than the placebo group. *P<0.05, **P<0.01 (Student's t-test); ***P<0.01 (covariance analysis). LFE, Lycii Fructus extract.

erythemas disappeared significantly faster in the LFE group compared with the placebo group (Table IV and Fig. 2). The reason that the C-Cube detected a significant difference where the other measures did not, may be due to the fact that the C-Cube measurement relies on a relatively large surface area (44.6 mm^2), resulting in greater detection sensitivity.

A previous rodent study demonstrated that LFE consumption inhibited UV-induced damage and suggested that LFE could have the same effect in humans (28). The same study also showed that LFE inhibited UV-induced immune responses with an upregulation in heme oxygenase-1 (HO-1) protein expression. Polysaccharides are a major active component of LFE (40). Another report showed that the polysaccharides contained in LFE protected epidermal cells from UV damage by inducing Nrf2 activity and eliminating reactive oxygen species (41). The effects of polysaccharides were also investigated in a study by Ding *et al* (42). However, that study failed to clarify whether polysaccharides are absorbed intact; if they are metabolized by the gut flora, they may be converted into an active agent (42).

Other than polysaccharides, polyphenols such as chlorogenic acid and rutin, which possess potent antioxidative effects, are abundantly present in LFE. These components may be absorbed during digestion intact directly or as metabolites, after being metabolized by gut microbiota. Previous studies suggest that chlorogenic acid is absorbed in the stomach or intestine intact as it is and as metabolites (43-45). Once absorbed, chlorogenic acid may spread to the skin via the bloodstream. A study using topically applied chlorogenic acid showed that the intradermal accumulation of chlorogenic acid inhibited UV-induced erythema (46). Quercetin is produced when rutin is metabolized by gut microbiota (47). Once produced in this manner, the aglycone is absorbed into the bloodstream (48), whereupon it may be transferred to and exert its antioxidative effects in the skin. In a study using epidermal cells, quercetin inhibited UV irradiation-induced release of certain inflammatory cytokines (49). Quercetin may protect against UV-induced cell damage as it blocks UV-induced production of reactive oxygen species and protects the mitochondria (50). Gut flora is suggested to mediate the effects of ingested photoprotective agents. For example, Gueniche *et al* (51) found that probiotic bacteria facilitated an earlier recovery following UV-induced immune response inhibition, while probiotic bacteria are associated with an increased MED (52).

Exposure to UV damages the epidermal cells. The causal factors include singlet oxygen production and DNA damage (53). Singlet oxygen produces 8-OHdG in DNA, which can be inhibited by antioxidants (54). As shown in Fig. 3, the present study demonstrated that UV-induced cytotoxicity was reduced by the pretreatment of the epidermal cells with LFE

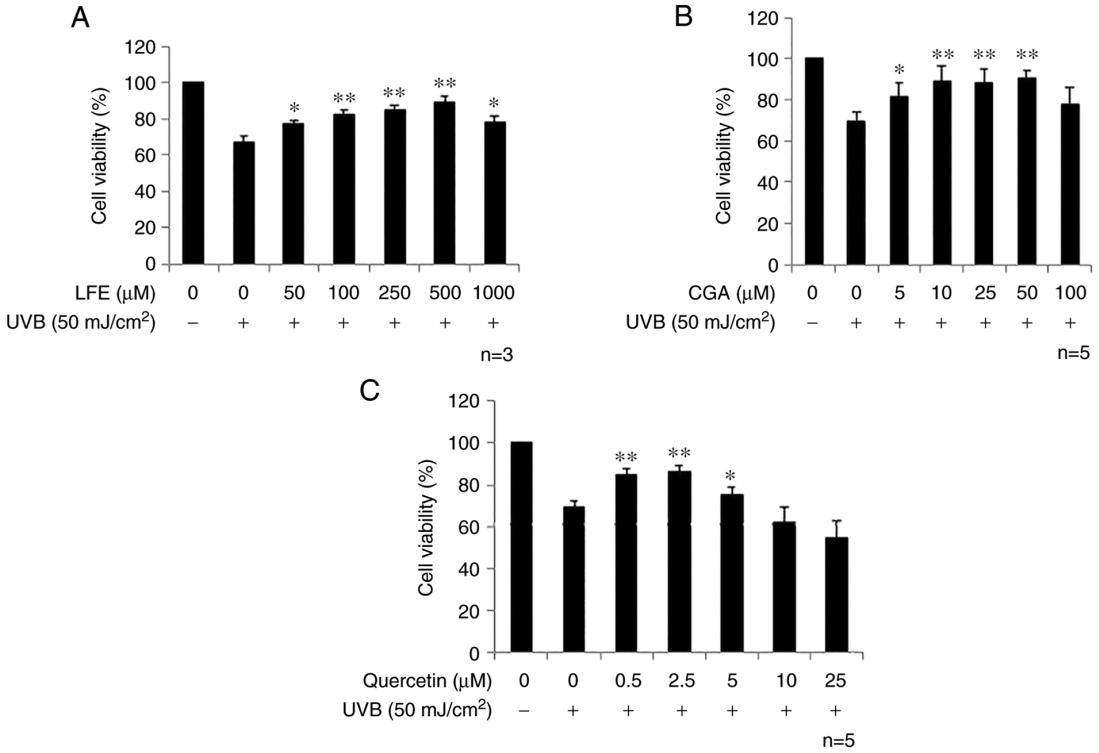

Figure 3. Effect of LFE and its components on UVB-induced toxicity in HaCaT cells. (A) Treatment with 50 μg/ml LFE significantly inhibited the UVB-induced toxicity in HaCaT cells. (B) Treatment with 5 μM CGA significantly inhibited the UVB-induced toxicity in HaCaT cells. (C) Treatment with 0.5 μM Quercetin significantly inhibited the UVB-induced toxicity in HaCaT cells, but increased cell death at a higher concentration (10 μM). *P<0.05, **P<0.01 vs. untreated control. LFE, Lycii Fructus extract; CGA, chlorogenic acid.

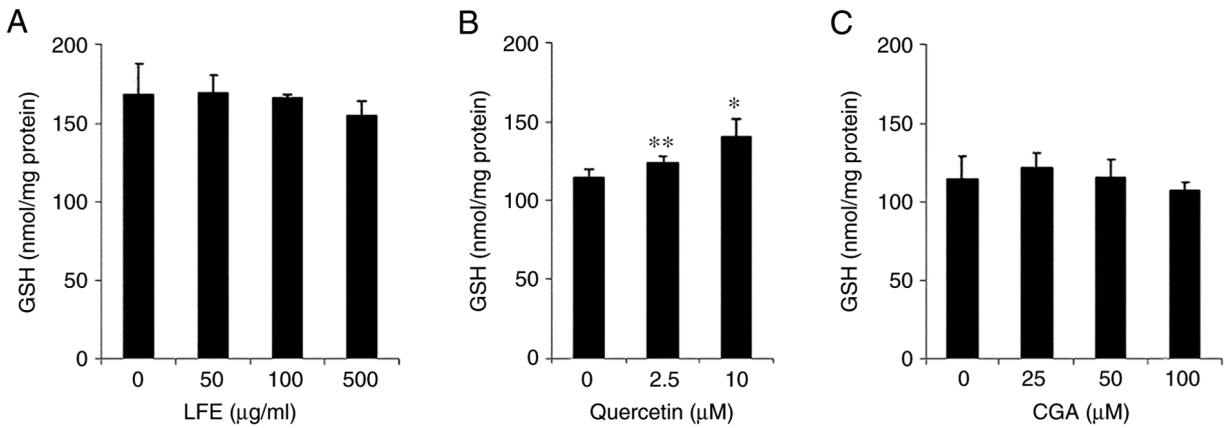

Figure 4. Effect of LFE on intracellular total GSH in HaCaT cells. (A) HaCaT cells were treated with DMSO or LFE (50, 100, or 500 μg/ml) for 24 h. LFE did not increase intracellular GSH in HaCaT cells. (B) HaCaT cells were treated with DMSO or Quercetin (2.5 or 10 μM) for 24 h; 2.5 and 10 μM Quercetin increased intracellular GSH levels significantly. (C) HaCaT cells were treated with DMSO or CGA (25, 50, or 100 μM) for 24 h. CGA did not increase intracellular GSH levels in HaCaT cells. *P<0.05, **P<0.01 vs. untreated control. LFE, Lycii Fructus extract; GSH, glutathione; CGA, chlorogenic acid.

or its components (chlorogenic acid and quercetin). Another polyphenol (Chafuroside B) can reduce UV damage, via the modulation of the inflammasome (55). Given this insight, the polyphenols in LFE may inhibit the inflammasome, which remains to be examined. The present study showed that, although LFE had no effect on intracellular GSH production, quercetin significantly increased total GSH levels in HaCaT cells (Fig. 4). Since LFE was reported to enhance the expression of HO-1, an enzyme involved in antioxidant responses, and GCLC (the catalytic subunit for the rate-limiting enzyme in glutathione biosynthesis) as well as NQO1, a Phase-II drug-metabolizing enzyme (30) in hepatocytes, similar mechanisms might be involved in the antioxidative cytoprotective effects of LFE in HaCaT cells. Although it is still unclear why an increase in the expression of these genes is not correlated with intracellular GSH level, quercetin is one of the possible components of LFE that, once absorbed into the body, travels to the skin via the bloodstream and enhances the antioxidative capacity in the epidermal cells. In addition, the redox state also plays an important role in intracellular antioxidant capacity, future efforts will be concerned with modulating the effects of antioxidative components of LFE on the GSSG/GSH ratio.

Other studies suggested that LFE ingestion was associated with increased superoxide dismutase (SOD) (56), and that goji extract administration reduced 8-OHdG levels in old mouse cavernosal tissue (57). More recently, our group demonstrated that LFE significantly enhanced the intracellular GSH levels in hepatocytes (30). However, as shown in Table V, LFE ingestion did not lead to increased antioxidative capacity in the blood as measured by total in-serum GSH levels. This discrepancy ay be attributable to the wide inter-individual variety in Japanese dietary habits. Fukushima *et al* (58) reported that Japanese women who consumed a substantial amount of coffee had fewer pigmented spots, and suggested that the volume of coffee consumed might affect in-serum antioxidative capacity. In an earlier study, Fukushima *et al* (59) claimed that tea and coffee are the typical beverages from which Japanese people obtain chlorogenic acid, catechines, and other antioxidants. LFE with antioxidants that inhibit UV-induced cytotoxicity, such as chlorogenic acid, can be expected to function to help protect the skin from UV irritation.

The related literature suggests that orally consumed components can affect the epidermis as well as the hypodermis. A systematic review by Wang *et al* (60), for example, revealed that eating fish oil rich in polyunsaturated fatty acids (docosahexaenoic acid and eicosapentaenoic acid) facilitates the expression of genes associated with basement membrane formation and skin cell division. Similarly, Li *et al* (16) reported that oral consumption of sulforaphane, which is abundant in cruciferous vegetables such as broccoli, inhibits inflammatory cytokines and enhances antioxidative activity in the retina. They concluded that sulforaphane both inhibits NLRP3 inflammasome and activates the antioxidative Nrf2 pathway. The above findings can support our findings of a reduction and accelerated decline in UV damage as represented by erythema formation by LFE supplementation. Taken together, three factors may play a pivotal role as follows: i) once absorbed, the LFE components may facilitate the GSH production in the liver; ii) once transferred to the epidermis through the bloodstream, they may enhance antioxidant gene expression and inhibit inflammatory responses in Nrf2-dependent manners; and iii) the antioxidative components may directly protect the epidermis.

Further research is needed to determine the extent to which the effect of LFE on the intestinal function affects the skin's photoprotective capacity.

A limitation of the study is the sample size. Sampling errors may occur when a survey is conducted using the probability sampling method.

In conclusion, this randomized, double-blind, placebo-controlled trial, combined with the *in vitro* research using the human HaCaT cells on the effects of LFE against UVB-induced damage, strongly suggests that LFE ingestion can protect epidermal cells from UVB-induced oxidative stress. These effects can, in turn, facilitate antioxidative capacity throughout the body, such as in the skin. LFE may augment the effect of sunscreen in protecting the skin from damage. However, the molecular mechanisms underlying the metabolism, absorption, and transportation to the skin of the LFE components are still unclear. Whether these components influence the skin function directly or indirectly through the actions of other organs is the subject of further study. In addition, to find an optimal combination of food ingredients having multiple action points, is another step in obtaining more effective remedies for skin health.

Acknowledgements

Not applicable.

Funding

This study was supported in part by MEXT KAKENHI (grant nos. 17H03818 and 20H02933).

Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

MT, WX, TN, and TK performed the experiments. TM, TN, and TK performed the data analysis. YN and OU conceived and designed the study. YN and OU confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This trial conformed to the Declaration of Helsinki and was approved by the Ethics Committee of Tactics (Hokkaido Activation Center) on December 14, 2016 (approval no. 2016-100). Written informed consent was obtained from all potential participants prior to participant selection.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Halliday GM, Damian DL, Rana S and Byrne SN: The suppressive effects of ultraviolet radiation on immunity in the skin and internal organs: Implications for autoimmunity. J Dermatol Sci 66: 176-182, 2012.
2. Heck DE, Gerecke DR, Vetrano AM and Laskin JD: Solar ultraviolet radiation as a trigger of cell signal transduction. Toxicol Appl Pharmacol 195: 288-297, 2004.
3. Ley RD and Reeve VE: Chemoprevention of ultraviolet radiation-induced skin cancer. Environ Health Perspect 105 (Suppl 4): S981-S984, 1997.
4. Yang B, Xu QY, Guo CY, Huang JW, Wang SM, Li YM, Tu Y, He L, Bi ZG, Ji C and Cheng B: MHY1485 ameliorates UV-induced skin cell damages via activating mTOR-Nrf2 signaling. Oncotarget 8: 12775-12783, 2017.
5. Martincorena I, Roshan A, Gerstung M, Ellis P, Van Loo P, McLaren S, Wedge DC, Fullam A, Alexandrov LB, Tubio JM, *et al*: Tumor evolution. High burden and pervasive positive selection of somatic mutations in normal human skin. Science 348: 880-886, 2015.
6. Gunn DA, de Craen AJ, Dick JL, Tomlin CC, van Heemst D, Catt SD, Griffiths T, Ogden S, Maier AB, Murray PG, *et al*: Facial appearance reflects human familial longevity and cardiovascular disease risk in healthy individuals. J Gerontol A Biol Sci Med Sci 68: 145-152, 2013.

7. Gunn DA, Larsen LA, Lall JS, Rexbye H and Christensen K: Mortality is written on the face. J Gerontol A Biol Sci Med Sci 71: 72-77, 2016.
8. Sveikata K, Balciuniene I and Tutkuviene J: Factors influencing face aging. Literature review. Stomatologija 13: 113-116, 2011.
9. Gordon JR and Brieva JC: Images in clinical medicine. Unilateral dermatoheliosis. N Engl J Med 366: e25, 2012.
10. Young AR, Sheehan JM, Chadwick CA and Potten CS: Protection by ultraviolet A and B sunscreens against in situ dipyrimidine photolesions in human epidermis is comparable to protection against sunburn. J Invest Dermatol 115: 37-41, 2000.
11. Kim HH, Cho S, Lee S, Kim KH, Cho KH, Eun HC and Chung JH: Photoprotective and anti-skin-aging effects of eicosapentaenoic acid in human skin in vivo. J Lipid Res 47: 921-930, 2006.
12. Seite S, Fourtanier A, Moyal D and Young AR: Photodamage to human skin by suberythemal exposure to solar ultraviolet radiation can be attenuated by sunscreens: A review. Br J Dermatol 163: 903-914, 2010.
13. Hasegawa T, Nakashima M and Suzuki Y: Nuclear DNA damage-triggered NLRP3 inflammasome activation promotes UVB-induced inflammatory responses in human keratinocytes. Biochem Biophys Res Commun 477: 329-335, 2016.
14. D'Errico M, Lemma T, Calcagnile A, Proietti De Santis L and Dogliotti E: Cell type and DNA damage specific response of human skin cells to environmental agents. Mutat Res 614: 37-47, 2007.
15. Kobayashi M and Yamamoto M: Nrf2-Keap1 regulation of cellular defense mechanisms against electrophiles and reactive oxygen species. Adv Enzyme Regul 46: 113-140, 2006.
16. Li S, Yang H and Chen X: Protective effects of sulforaphane on diabetic retinopathy: Activation of the Nrf2 pathway and inhibition of NLRP3 inflammasome formation. Exp Anim 68: 221-231, 2019.
17. Swindells K and Rhodes LE: Influence of oral antioxidants on ultraviolet radiation-induced skin damage in humans. Photodermatol Photoimmunol Photomed 20: 297-304, 2004.
18. Yimcharoen M, Kittikunnathum S, Suknikorn C, Nak-On W, Yeethong P, Anthony TG and Bunpo P: Effects of ascorbic acid supplementation on oxidative stress markers in healthy women following a single bout of exercise. J Int Soc Sports Nutr 16: 2, 2019.
19. Hanson KM and Clegg RM: Bioconvertible vitamin antioxidants improve sunscreen photoprotection against UV-induced reactive oxygen species. J Cosmet Sci 54: 589-598, 2003.
20. Jeon HY, Kim JK, Kim WG and Lee SJ: Effects of oral epigallocatechin gallate supplementation on the minimal erythema dose and UV-induced skin damage. Skin Pharmacol Physiol 22: 137-141, 2009.
21. Puglia C, Offerta A, Saija A, Trombetta D and Venera C: Protective effect of red orange extract supplementation against UV-induced skin damages: Photoaging and solar lentigines. J Cosmet Dermatol 13: 151-157, 2014.
22. Heinrich U, Moore CE, De Spirt S, Tronnier H and Stahl W: Green tea polyphenols provide photoprotection, increase microcirculation, and modulate skin properties of women. J Nutr 141: 1202-1208, 2011.
23. Rizwan M, Rodriguez-Blanco I, Harbottle A, Birch-Machin MA, Watson RE and Rhodes LE: Tomato paste rich in lycopene protects against cutaneous photodamage in humans in vivo: A randomized controlled trial. Br J Dermatol 164: 154-162, 2011.
24. Juturu V, Bowman JP and Deshpande J: Overall skin tone and skin-lightening-improving effects with oral supplementation of lutein and zeaxanthin isomers: A double-blind, placebo-controlled clinical trial. Clin Cosmet Investig Dermatol 9: 325-332, 2016.
25. Ulbricht C, Bryan JK, Costa D, Culwell S, Giese N, Isaac R, Nummy K, Pham T, Rapp C, Rusie E, *et al*: An evidence-based systematic review of Goji Lycium spp.) by the Natural Standard Research Collaboration. J Diet Suppl 12: 184-240, 2015.
26. Amagase H and Farnsworth NR: A review of botanical characteristics, phytochemistry, clinical relevance in efficacy and safety of Lycium barbarum fruit (Goji). Food Res Int 44: 1702-1717, 2011.
27. Gomez-Bernal S, Rodriguez-Pazos L, Martinez FJ, Ginarte M, Rodríguez-Granados MT and Toribio J: Systemic photosensitivity due to Goji berries. Photodermatol Photoimmunol Photomed 27: 245-247, 2011.
28. Reeve VE, Allanson M, Arun SJ, Domanski D and Painter N: Mice drinking goji berry juice (Lycium barbarum) are protected from UV radiation-induced skin damage via antioxidant pathways. Photochem Photobiol Sci 9: 601-607, 2010.
29. Kuwazuru S, Nakashima M, Honda E and Fukaya Y: Effects of oral lycii fructus extracts on skin erythema and skin pigmentation induced by solar-simulated ultraviolet irradiation in healthy men. Pharmacometrics 83: 39-45, 2012.
30. Xu W, Saiki S, Myojin T, Liu Y, Zhu B, Murata Y, Ashida H, Tsunenaga M and Nakamura Y: Lycii fructus extract ameliorates hydrogen peroxide-induced cytotoxicity through indirect antioxidant action. Biosci Biotechnol Biochem 82: 1812-1820, 2018.
31. World Medical Association: World Medical Association Declaration of Helsinki: Ethical principles for medical research involving human subjects. JAMA 310: 2191-2194, 2013.
32. Fitzpatrick TB: The validity and practicality of sun-reactive skin types I through VI. Arch Dermatol 124: 869-871, 1988.
33. Kobayashi S, Murakami K, Sasaki S, Okubo H, Hirota N, Notsu A, Fukui M and Date C: Comparison of relative validity of food group intakes estimated by comprehensive and brief-type self-administered diet history questionnaires against 16 d dietary records in Japanese adults. Public Health Nutr 14: 1200-1211, 2011.
34. Delalleau A, Lagarde JM and George J: An a priori shading correction technique for contact imaging devices. IEEE Trans Image Process 20: 2876-2885, 2011.
35. Lim SH, Kim SM, Lee YW, Ahn KJ and Choe YB: Change of biophysical properties of the skin caused by ultraviolet radiation-induced photodamage in Koreans. Skin Res Technol 14: 93-102, 2008.
36. Ambach W and Blumthaler M: Biological effectiveness of solar UV radiation in humans. Experientia 49: 747-753, 1993.
37. Stahl W, Heinrich U, Aust O, Tronnier H and Sies H: Lycopene-rich products and dietary photoprotection. Photochem Photobiol Sci 5: 238-242, 2006.
38. Carrascosa JM, Floriach N, Sala E and Aguilera J: Increase in minimal erythemal dose following oral administration of an antioxidant complex based on a mix of carotenoids: Double-blind, placebo-controlled trial. Photodermatol Photoimmunol Photomed 33: 284-286, 2017.
39. Koyama YI, Kuwaba K, Kondo S and Tsukada Y: Supplemental ingestion of collagen peptide suppresses uitraviolet-induced erythema. Jpn Pharmacol Ther 42: 781-790, 2014.
40. Pengjiao Z, Juan L, Yulong C and Lijuan Z: The structures and biological functions of polysaccharides from traditional Chinese herbs. Prog Mol Biol Transl Sci 163: 423-444, 2019.
41. Li H, Li Z, Peng L, Jiang N, Liu Q, Zhang E, Liang B, Li R and Zhu H: Lycium barbarum polysaccharide protects human keratinocytes against UVB-induced photo-damage. Free Radic Res 51: 200-210, 2017.
42. Ding Y, Yan Y, Peng Y, Chen D, Mi J, Lu L, Luo Q, Li X, Zeng X and Cao Y: In vitro digestion under simulated saliva, gastric and small intestinal conditions and fermentation by human gut microbiota of polysaccharides from the fruits of Lycium barbarum. Int J Biol Macromol 125: 751-760, 2019.
43. Nakamura S, Matsui Y, Watanabe T, Kondou N and Masukawa Y: Pharmacokinetics of chlorogenic acids absorbed in human plasma and their metabolites following oral ingestion of coffee drink. 34: 1239-1246, 2006.
44. Lafay S, Gil-Izquierdo A, Manach C, Morand C, Besson C and Scalbert A: Chlorogenic acid is absorbed in its intact form in the stomach of rats. J Nutr 136: 1192-1197, 2006.
45. Renouf M, Marmet C, Giuffrida F, Lepage M, Barron D, Beaumont M, Williamson G and Dionisi F: Dose-response plasma appearance of coffee chlorogenic and phenolic acids in adults. Mol Nutr Food Res 58: 301-309, 2014.
46. Kitagawa S, Yoshii K, Morita SY and Teraoka R: Efficient topical delivery of chlorogenic acid by an oil-in-water microemulsion to protect skin against UV-induced damage. Chem Pharm Bull (Tokyo) 59: 793-796, 2011.
47. Yang J, Qian D, Jian S, Shang EX, Guo J and Duan JA: Identification of rutin deglycosylated metabolites produced by human intestinal bacteria using UPLC-Q-TOF/MS. J Chromatogr B Analyt Technol Biomed Life Sci 898: 95-100, 2012.
48. Erlund I, Kosonen T, Alfthan G, Mäenpää J, Perttunen K, Kenraali J, Parantainen J and Aro A: Pharmacokinetics of quercetin from quercetin aglycone and rutin in healthy volunteers. Eur J Clin Pharmacol 56: 545-553, 2000.
49. Vicentini FT, He T, Shao Y, Fonseca MJ, Verri WA Jr, Fisher GJ and Xu Y: Quercetin inhibits UV irradiation-induced inflammatory cytokine production in primary human keratinocytes by suppressing NF-kappaB pathway. J Dermatol Sci 61: 162-168, 2011.
50. Zhu X, Li N, Wang Y, Ding L, Chen H, Yu Y and Shi X: Protective effects of quercetin on UVB irradiationinduced cytotoxicity through ROS clearance in keratinocyte cells. Oncol Rep 37: 209-218, 2017.

51. Gueniche A, Philippe D, Bastien P, Blum S, Buyukpamukcu E and Castiel-Higounenc I: Probiotics for photoprotection. Dermatoendocrinol 1: 275-279, 2009.
52. Bouilly-Gauthier D, Jeannes C, Maubert Y, Duteil L, Queille-Roussel C, Piccardi N, Montastier C, Manissier P, Piérard G and Ortonne JP: Clinical evidence of benefits of a dietary supplement containing probiotic and carotenoids on ultraviolet-induced skin damage. Br J Dermatol 163: 536-543, 2010.
53. Gueranger Q, Li F, Peacock M, Larnicol-Fery A, Brem R, Macpherson P, Egly JM and Karran P: Protein oxidation and DNA repair inhibition by 6-thioguanine and UVA radiation. J Invest Dermatol 134: 1408-1417, 2014.
54. Hakozaki T, Date A, Yoshii T, Toyokuni S, Yasui H and Sakurai H: Visualization and characterization of UVB-induced reactive oxygen species in a human skin equivalent model. Arch Dermatol Res 300 (Suppl 1): S51-S56, 2008.
55. Hasegawa T, Shimada S, Ishida H and Nakashima M: Chafuroside B, an Oolong tea polyphenol, ameliorates UVB-induced DNA damage and generation of photo-immunosuppression related mediators in human keratinocytes. PLoS One 8: e77308, 2013.
56. Amagase H, Sun B and Borek C: Lycium barbarum (goji) juice improves in vivo antioxidant biomarkers in serum of healthy adults. Nutr Res 29: 19-25, 2009.
57. Moon HW, Park JW, Lee KW, Jeong HC, Choi JB, Choi SW, Bae WJ, Cho HJ, Ha US, Hong SH, *et al*: Administration of Goji (lycium chinense mill.) extracts improves erectile function in old aged rat model. World J Mens Health 35: 43-50, 2017.
58. Fukushima Y, Takahashi Y, Hori Y, Kishimoto Y, Shiga K, Tanaka Y, Masunaga E, Tani M, Yokoyama M and Kondo K: Skin photoprotection and consumption of coffee and polyphenols in healthy middle-aged Japanese females. Int J Dermatol 54: 410-418, 2015.
59. Fukushima Y, Tashiro T, Kumagai A, Ohyanagi H, Horiuchi T, Takizawa K, Sugihara N, Kishimoto Y, Taguchi C, *et al*: Coffee and beverages are the major contributors to polyphenol consumption from food and beverages in Japanese middle-aged women. J Nutr Sci 3: e48, 2014.
60. Wang P, Sun M, Ren J, Aslam MN, Xu Y, Fisher GJ, Voorhees JJ, Wang X and Li Y: Dietary fish oil supplementation enhances expression of genes involved in cornified cell envelope formation in rat skin. J Invest Dermatol 138: 981-983, 2018.