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Molecular evidence that oral supplementation with lycopene or lutein protects human skin against ultraviolet radiation: results from a double-blinded, placebo-controlled, crossover study*

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Summary

Background Increasing evidence suggests photoprotection by oral supplementation with β-carotene and lycopene.
Objectives To examine the capacity of lycopene-rich tomato nutrient complex (TNC) and lutein, to protect against ultraviolet (UV)A/B and UVA1 radiation at a molecular level.
Methods In a placebo-controlled, double-blinded, randomized, crossover study two active treatments containing either TNC or lutein were assessed for their capacity to decrease the expression of UVA1 the radiation-inducible genes *HO1*, *ICAM1* and *MMP1*. Sixty-five healthy volunteers were allocated to four treatment groups and subjected to a 2-week washout phase, followed by two 12-week treatment phases separated by another 2 weeks of washout. Volunteers started either with active treatment and were then switched to placebo, or vice versa. At the beginning and at the end of each treatment phase skin was irradiated and 24 h later biopsies were taken from untreated, UVA/B- and UVA1-irradiated skin for subsequent reverse transcriptase polymerase chain reaction analysis of gene expression. Moreover, blood samples were taken after the washout and the treatment phases for assessment of carotenoids.
Results TNC completely inhibited UVA1- and UVA/B-induced upregulation of heme-oxygenase 1, intercellular adhesion molecule 1 and matrix metallopeptidase 1 mRNA, no matter the sequence (ANOVA, $P < 0{\cdot}05$). In contrast, lutein provided complete protection if it was taken in the first period but showed significantly smaller effects in the second sequence compared with TNC.
Conclusions Assuming the role of these genes as indicators of oxidative stress, photodermatoses and photoageing, these results might indicate that TNC and lutein could protect against solar radiation-induced health damage.

What's already known about this topic?

- Previous studies have provided evidence for photoprotection by β-carotene.
- Virtually all previous studies assessed photoprotection as the reduction in ultraviolet (UV) radiation-induced erythema formation in human skin.

What does this study add?

- Lycopene-rich tomato nutrient complex (TNC) and lutein protect from UVA/B- and UVA1-induced gene expression in human skin.

- Molecular markers for UVA1-inducible genes may be a suitable tool with which to address photodamage in human skin *in vivo*.

What is the translational message?

- Dietary strategies may help to protect human skin from UV radiation.

There is growing evidence that dietary intervention can protect human skin against the detrimental effects caused by solar ultraviolet (UV) radiation.[1] In this regard, carotenoids are of particular interest.[2] The vast majority of published studies have focused on β-carotene. Accordingly, based on the outcome of seven human intervention studies,[3–9] we previously performed a meta-analysis which showed that supplementation with β-carotene is associated with protection against the development of a sunburn reaction.[10] However, it should be noted that safety concerns were raised with regard to β-carotene supplements when they are taken over longer periods of time (i.e. years) at nonphysiological levels. Accordingly, in smokers and asbestos workers, a higher cumulative index for lung cancer has been observed.[11]

As a consequence, more recent studies on oral photoprotection have included other carotenoids, as well as β-carotene. It was reported that a carotenoid mixture consisting of 8 mg each of β-carotene, lycopene and lutein provided a level of photoprotection similar to that achieved with 24 mg β-carotene.[5] Also, in an intervention study with lycopene-rich tomato paste, which provided 16 mg lycopene daily, a significant reduction of solar simulator-induced erythema was observed in human volunteers after 10 weeks of supplementation.[12] Of note, a combination of tomato phytonutrients from a tomato-based supplement resulted in better protection from UV-induced radiation than the effect of synthetic lycopene as a standalone treatment.[13] A randomized controlled trial showed that lycopene rich-tomato paste was able to prevent UV-induced effects at the molecular level.[14] The beneficial effect of the colourless tomato carotenoids phytoene and phytofluene in the protection of skin cells from UV damage was further supported *in vitro*.[15] All these *in vivo* studies used the UV-induced erythema response, which was induced by a solar simulator and consisted of a combination of UVB and UVA radiation as the readout system for photoprotection efficacy. This is somewhat surprising because (i) carotenoids are thought to provide photoprotection by acting as antioxidants (at least primarily); and because (ii) the solar simulator radiation-induced erythema response, which results from the UVB part of the emission spectrum, is mainly the consequence of the UVB radiation-induced formation of DNA photoproducts such as cyclobutane pyrimidine dimers,[16] that is, a photobiological event, which cannot be, or can only slightly be, reduced by antioxidants. However, it should be stated that shortwave UVB radiation and UVA radiation can cause oxidative stress.[17] Longwave UV radiation (UVA1)-induced gene expression might be a choice for readout, to show beneficial effects of antioxidants.

Whether nutritional supplementation with lycopene and/or lutein can protect against UVA1 radiation-induced gene expression is not known. In the present placebo-controlled, double-blinded, randomized, crossover study, we assessed the capacity of two different nutritional supplements, that is, a lycopene-rich tomato nutrient complex (TNC) and a lutein containing one, to prevent not only UVB/A radiation (emitted from a solar simulator), but also UVA1 radiation-induced gene expression in human skin. Specifically, we analysed the mRNA expression of genes known to be UVA1-inducible, as well as UVB/A-inducible, and which are functionally involved in solar radiation-induced skin damage. These genes included *HO1* as an indicator gene for oxidative stress (reviewed by Nisar *et al.*),[18] *MMP1* (reviewed by Krutmann and Gilchrest),[19] which is critically involved in collagen breakdown and thus photoageing of human skin, and *ICAM1*, which plays an important role in skin inflammation and is expressed at increased levels in lesional skin of patients with polymorphic light eruption (PLE; reviewed in Vandergriff and Bergstresser).[20]

Materials and methods

Materials

TNC capsules and lutein-containing capsules, as well as placebo capsules, were provided by Lycored (Be'er Sheva, Israel). Lycopene-rich TNC softgel capsules contained 5 mg lycopene, as well as other tomato phytonutrients, such as phytoene and phytofluene, tocopherols and phytosterols. Lutein softgel capsules contained 10 mg free lutein stabilized by 10% carnosic acid, whereas placebo softgel capsules contained soybean oil. The latter proved to be an acceptable placebo in clinical trials addressing ω3 fatty acid supplementation.[21] The UV blocking capacity of most natural oils was shown to be insufficient to obtain significant protection.[22] Specifically, while soybean oil is rich in ω3 fatty acids, for example α-linolenic acid, its conversion into photoprotective long-chain ω3 polyunsaturated fatty acids is poor in mammals.[23,24] Correspondingly, no significant effect on UV responses could be detected for the biomarkers assessed under placebo treatment (see Figs 3 and 4). There was a slight colour difference between the placebo capsules and the capsules containing lycopene (which looked darker), but this difference was only recognizable during direct

comparison. Dosage for the two different treatment arms was as follows: two capsules of TNC twice daily; one lutein capsule twice daily. All supplements were supplied in identically packed, coded containers.

Volunteers

The study was approved by the local ethics committee of the Heinrich-Heine University, Düsseldorf, Germany (project no. 2869), and conducted at the IUF – Leibniz Research Institute for Environmental Medicine, Düsseldorf, Germany, according to the ethical rules stated in the principles of the Declaration of Helsinki, and the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use Good Clinical Practice guidelines were adhered to, as applicable. Sixty-five healthy participants (52 men, 13 women) were enrolled, based on a power calculation with the following assumptions: based on a within-subject SD of the response variable (biomarker) of 30%, the probability was 80% that the study would detect a treatment difference at a two-sided 5% significance level, if the true difference between the treatments was > 20%.

The study was conducted between November 2008 and October 2009. The age of the volunteers ranged from 18 to 60 years; all individuals were nonsmokers, had normal eating habits and no history of any skin disease or skin cancer. Further exclusion criteria were: pregnant or lactating women, intensely tanned skin and skin abnormalities in the test areas, sunbed use or sunbathing, and unusual eating habits (e.g. vegetarian). Participants were urged to keep a balanced diet without consuming tomatoes, tomato products and other dietary supplements.

Study design

The overall study design was that of a placebo-controlled, double-blinded, randomized crossover intervention study. For each nutritional supplement, two consecutive periods consisting of a washout phase of 2 weeks followed by a treatment phase of 12 weeks were carried out (Fig. 1). The study consisted of two arms, a lycopene-rich TNC arm, and a lutein treatment arm. The participants enrolled at the IUF were distributed according to an online block randomization service by the sponsor; block size was four with an allocation ratio of 1: 1 per study arm: lycopene active group (TGA); lycopene placebo group (TGB); lutein active group (TGC); lutein placebo group (TGD). After the first treatment phase volunteers within one treatment arm were crossed over and the second washout phase and treatment phase were conducted. At the beginning of the study and at the end of each treatment phase participants were locally exposed to 1·5 minimal erythemal dose (MED) of UVB/A using a solar simulator (Dr. K. Höhnle GmbH, Germany) with a lamp filter combination according to COLIPA standards, or UVA1 radiation (100 J cm^{-2}) using a Sellamed 2000 device (Dr Sellmeier, Gevelsberg, Germany) with an emission spectrum of 340–400 nm as previously described.[25,26] The individual MED was determined 24 h after irradiation of six skin areas on the upper buttock with increasing amounts of UVB/A as the lowest dose causing visible erythema. Blood samples drawn from the cubital vein were collected four times: at the end of the first washout phase, at the end of the first treatment phase, at the end of the second washout phase and at the end of the second treatment phase. Serum samples were prepared and stored at −80 °C until high-performance liquid chromatography (HPLC) analysis for lycopene and lutein contents. Three 6-mm punch biopsies were obtained from buttock skin of each volunteer 24 h after irradiation. Irradiations were given at the end of the first washout phase, at the end of the first treatment phase and at the end of the second treatment phase. At each time point, one biopsy was taken from nonirradiated skin, one biopsy from UVB/A-irradiated skin and one biopsy from UVA1-irradiated skin.

Assessment of blood samples

Blood samples were collected in heparinized tubes and immediately centrifuged. Plasma was stored at −80 °C until analysis. Carotenoids (lutein, zeaxanthin, β-cryptoxanthin, lycopene, α-and β-carotene) were analysed by HPLC with UV/visible detection as previously described.[27,28]

Assessment of gene expression

Biopsies were snap frozen in liquid nitrogen and stored at −80 °C until further analysis, which was done as previously described.[29,30] For reverse transcriptase polymerase chain reaction (PCR) analysis of mRNA expression the following primer pairs were used: heme-oxygenase 1 (HO-1; forward 5′-CT GCGTTCCT-GCTCAACATC-3′, reverse 5′-GCAGAATCTTGCAC TTTGTTGCT-3′;[31] matrix metallopeptidase 1 (MMP-1; forward 5′-GGGAGATCATCGGGACAACTC-3′, reverse 5′-GGGCCTGGT T-GAAAAGCAT-3′;[32] intercellular adhesion molecule 1 (ICAM-1; forward 5′-CCTGGCACCCAGCACAAT-3′, reverse 5′-GCCGA TCCACACGGAGTACT-3′);[33] and 18S rRNA as housekeeping gene (forward 5′-GCCGCTAGAGGTGAAATTCTTG-3′, reverse 5′-CATTCTTGGCAAATGCTTTCG-3′).[34]

For comparison of relative gene expression in real-time PCR the $2^{(-\Delta\Delta C(T))}$ method was used.[35] Gene expression is indicated as x-fold induction vs. an unirradiated control.

Statistical analysis

Normality of the data was tested using the Shapiro–Wilk test. For comparison of significant differences, a paired test, t-test or ANOVA was used. In case of normality failure, the corresponding rank test, for example Wilcoxon signed rank test, Mann–Whitney rank sum test or Kruskal–Wallis ANOVA on ranks were employed. As post hoc analysis, the Tukey test was used as otherwise stated. A P-value < 0·05 was considered significant. Data are presented as point or box plots with median and corresponding percentiles as indicated in the figure legends.

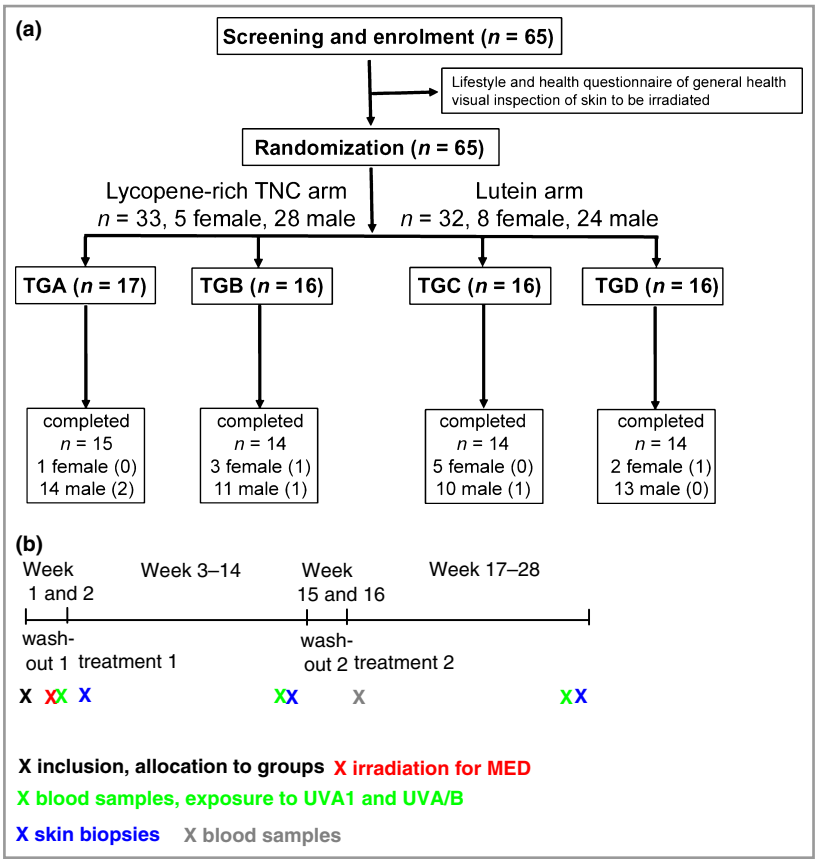

Fig 1. (a) Overall trial design showing number of volunteers (dropouts in parentheses); (b) details for one of the two arms of the placebo-controlled, double blinded, randomized crossover study testing the efficacy of the oral supplements lycopene-rich tomato nutrient complex (TNC) or lutein. During enrolment, demographic data were obtained and volunteers were allocated to the four treatment groups: lycopene active group (TGA), lycopene placebo group (TGB), lutein active group (TGC) or lutein placebo group (TGD). At the end of the first 2-week washout phase, at day 14, individual minimal erythemal doses (MEDs) of the volunteers were determined. A day later (day 15), blood samples were taken, and ultraviolet (UV)A1 and UVA/B irradiations were performed. After 24 h, at day 16, three biopsies were taken. The first treatment phase began and lasted 12 weeks. At the end of the first treatment phase (day 100), blood samples were taken and UVA1 and UVA/B irradiations were performed. One day later (day 101), biopsies were taken and the second washout phase was started. At day 115, the second washout was finished, blood samples were taken and the second treatment phase started. After another 12 weeks (day 199), blood samples were taken and UVA1 and UVA/B irradiations were performed. One day later (day 200) biopsies were taken. Blood samples were used for determination of lycopene and lutein content. Biopsies were used for analysis of molecular markers.

Results

For the lycopene-rich TNC arm of the study, 33 participants were recruited. Of these 29 [four women (mean ± SEM age 40·0 ± 7·9 years), 25 men (mean ± SEM age 43·0 ± 2·3 years)] completed the study. For the lutein arm of the study, 32 participants were recruited, of whom 30 completed the study [seven women (mean ± SEM age 47·0 ± 3·2 years), 23 men (mean ± SEM age 37·0 ± 2·3 years)]. Compliance was good (98–100 %) in both treatment arms. This was also documented by the development of lycopene and lutein blood levels, as shown in Figure 2. In both treatment arms the two treatment groups did not significantly differ in regard to blood levels of lycopene [TGA_{Day15}: 0·609 nmol mL^{-1} and TGB_{Day15}: 0·554 nmol mL^{-1} (median, Mann–Whitney rank sum test $P = 0·844$)] and lutein [TGC_{Day15}: 0·216 nmol mL^{-1} and TGD_{Day15}: 0·221 nmol mL^{-1} (mean, t-test $P = 0·852$)] prior to the intervention. Both carotenoids significantly increased upon intake of the lycopene- or lutein-containing capsules, but decreased to background levels or did not increase, respectively, when participants were treated with placebo. Lutein levels in the TGC group did not completely decrease to baseline after the second 2-week washout phase (Fig. 2b).

Nutritional intervention was well tolerated in all participants. With regard to safety, only one adverse event was reported by a volunteer from the lycopene arm of the study who suffered from diarrhoea a few days after starting with treatment. An external physician diagnosed the participant suffering from a viral infection of the upper intestine. The volunteer continued the study after a few days.

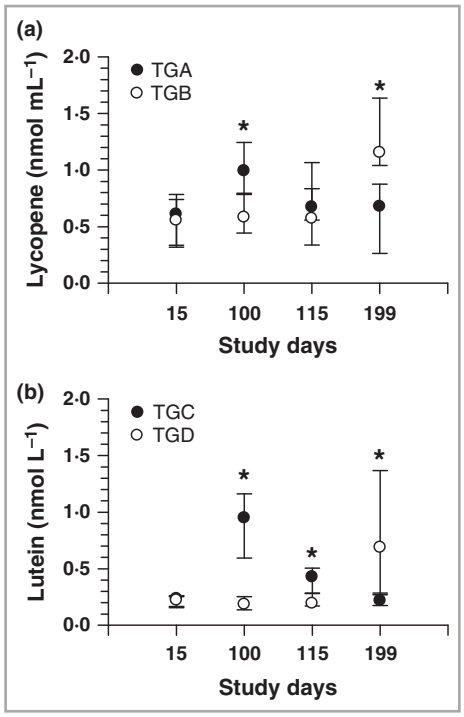

Fig 2. (a) Lycopene or (b) lutein content in blood samples taken at the indicated time points was determined as described in the 'Materials and methods'. The following treatment regimes are shown: (a) TGA – tomato nutrient complex (TNC)/placebo, TGB – placebo/TNC; (b) TGC – lutein/placebo; TGD – placebo/lutein. Given are medians, and 75th and 25th percentiles. Significance was determined by Kruskal–Wallis one-way ANOVA on ranks (Tukey) for each time point compared with the starting level at day 15. *$P < 0{\cdot}05$ vs. day 15.

Assessment of gene expression revealed that UVB/A, as well as UVA1, radiation significantly upregulated steady-state levels of HO-1, ICAM-1 and MMP-1 mRNA in skin of volunteers who were either untreated or had been treated with placebo. In marked contrast, TNC (Fig. 3), as well as lutein (Fig. 4), treatment significantly inhibited UVB/A and UVA1 radiation-induced gene expression.

As the factors for UVA1 and UVB/A induction of gene expression differed, we calculated, for further comparison, the remaining gene expression under active treatment as percentage of gene induction under nonprotected conditions, which had been set to 100%. The photoprotective effect of the active treatment did not differ between UVB/A- and UVA1-irradiated skin areas in both treatment arms (Table 1). In addition, we observed that the photoprotective effect of the active treatment did not differ in participants who were first treated with active agent and then crossed over to placebo, or who were first treated with placebo and then crossed over to active treatment in the lycopene-rich TNC arm of the study (Table 2). In contrast, we observed differences for the lutein arm (Table 2). When lutein was given in the second phase of the treatment significantly lower photoprotection against UVA-induced ICAM-1 expression was detected, while the change for UVA-induced protection against HO-1 and MMP-1 was not significant (Table 2). For protection from UVB/A-induced gene expression, significantly lower protection was observed for all three markers studied (Table 2).

Lycopene-rich TNC and lutein supplementation did not significantly differ in their capacity to prevent UVB/A and UVA1 radiation-induced gene expression, when both active agents had been given in the first treatment phase (Table 2). When lutein was given in the second treatment phase (TGD group) a decreased capacity to prevent UVA-induced upregulation of ICAM-1 or HO-1, and all UVA/B-induced markers assessed was determined (Table 3).

Discussion

In this placebo-controlled, double-blinded, randomized, crossover study we assessed the capacity of a lycopene-rich TNC and a lutein-containing nutritional supplement to protect human skin against UV radiation (UVR). To the best of our knowledge we show here, for the first time, that (i) TNC and lutein do not only protect healthy human skin against UVB/A, but also against longwave UVA1 radiation; and (ii) that oral photoprotection of healthy human skin can be demonstrated at the level of *HO1*, *ICAM1* and *MMP1* gene expression.

This conclusion is based on the observation that intake of lycopene-rich TNC and lutein-containing capsules, in contrast to placebo capsules, was associated with a significant reduction of UVR-induced mRNA expression of HO-1, MMP-1 and ICAM-1 (Figs 3 and 4).

These results corroborate and extend results from a previous study in which oral supplementation with a nutritional supplement containing TNC, β-carotene and *Lactobacillus johnsonii* was found to mitigate the development of skin lesions, as well as the concomitant upregulation of ICAM-1 mRNA in UVA1-irradiated lesional skin of patients with PLE.[29,30] Taken together, these studies imply that oral photoprotection with selected carotenoids not only provides protection against shortwave UVB/A radiation, but also against longwave UVA1 radiation. The precise mechanism by which lycopene and lutein inhibit UVA1 radiation-induced gene expression is currently not known but most likely involves antioxidative mechanisms.

A strength of the study is the double-blind, randomized, controlled design. In addition, a sufficient number of volunteers was included based on a reasonable sample size. The crossover design increased the power and decreased the confounding covariates as each volunteer was his/her own control.

Frequently, a weakness of crossover studies is inappropriate washout phases. In the case of lycopene, a 2-week washout period seemed to be reasonable, as previously shown for serum levels.[36] Similarly, washout phases ≥ 2 weeks were sufficient to change serum lutein levels by supplementation from egg yolk.[37] A weakness of the crossover design was observed in the lutein arm, where we determined lower protection level if the lutein intake was preceded by 16 weeks of restricted

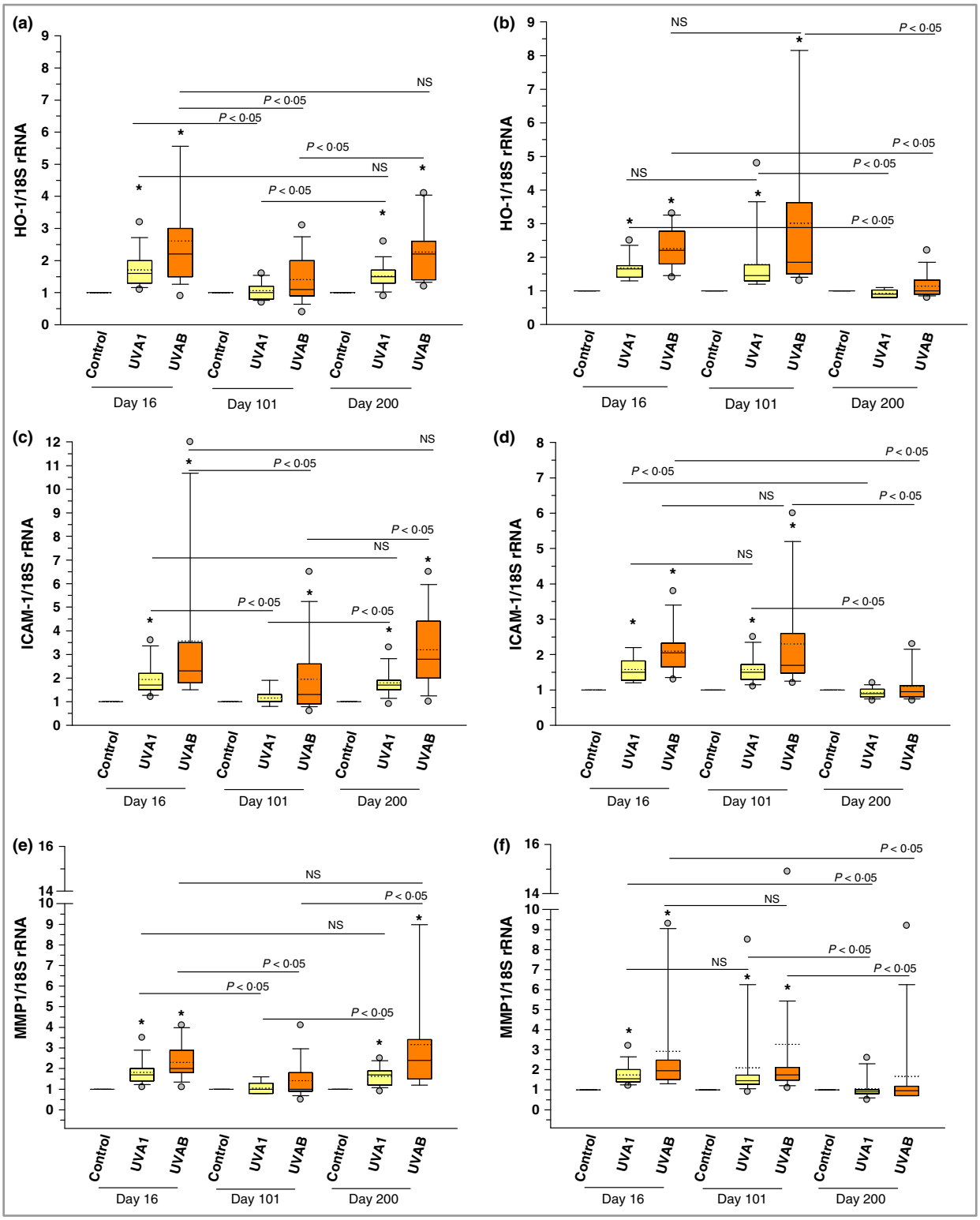

Fig 3. Effect of tomato nutrient complex (TNC) or placebo intake on ultraviolet (UV)A1- or UVA/B-induced gene expression in the (a, c, e) lycopene active group (TGA) or (b, d, f) lycopene placebo group (TGB) for *HO1*, *ICAM1* or *MMP1*. The results are summarized as horizontal boxes. The boundary of the box closest to zero indicates the 25th percentile, a line within the box marks the median and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers above and below the box indicate the 90th and 10th percentiles. A dotted line represents the mean, and grey circles outlying points. Significance was determined by Kruskal–Wallis one-way ANOVA on ranks Tukey or Student–Newman–Keuls [for (c) *ICAM1*, UVA/B]. *$P < 0{\cdot}05$ vs. the corresponding control, and between treatments as indicated. ns, not significant

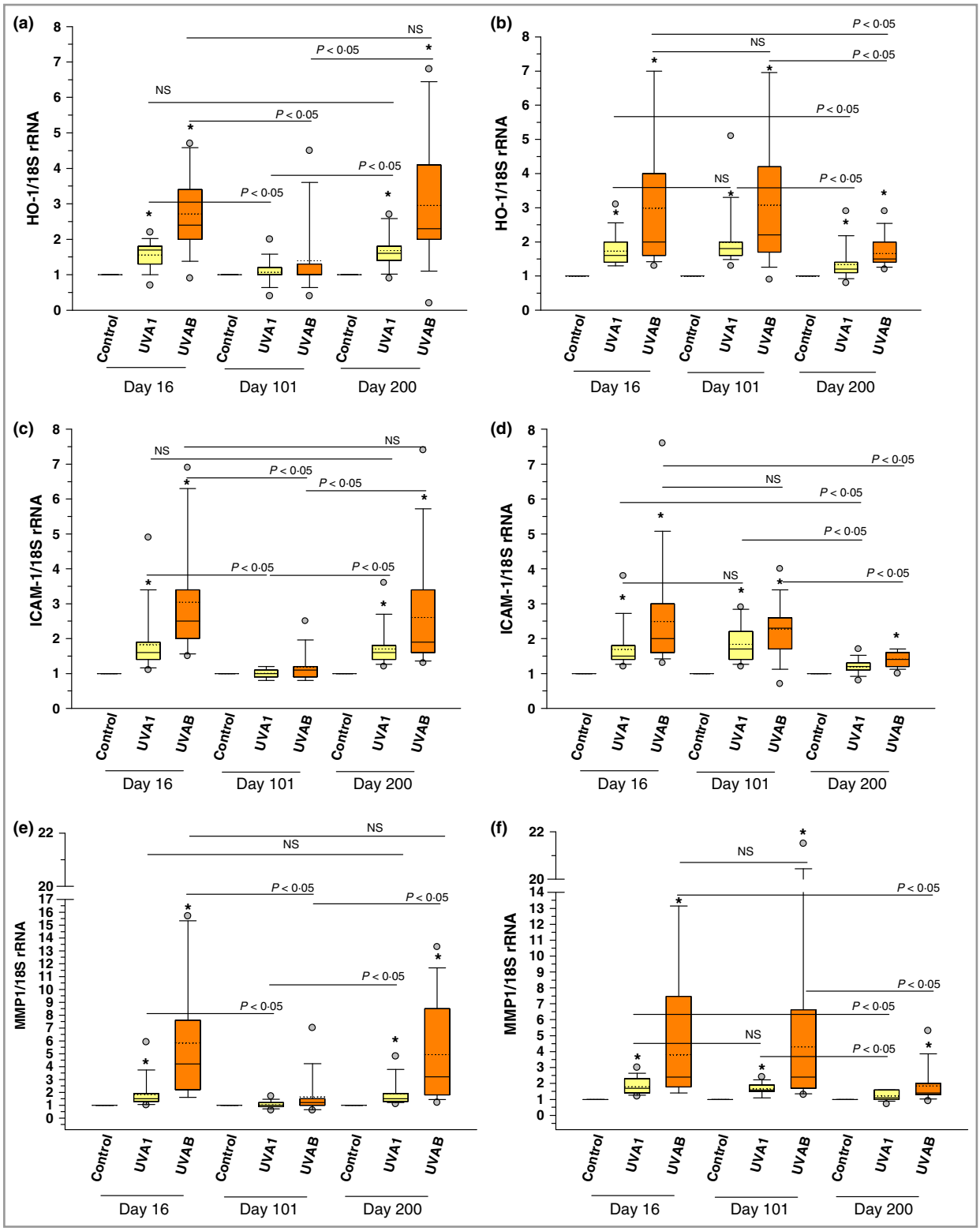

Fig 4. Effect of lutein or placebo intake on ultraviolet (UV)A1- or UVA/B-induced gene expression in the (a, c, e) lutein active group (TGC) or (b, d, f) lutein placebo group for *HO1*, *ICAM1* or *MMP1*. The results are summarized as horizontal boxes. The boundary of the box closest to zero indicates the 25th percentile, a line within the box marks the median and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers above and below the box indicate the 90th and 10th percentiles. A dotted line represents the mean, and grey circles outlying points. Significance was determined by Kruskal–Wallis one-way ANOVA on ranks Tukey or Student-Newman-Keuls [for (b) HO-1 UVA1, UVA/B; for (d) ICAM-1 UVA/B; for (f) MMP1 UVA/B]. *P < 0·05 vs. the corresponding control and between treatments as indicated. ns, not significant.

Table 1 Comparison of efficacy (remaining gene expression in percentage) in active treated volunteers after irradiation with ultraviolet (UV)A1 or UVA/B according to Wilcoxon signed rank test

	Regime/ marker	Median UVA1 active	Median UVA/B active	Protection by active % UVA vs. UVA/B (P-value)
TNC	TGA ICAM-1	0	29	0·365
	TGA HO-1	0	14	0·844
	TGA MMP-1	0	0	1·000
	TGB ICAM-1	0	0	0·438
	TGB HO-1	0	0	0·219
	TGB MMP-1	0	0	0·219
Lutein	TGC ICAM-1	0	3	0·652
	TGC HO-1	0	0	0·250
	TGC MMP-1	0	8	0·846
	TGD ICAM-1	33	29	0·326
	TGD HO-1	33	50	0·326
	TGD MMP-1	29	25	0·489

TNC, tomato nutrient complex; TGA, lycopene active group; ICAM-1, intercellular adhesion molecule 1; HO-1, heme-oxygenase 1; MMP-1, matrix metallopeptidase 1; TGB, lycopene placebo group; TGC, lutein active group; TGD, lutein placebo group.

Table 2 Comparison of efficacy (remaining gene expression in percentage) in active treated crossover groups according to Mann–Whitney rank sum test

		Marker	Regime	Median active first	Median active second	Protection by active % active first vs. active second (P-value)
TNC	UVA1	ICAM-1	TGA/TGB	0	0	0·252
		HO-1	TGA/TGB	0	0	0·165
		MMP-1	TGA/TGB	0	0	0·817
	UVA/B	ICAM-1	TGA/TGB	29	0	0·115
		HO-1	TGA/TGB	14	0	0·291
		MMP-1	TGA/TGB	0	0	0·169
Lutein	UVA1	ICAM-1	TGC/TGD	0	33	0·023
		HO-1	TGC/TGD	0	33	0·066
		MMP-1	TGC/TGD	0	29	0·052
	UVA/B	ICAM-1	TGC/TGD	3	29	0·003
		HO-1	TGC/TGD	0	50	< 0·001
		MMP-1	TGC/TGD	8	25	0·031

TNC, tomato nutrient complex; UV, ultraviolet; ICAM-1, intercellular adhesion molecule 1; TGA, lycopene active group; TGB, lycopene placebo group; HO-1, heme-oxygenase 1; MMP-1, matrix metallopeptidase 1; TGC, lutein active group; TGD, lutein placebo group.

diet due to washout and placebo treatment (Tables 2 and 3). Obviously, the 12-week intake phase in the TGD group was not sufficient to reach similar blood levels as the patients in the TGC group (Fig. 2b), as the lutein was accumulated somewhere else.[38] Accordingly, UVA1 radiation-induced gene expression was previously shown by us and others to involve, critically, the generation of singlet oxygen as an initiating event,[39–41] which most likely can be targeted by the well-known capacity of carotenoids and of lycopene, in particular, to quench singlet oxygen.[2]

These findings are of obvious clinical relevance because UVA1 radiation is well known to contribute to photoageing and photocarcinogenesis in healthy human skin, and to be an important trigger for the most frequent photodermatosis, that is, PLE. In this regard it is of interest that the genes we found to be reduced in their UVA1 inducibility are critically involved in these pathological conditions. Therefore, we suggest that oral supplementation with lycopene-rich TNC and lutein may be efficient in inhibiting UVA1 radiation-induced oxidative stress responses in general, and gene regulatory events involved in photoageing, photocarcinogenesis and photodermatoses specifically.

Notably, the TNC used in this study contained a combination of tomato phytonutrients. Previous studies suggest that the combined effect of tomato phytonutrients in protection from UV-induced erythema is stronger than the effect of lycopene as a standalone treatment.[13] Other tomato carotenoids, namely phytoene and phytofluene, have been suggested to have a protective effect of skin cells from UV damage.[15] The combination of lycopene with phytoene and phytofluene was

Table 3 Comparison of efficacy (remaining gene expression in percentage) between lycopene and lutein treatment after irradiation with ultraviolet (UV)A1 or UVA/B according to Kruskall–Wallis ANOVA on ranks (Dunn's method)

	Marker	Regime	TNC median active	Regime	Lutein median active	P-value
UVA1	ICAM-1	TGA	0	TGC	0	NS
	HO-1	TGA	0	TGC	0	NS
	MMP-1	TGA	0	TGC	0	NS
	ICAM-1	TGB	0	TGD	33	< 0·05
	HO-1	TGB	0	TGD	33	< 0·05
	MMP-1	TGB	0	TGD	29	NS
UVA/B	ICAM-1	TGA	29	TGC	3	NS
	HO-1	TGA	14	TGC	0	NS
	MMP-1	TGA	0	TGC	8	NS
	ICAM-1	TGB	0	TGD	29	< 0·05
	HO-1	TGB	0	TGD	50	< 0·05
	MMP-1	TGB	0	TGD	25	< 0·05

TNC, tomato nutrient complex; ICAM-1, intercellular adhesion molecule 1; TGA, lycopene active group; TGC, lutein active group; NS, not significant; HO-1, heme-oxygenase 1; MMP-1, matrix metallopeptidase 1; TGB, lycopene placebo group; TGD, lutein placebo group.

recently suggested to be synergistic in another cellular model of prostate cells.[42] We suggest that the protective effect of the TNC in this study is the result of the combined effect of the tomato phytonutrients. To ingest 20 mg lutein and 20 mg lycopene, a cup of chopped kale (130 g) and a serving (242 g) of tomato juice would be enough.[43]

Exposure to UVR is, to date, considered the primary preventable cause of skin ageing, a process that includes loss of elasticity, and drying and wrinkling. The specific genes found to be regulated in the current study might reflect on skin appearance. Accordingly, a significant correlation was found between forehead skin roughness and lycopene concentration in the skin of 40–50-year-old men and women.[44] Moreover, an antioxidant supplement containing TNC in combination with other phytonutrients was previously shown to support parameters related to skin structure and appearance, such as skin density, thickness, and roughness and scaling.[45]

It should also be noted that inhibition of UVA1- and also of UVA/B-induced gene expression by lycopene-rich TNC and lutein supplementation was shown in relatively small groups of only 30 study participants. This observation emphasizes that a combination of a crossover design together with the assessment of gene expression as a highly sensitive biological end point represents a study design well suited to examine the efficacy of nutritional strategies for skin health, including photoprotection.

Further clinical trials addressing *in vivo* effects, such as an increase in the MED, are necessary to verify the impact of the molecular findings on human skin.

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References

1 Krutmann J, Humbert P (eds). *Nutrition for Healthy Skin: Strategies for Clinical and Cosmetic Practice*. Berlin, Heidelberg: Springer, 2011.
2 Stahl W. Systemic photoprotection by carotenoids. In: *Nutrition for Healthy Skin: Strategies for Clinical and Cosmetic Practice* (Krutmann J, Humbert P, eds). Berlin, Heidelberg: Springer, 2011; 65–70.
3 Garmyn M, Ribaya-Mercado JD, Russel RM *et al*. Effect of beta-carotene supplementation on the human sunburn reaction. *Exp Dermatol* 1995; **4**:104–11.
4 Gollnick HPM, Hopfenmüller W, Hemmmes C *et al*. Systemic beta carotene plus topical UV-sunscreen are an optimal protection against harmful effects of natural UV-sunlight: results of the Berlin-Eilath study. *Eur J Dermatol* 1996; **6**:200–5.
5 Heinrich U, Gartner C, Wiebusch M *et al*. Supplementation with beta-carotene or a similar amount of mixed carotenoids protects humans from UV-induced erythema. *J Nutr* 2003; **133**:98–101.
6 Lee J, Jiang S, Levine N *et al*. Carotenoid supplementation reduces erythema in human skin after simulated solar radiation exposure. *Proc Soc Exp Biol Med* 2000; **223**:170–4.
7 Mathews-Roth MM, Pathak MA, Parrish J *et al*. A clinical trial of the effects of oral beta-carotene on the responses of human skin to solar radiation. *J Invest Dermatol* 1972; **59**:349–53.
8 McArdle F, Rhodes LE, Parslew RA *et al*. Effects of oral vitamin E and beta-carotene supplementation on ultraviolet radiation-induced oxidative stress in human skin. *Am J Clin Nutr* 2004; **80**:1270–5.
9 Stahl W, Heinrich U, Jungmann H *et al*. Carotenoids and carotenoids plus vitamin E protect against ultraviolet light-induced erythema in humans. *Am J Clin Nutr* 2000; **71**:795–8.
10 Kopcke W, Krutmann J. Protection from sunburn with beta-Carotene – a meta-analysis. *Photochem Photobiol* 2008; **84**:284–8.
11 Krinsky NI, Johnson EJ. Carotenoid actions and their relation to health and disease. *Mol Aspects Med* 2005; **26**:459–516.
12 Stahl W, Heinrich U, Wiseman S *et al*. Dietary tomato paste protects against ultraviolet light-induced erythema in humans. *J Nutr* 2001; **131**:1449–51.
13 Aust O, Stahl W, Sies H *et al*. Supplementation with tomato-based products increases lycopene, phytofluene, and phytoene levels in

human serum and protects against UV-light-induced erythema. *Int J Vitam Nutr Res* 2005; **75**:54–60.
14 Rizwan M, Rodriguez-Blanco I, Harbottle A *et al.* Tomato paste rich in lycopene protects against cutaneous photodamage in humans *in vivo*: a randomized controlled trial. *Br J Dermatol* 2011; **164**:154–62.
15 Fuller B, Smith D, Howerton A *et al.* Anti-inflammatory effects of CoQ10 and colorless carotenoids. *J Cosmet Dermatol* 2006; **5**:30–8.
16 Young AR, Chadwick CA, Harrison GI *et al.* The similarity of action spectra for thymine dimers in human epidermis and erythema suggests that DNA is the chromophore for erythema. *J Invest Dermatol* 1998; **111**:982–8.
17 Swindells K, Rhodes LE. Influence of oral antioxidants on ultraviolet radiation-induced skin damage in humans. *Photodermatol Photoimmunol Photomed* 2004; **20**:297–304.
18 Nisar MF, Parsons KS, Bian CX *et al.* UVA irradiation induced heme oxygenase-1: a novel phototherapy for morphea. *Photochem Photobiol* 2015; **91**:210–20.
19 Krutmann J, Gilchrest B. Photoaging of skin. In: *Skin Aging* (Gilchrest B, Krutmann J, eds). Berlin, Heidelberg: Springer, 2006; 33–44.
20 Vandergriff T, Bergstresser P. Abnormal responses to ultraviolet radiation: idipathic, probably immunologic, and photoexacerbated. In: *Fitzpatrick's Dermatology in General Medicine* (Goldsmith LA, Katz SI, Gilchrest BA, Paller AS, Leffell DJ, Wolff K, eds), 8th edn. New York: McGraw Hill, 2012; 1049–53.
21 Warstedt K, Furuhjelm C, Duchen K *et al.* The effects of omega-3 fatty acid supplementation in pregnancy on maternal eicosanoid, cytokine, and chemokine secretion. *Pediatr Res* 2009; **66**:212–17.
22 Gause S, Chauhan A. UV-blocking potential of oils and juices. *Int J Cosmet Sci* 2016; **38**:354–63.
23 Simopoulos AP. Omega-3 fatty acids in wild plants, nuts and seeds. *Asia Pacific J Clin Nutr* 2002; **11**:S163–73.
24 Pilkington SM, Watson RE, Nicolaou A *et al.* Omega-3 polyunsaturated fatty acids: photoprotective macronutrients. *Exp Dermatol* 2011; **20**:537–43.
25 Vielhaber G, Grether-Beck S, Koch O *et al.* Sunscreens with an absorption maximum of ≥ 360 nm provide optimal protection against UVA1-induced expression of matrix metalloproteinase-1, interleukin-1, and interleukin-6 in human dermal fibroblasts. *Photochem Photobiol Sci* 2006; **5**:275–82.
26 Tzaneva S, Seeber A, Schwaiger M *et al.* High-dose versus medium-dose UVA1 phototherapy for patients with severe generalized atopic dermatitis. *J Am Acad Dermatol* 2001; **45**:503–7.
27 Polidori MC, Carrillo JC, Verde PE *et al.* Plasma micronutrient status is improved after a 3-month dietary intervention with 5 daily portions of fruits and vegetables: implications for optimal antioxidant levels. *Nutr J* 2009; **8**:10.
28 Stahl W, Sundquist AR, Hanusch M *et al.* Separation of beta-carotene and lycopene geometrical isomers in biological samples. *Clin Chem* 1993; **39**:810–14.
29 Marini A, Grether-Beck S, Jaenicke T *et al.* Pycnogenol® effects on skin elasticity and hydration coincide with increased gene expressions of collagen type I and hyaluronic acid synthase in women. *Skin Pharmacol Physiol* 2012; **25**:86–92.
30 Marini A, Jaenicke T, Grether-Beck S *et al.* Prevention of polymorphic light eruption by oral administration of a nutritional supplement containing lycopene, beta-carotene, and *Lactobacillus johnsonii*: results from a randomized, placebo-controlled, double-blinded study. *Photodermatol Photoimmunol Photomed* 2014; **30**:189–94.
31 Yoshida T, Biro P, Cohen T *et al.* Human heme oxygenase cDNA and induction of its mRNA by hemin. *Eur J Biochem* 1988; **171**:457–61.
32 Goldberg GI, Wilhelm SM, Kronberger A *et al.* Human fibroblast collagenase. Complete primary structure and homology to an oncogene transformation-induced rat protein. *J Biol Chem* 1986; **261**:6600–5.
33 Staunton DE, Marlin SD, Stratowa C *et al.* Primary structure of ICAM-1 demonstrates interaction between members of the immunoglobulin and integrin supergene families. *Cell* 1988; **52**:925–33.
34 McCallum FS, Maden BE. Human 18S ribosomal RNA sequence inferred from DNA sequence. Variations in 18S sequences and secondary modification patterns between vertebrates. *Biochem J* 1985; **232**:725–33.
35 Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 2001; **25**:402–8.
36 McLaughlin JM, Olivo-Marston S, Vitolins MZ *et al.* Effects of tomato- and soy-rich diets on the IGF-I hormonal network: a crossover study of postmenopausal women at high risk for breast cancer. *Cancer Prev Res (Phila)* 2011; **4**:702–10.
37 Handelman GJ, Nightingale ZD, Lichtenstein AH *et al.* Lutein and zeaxanthin concentrations in plasma after dietary supplementation with egg yolk. *Am J Clin Nutr* 1999; **70**:247–51.
38 Widomska J, Zareba M, Subczynski WK. Can xanthophyll-membrane interactions explain their selective presence in the retina and brain? *Foods* 2016; **5**:7.
39 Basu-Modak S, Tyrrell RM. Singlet oxygen: a primary effector in the ultraviolet A/near-visible light induction of the human heme oxygenase gene. *Cancer Res* 1993; **53**:4505–10.
40 Wlaschek M, Briviba K, Stricklin GP *et al.* Singlet oxygen may mediate the ultraviolet A-induced synthesis of interstitial collagenase. *J Invest Dermatol* 1995; **104**:194–8.
41 Grether-Beck S, Olaizola-Horn S, Schmitt H *et al.* Activation of transcription factor AP-2 mediates UVA radiation- and singlet oxygen-induced expression of the human intercellular adhesion molecule 1 gene. *Proc Natl Acad Sci U S A* 1996; **93**:14586–91.
42 Linnewiel-Hermoni K, Khanin M, Danilenko M *et al.* The anti-cancer effects of carotenoids and other phytonutrients resides in their combined activity. *Arch Biochem Biophys* 2015; **572**:28–35.
43 US Department of Agriculture ARS, Nutrient Data Laboratory. USDA National Nutrient Database for Standard Reference, Release 28. Current version: May 2016. Available at: https://www.ars.usda.gov/northeast-area/beltsville-md/beltsville-human-nutrition-research-center/nutrient-data-laboratory/docs/usda-national-nutrient-database-for-standard-reference/ (last accessed 30 January 2017).
44 Darvin M, Patzelt A, Gehse S *et al.* Cutaneous concentration of lycopene correlates significantly with the roughness of the skin. *Eur J Pharm Biopharm* 2008; **69**:943–7.
45 Heinrich U, Tronnier H, Stahl W *et al.* Antioxidant supplements improve parameters related to skin structure in humans. *Skin Pharmacol Physiol* 2006; **19**:224–31.