Eur J Nutr (2005) 44 : 365–370
DOI 10.1007/s00394-004-0536-5

ORIGINAL CONTRIBUTION

Shu-Lan Yeh
Chin-Shiu Huang
Miao-Lin Hu

Lycopene enhances UVA-induced DNA damage and expression of heme oxygenase-1 in cultured mouse embryo fibroblasts

Summary *Background* It has been suggested that carotenoids including lycopene may reduce the risk of photodamage. However, carotenoids are unstable under light exposure and may produce prooxidative effects under certain circumstances. *Aim of the study* We examined whether lycopene inhibits ultraviolet A (UVA)-induced DNA damage and the expression of heme oxygenase-1 (HO-1). We hypothesized that the breakdown of lycopene by UVA irradiation, rather than intact lycopene itself, causes oxidative damage. *Methods* Mouse fibroblasts, C3H10T1/2 (C3H), were first enriched with 10 μM of lycopene in the dark for 2 h before exposure to UVA (22.5 KJ/m^2). Then, DNA damage measured by the single-cell gel electrophoretic assay (comet assay) and the expression of HO-1 measured by western blotting were determined. In addition, we exposed lycopene powder to UVA (22.5 KJ/m^2) to prepare pre-irradiated lycopene (ILP). Then, C3H cells were incubated with ILP for 2 h, and DNA damage and the expression of HO-1 also were determined. *Results* We found that lycopene enrichment did not cause damage to DNA in C3H cells not irradiated with UVA. However, lycopene enrichment strongly induced DNA damage when cells were irradiated with UVA (by ca. 2-fold as compared to control). In addition, lycopene enhanced UVA-induced HO-1 expression by ca. 2.5-fold. UVA irradiation led to a significant loss of lycopene that had been pre-incorporated into C3H cells. When cells were incubated with lycopene that had been pre-irradiated with UVA without subjecting the cells to further UVA irradiation, cellular DNA damage and expression of HO-1 were markedly increased, and these effects of irradiated lycopene were concentration-dependent. *Conclusions* These results demonstrate that lycopene enhances UVA-induced oxidative stress in C3H cells, and they suggest that under UVA irradiation, lycopene may produce oxidative products that are responsible for the prooxidant effects.

Key words lycopene – UVA – DNA damage – HO-1

Received: 4 March 2004
Accepted: 21 July 2004
Published online: 18 November 2004

S.-L. Yeh, PhD
Graduate Institute of Nutritional Science
Chung Shan Medical University
Taichung, Taiwan, ROC

M.-L. Hu, PhD (✉) · C.-S. Huang, MSc
Dept. of Food Science
National Chung-Hsing University
250 Kuo-Kuang Road
Taichung, Taiwan, ROC
Tel./Fax: +886-4/2281-2363
E-Mail: mlhuhu@dragon.nchu.edu.tw

Introduction

Lycopene, an acyclic non-provitamin A carotenoid with 11 linearly arranged conjugated double bounds and found in relatively few foods, has received much research attention because of its potential antioxidant and anticarcinogenic properties [1–3]. Some in vitro studies have shown that lycopene is more efficient than β-carotene, another important carotenoid, in scavenging singlet oxygen [4] and other reactive oxygen species (ROS) [5]. Epidemiological studies have indicated that elevated consumption of tomato significantly reduces mortality from several cancer sites, especially prostate cancer [2, 6]. The beneficial effects of tomato-rich diets are generally attributed to lycopene [1, 2, 6]. Lycopene is thought to play a role in protecting against ultraviolet (UV) radiation because it is destroyed to a *greater* extent than β-carotene in human skin irradiated with UV [7]. Stahl et al. [8] demonstrated that diets rich in tomato paste dramatically reduce UV-induced skin damage. They found that after a 10-week supplementation of 40 g

European Journal of Nutrition (2005) Vol. 44, Number 6

tomato paste per day (containing 16 mg lycopene), the serum levels of lycopene increase significantly in supplemented subjects. At week 10, dorsal erythema formation is lower in the group supplemented with tomato paste than in the control group.

Lycopene has recently been utilized as a component of cosmetics for reducing skin damage induced by UV [9]. However, the results of in vitro studies on the protection of lycopene against ultraviolet A (UVA) damage are inconsistent [10–12]. The UVA (320–380 nm) component of sunlight is particularly associated with oxidative damage involved in photoaging [13–15]. Neither β-carotene nor lycopene alone was found to prevent oxidative stress induced by UVA in human skin cell line [10, 11]. Indeed, both β-carotene and lycopene (0.1–1.0 μM) stimulate UVA-induced mRNA expression of metalloproteinase 1 (MMP-1) and heme oxygenase-1 (HO-1), both of which are associated with UVA-induced skin photoaging and with oxidative stress [10, 11]. In addition, little is known regarding the in vitro effect of lycopene on UVA-induced cellular DNA damage.

In this study, we therefore examined the effect of lycopene on DNA damage induced by UVA and the possibility that UVA-irradiated lycopene (ILP) rather than lycopene itself causes the prooxidative effects. The C3H10T1/2 (C3H) cells, a mouse embryo fibroblast cell line, were incubated with lycopene before exposure to UVA. In addition, we exposed lycopene to UVA in open air for 30 min before incubation with C3H cells. Because the expression of HO-1 is a sensitive marker of cellular oxidative stress [16–19], we measured both DNA damage and HO-1 protein levels to evaluate the prooxidant effect of lycopene with and without UVA irradiation.

Materials and methods

Chemicals

All chemicals used were of reagent or higher grade. Lycopene and tetrahydrofuran (THF; without BHT) was from Wako Co. (Japan). Basal Medium of Eagle, fetal bovine serum, trypsin, penicillin, streptomycin, sodium pyruvate, and non-essential amino acids were from GIBCO/BRL (Maryland, USA).

Cell culture and lycopene incorporation

C3H cells were grown in Basal Medium of Eagle containing 10 % (v/v) FBS, 0.37 % (w/v) $NaHCO_3$, penicillin (100 unit/ml), streptomycin (100 unit/ml) in a humidified incubator under 5 % CO_2 and 95 % air at 37 °C. The cells were harvested at ca. 90 % confluence (10^6 cells/dish). The survival rate of cells was always higher than 95 % by Trypan-blue assay [20]. A stock THF-lycopene solution (10 mM) was prepared freshly before each experiment. An aliquot (10 μl) of THF or the THF-lycopene stock solution was added to C3H cells (ca. 10^6 cells/dish) containing 10 ml medium just before incubation at 37 °C in the dark for 2 h. The cells were then washed three times in phosphate buffered saline (PBS, pH 7.4), and the concentrations of lycopene were determined by HPLC with detection at 470 nm following extraction with ethanol and hexane (1:2;v/v) [21].

UVA irradiation

In brief, cells in 10-cm^2 dishes were washed three times and then covered with 10 ml Hanks balanced salt solution (HBSS) before irradiation with UVA (22.5 KJ/m^2, main output at 365 nm) using a UV irradiation chamber (XL-1000 UV crosslinker, Spectronics Corporation, USA). After irradiation, cells were incubated with the fresh Basal Medium of Eagle. Sham-irradiated cells were treated in the same manner except that they were not irradiated. To prepare pre-irradiated lycopene (ILP), an aliquot (100 μl) of the stock lycopene was evaporated to form dry powder followed by UVA irradiation (22.5 KJ/m^2). At this point, the ILP powder was re-dissolved in the original volume of THF and was referred to as 10 mM ILP stock, which contained only 30 ± 3 % residual LP, as determined by HPLC with detection at 470 nm.

Comet assay

Immediately after irradiation or incubation with ILP for 2 h, cell viability was determined using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) colorimetric assay [22] and Trypan-blue assay. The C3H cells were then harvested for the examination of DNA damage by using the comet assay adapted from the method of Singh et al. [23]. Briefly, after oxidant treatment, cells were suspended in a low-melting-point agarose in PBS at 37 °C and pipetted onto a frosted glass microscope slide pre-coated with a layer of 1 % normal-melting-point agarose, the slides were immersed in cold-lysing solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1 % sodium lauryl sarcosinate, 1 % Triton X-100) for 1 h at 4 °C. The slides were then placed in an electrophoresis tank, allowing the DNA to unwind for 15 min in the alkaline solution (300 mM NaOH and 1 mM EDTA). Electrophoresis was then performed at 300 mA for 20 min in the same alkaline solution at room temperature. The slides were then neutralized with 0.4 M Tris-HCl buffer (pH 7.4) and stained with ethidium bromide. Each data pint was determined by 3 experiments, 2 slides for each experiment, and 30 comets on each slide. The images were analyzed by computer using the

Image Pro Plus software (Media Cybernetics, USA) as %DNA in tail [24, 25].

Western blots of HO-1

Our preliminary data show that UVA irradiation of C3H cells markedly increased HO-1 protein between 3 and 6 h post-irradiation (data not shown). Therefore, we harvested the cells at 3 h post UVA irradiation. In some experiments the cells were incubated with LP or ILP at 37 °C for 2 h without UVA irradiation. Briefly, the medium was removed and cells were lysed with 20 % sodium dodecyl sulfate (SDS) containing 1 mM phenylmethylmethylsulfonyl fluoride (PMSF). The lysate was sonicated for 30 seconds on ice, followed by centrifugation for 30 min at 4 °C. An amount of protein (40 μg) from the supernatant was resolved by SDS-polyacrylamide gel electrophoresis and transferred onto a nitrocellulose membrane. After blocking with TBS buffer (20 mM Tris-HCl, 150 mM NaCl, pH 7.4) containing 5 % nonfat milk, the membrane was incubated with anti-heme-oxygenase-1 monoclonal antibody (StressGen Biotechnologies Co, Victoria, BC Canada) followed by horseradish peroxidase-conjugated antimouse IgG, and then visualized using the ECL chemiluminescent detection kit (Amersham Co, Bucks, UK).

Data analysis

Values are expressed as means ± SD of three replicates and analyzed by using Student's *t*-test for two-group comparison or using one-way ANOVA followed by Duncan's multiple range test for comparisons of group means. A P value < 0.05 was considered statistically significant.

Results

Lycopene uptake and consumption

After incubation with 10 μM lycopene for 2 h at 37 °C in the dark, C3H cells incorporated ca. 1.4 nmol lycopene/10^6 cells (data not shown). UVA exposure led to rapid consumption of lycopene of the cells (Fig. 1). After UVA exposure for 30 min (22.5 KJ/m^2), ca. 20 % of incorporated lycopene remained in cells, whereas 70 % of the lycopene remained in cells without UVA irradiation.

DNA damage and cell viability

Comet images of DNA damage in C3H were determined after irradiation with 22.5 KJ/m^2 UVA. UVA-irradiation

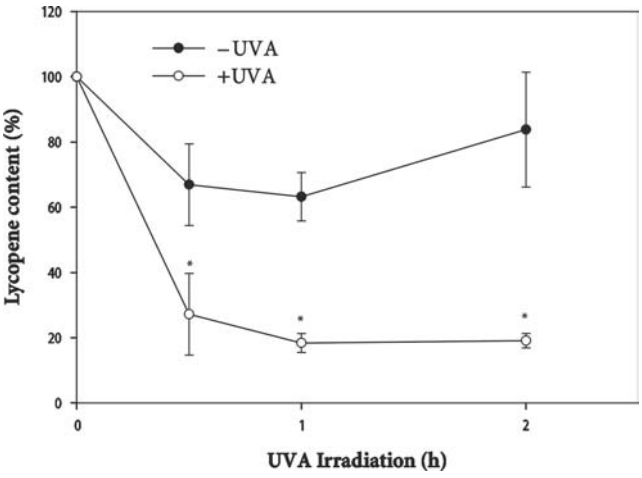

Fig. 1 Consumption of incorporated lycopene in C3H cells by UVA irradiation for 0.5, 1 and 2 h (equivalent to 22.5, 45 and 90 KJ/m^2, respectively). Values (means ± SD, n = 3) with * are significantly different from the group without UVA irradiation at the same irradiation time (student's *t* test $P < 0.05$)

significantly led to comet formation in cellular DNA. Pre-incubation with lycopene significantly enhanced % DNA in tail of cells over the control by about 2-fold (Fig. 2). To examine the attribution of degraded products of lycopene, ILP was incubated with C3H cells for 2 h without subsequent UVA irradiation. The results showed that ILP itself led to DNA damage in C3H cells in a dose-dependent manner (Fig. 3). However, using MTT assay to determine the viability after various treatments, we found no significant differences between the control (without any treatment) and various treatments (Fig. 4). We also used Trypan-blue assay to determine the viability, and the result was consistent with that of

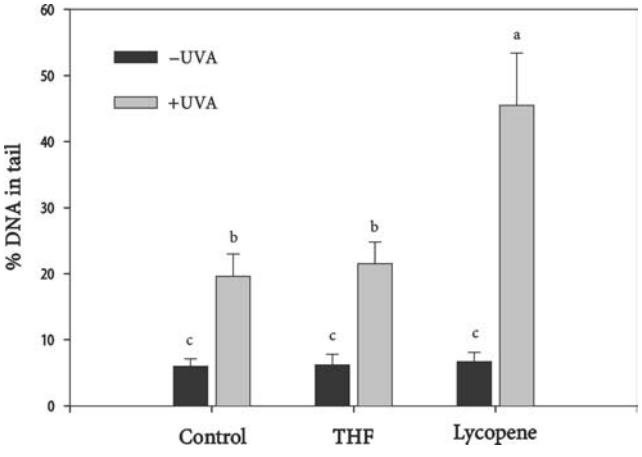

Fig. 2 Effect of lycopene incubation (10 μM) on DNA damage in C3H cells irradiated with 22.5 KJ/m^2 UVA. Lycopene was dissolved in tetrahydrofuran (THF), which serves as the solvent control. Values (mean ± SD, n ≥ 3) with different superscript letters are significantly different ($P < 0.05$)

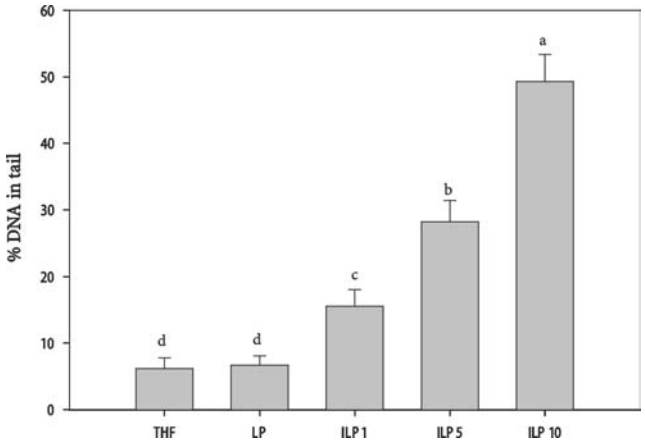

Fig. 3 Concentration effect of pre-irradiated lycopene (ILP) on DNA damage of C3H cells. ILP1, ILP5 and ILP10 represent 1, 5, and 10 μM of ILP. Lycopene was dissolved in tetrahydrofuran (THF), which serves as the solvent control. Values (mean ± SD, n = 3) with different letters are significantly different ($P < 0.05$)

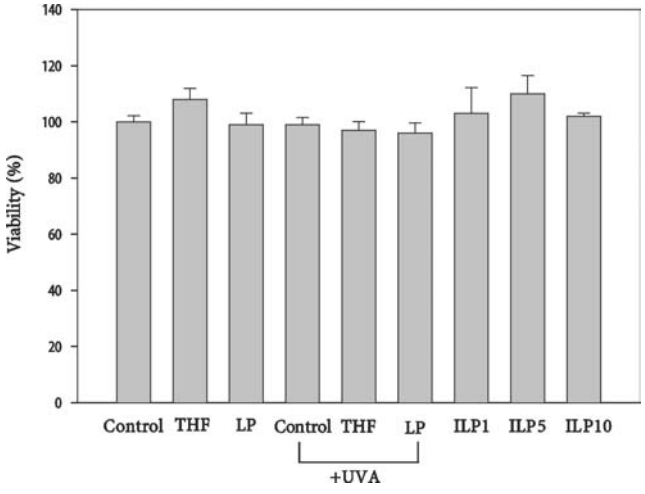

Fig. 4 Effects of lycopene (LP), UVA irradiation (UVA) and pre-irradiated lycopene (ILP) on the viability of C3H cells. LP and ILP were dissolved in tetrahydrofuran (THF), which serves as the solvent control. ILP1, ILP5 and ILP10 represent 1, 5, and 10 μM of ILP. Values (mean ± SD, n = 3) are not significantly different ($P > 0.05$)

MTT assay (data not shown). In addition, as shown in Fig. 5, UVA exposure (11.25 KJ/m^2 and 22.5 KJ/m^2) led to a dose-dependent loss of absorption of lycopene between 300 and 550 nm, with little changes below 300 nm.

HO-1 protein expression

The expression of HO-1 protein in C3H cells was strongly induced by UVA irradiation (Fig. 6). Lycopene enrichment markedly enhanced the level of HO-1 protein induced by UVA by ca. 2.5-fold. Incubation of cells with 10 μM lycopene without subsequent UVA irradia-

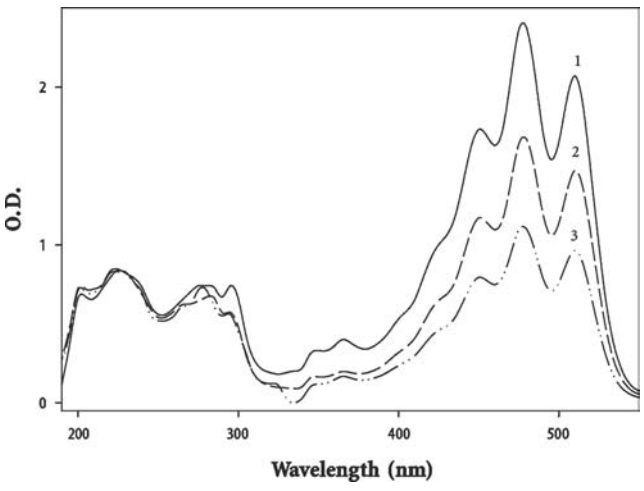

Fig. 5 Bleaching of lycopene (dissolved in tetrahydrofuran) by UVA. Numbers 1, 2 and 3 denote absorption spectra recorded after UVA irradiation at 0, 11.2 and 22.5 KJ/m^2

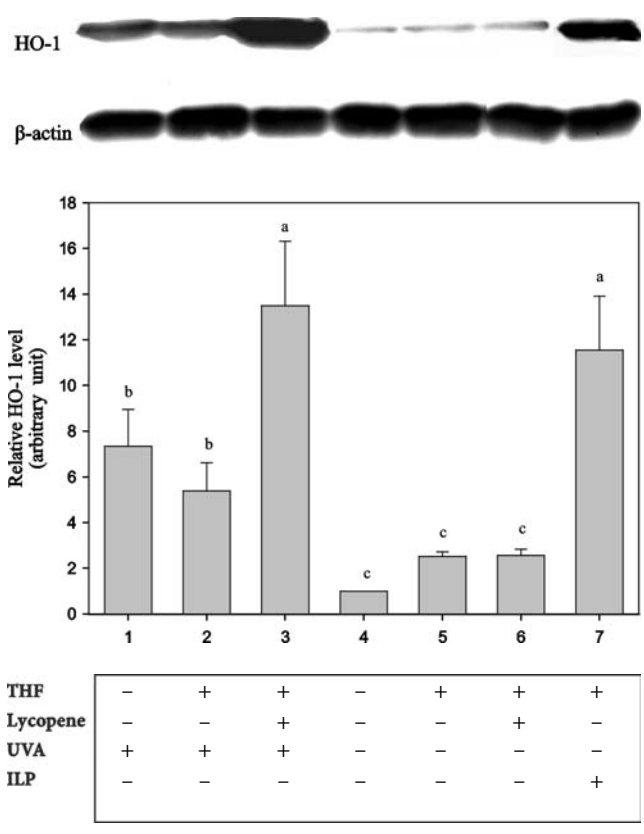

Fig. 6 Western blots showing the effect of 10 μM lycopene on HO-1 protein induced by UVA and the effect of 10 μM pre-irradiated lycopene (ILP) itself on HO-1 protein. C3H cells were pre-incubated with (+) and without (–) lycopene or ILP before irradiation with and without UVA (22.5 KJ/m^2). Cells were harvested 3 h post UVA irradiation (lane 1–3) or immediately after incubation with test compounds for 2 h (lane 4–7). For loading control, expression levels of β-actin were analyzed using the same lysate. The relative HO-1 levels were quantitated by Matrox Inspector 2.1 software. Values (mean ±SD, n = 3) with different letters are significantly different ($P < 0.05$)

tion did not affect the HO-1 protein level. In contrast, enrichment with 10 μM ILP markedly increased the level of HO-1 protein.

Discussion

The present study employed C3H cells enriched with lycopene followed by exposure to UVA to investigate the effects of lycopene on UVA-induced DNA damage, as it has been suggested that lycopene may be a better antioxidant than β-carotene, including the photoprotective effect [7]. Under the present experimental conditions, we showed that lycopene enrichment actually enhanced DNA damage in C3H cells exposed to UVA. The results indicate that lycopene is prooxidative under UVA irradiation, and they complement the finding by Offord et al. [11] that lycopene promotes HO-1 and MMP-1 expression induced by UVA. These properties of lycopene are similar to those of β-carotene, as Obermuller-Jevic et al. [10] had reported that β-carotene or its degradation product enhances UVA-induced lipid peroxidation and the subsequent activation of the HO-1 cascade.

We further demonstrated that, when C3H cells were incubated with pre-irradiated lycopene without subsequent UVA irradiation, DNA damage and the induction of HO-1 protein were markedly enhanced, whereas the non-irradiated lycopene had no effect. These results suggest that the degradation products of lycopene induced by UVA are at least partially responsible for the prooxidative effect of lycopene in C3H cells subsequently exposed to UVA. Indeed, Fuhrman et al. [26] have suggested that the oxidative products of lycopene are not stable and are capable of inducing oxidation. They found that lycopene in combination with vitamin E, glabridin, rosmarinic acid or carnosic acid synergistically inhibits the oxidation of LDL, and they suggested that the synergistic effects may be attributed to the interaction of these antioxidants with lycopene-derived radicals and/or with oxidative products of lycopene. Indeed, it has been envisioned that a combination of natural antioxidants, including lycopene, may be required for effective photoprotection [11]. Although we did not identify the oxidative products of UVA-exposed lycopene, it has been reported that Apo-6'-lycopene, 2-methyl-hepte-6-one, as well as further reaction products, are formed during irradiation of lycopene [9, 27].

A question that may be asked is whether the doses of lycopene and UVA used in the present in vitro study has any bearing in vivo. The mean human concentrations, which vary widely in different populations, range from 50 to 900 nM. Thus, the concentration (10 μM) used in the present study seems rather high. However, it is possible that the serum or skin lycopene concentration may be greatly increased by high intake of the products of tomato and by using of lycopene-enriched cosmetics [9, 28]. Thus, it is possible that the lycopene doses used here are not exceedingly high and can be reached in vivo under certain circumstances. Regarding the dose of UVA, our irradiation dose (22.5 KJ/m^2), which significantly induced DNA damage in C3H cells, is much lower than the average dose (500 KJ/m^2) capable of inducing minimal erythema in human skin [29].

In summary, the present study in C3H cells demonstrates that lycopene possesses prooxidant activity when exposed to UVA and that the oxidative derivatives of lycopene rather than itself may be responsible for the increased oxidative stress. The present results suggest that it may be premature to use lycopene as a component of cosmetics and that further studies are needed to ascertain the safety of using lycopene, and perhaps other carotenoids in cosmetics.

Acknowledgment This research was supported by a grant (NSC-92–2320-B005–003) from the National Science Council, ROC.

References

1. Stahl W, Sies H (1996) Lycopene: a biologically important carotenoid for humans? Arch Biochem Biophys 336:1–9
2. Clinton SK (1998) Lycopene: chemistry, biology, and implication for human health and disease. Nutr Rev 56:35–51
3. Bramley PM (2000) Is lycopene beneficial to human health? Phytochemistry 54:233–236
4. Di Mascio P, Kaiser S, Sies H (1989) Lycopene as the most efficient biological carotenoid singlet oxygen quencher. Arch Biochem Biophys 274:532–538
5. Miller NJ, Sampson J, Candeias LP, Bramley PM, Rice-Evans CA (1996) Antioxidant activities of carotenes and xanthophylls. FEBS Lett 384:240–242
6. Rao AV, Agarwal S (1999) Role of lycopene as an antioxidant carotenoid in the prevention of chronic disease: a review. Nutr Res 19:305–323
7. Ribaya-Mercado JD, Garmyn M, Gilchrest BA, Russell RM (1995) Skin lycopene is destroyed preferentially over β-carotene during ultraviolet irradiation in humans. J Nutr 125:1854–1858
8. Stahl W, Heinrich U, Wiseman S, Eichler O, Sies H, Tronnier H (2001) Dietary tomato paste protects against ultraviolet light-induced erythema in humans. J Nutr 131:1449–1451
9. Harang B (2000) Composition having tanning and photoprotective activity, and its cosmetic applications. Official Gazette of the United States Patent and Trademark Office Patents 1237(5)
10. Obermuller-Jevic UC, Francz PI, Frank J, Flaccus A, Biesalski HK (1999) Enhancement of the UVA induction of haem oxygenase-1 expression by beta-carotene in human skin fibroblasts. FEBS Lett 460:212–216
11. Offord EA, Gautier JC, Avanti O, Scaletta C, Runge F, Kramer K, Applegate LA (2002) Photoprotective potential of lycopene, vitamin E, vitamin C and carnosic acid in UVA-irradiated human skin fibroblasts. Free Radic Biol Med 32:1293–1303

12. Trekli MC, Riss G, Goralczyk R, Tyrrell RM (2003) Beta-carotene suppresses UVA-induced HO-1 gene expression in cultured FEK4. Free Radic Biol Med 34:456–464
13. Tyrrell RM (1991) UVA (320–380 nm) radiation as an oxidative stress. In: Sies H (ed) Oxidative stress: oxidants and antioxidants. Academic Press, London, pp 57–83
14. Scharffetter K, Wlaschek M, Hogg A, Bolsen K, Schothorst A, Goerz G, Krieg T, Plewig G (1991) UVA irradiation induces collagenase in human dermal fibroblasts in vitro and in vivo. Arch Dermatol Res 283:506–511
15. Keyse SM, Tyrrell RM (1989) Heme oxygenase is the major 32-kDa stress protein induced in human skin fibroblasts by UVA radiation, hydrogen peroxide, and sodium arsenite. Proc Natl Acad Sci USA 86:99–103
16. Lundby C, Pilegaard H, van Hall G, Sander M, Calbet J, Loft S, Moller P (2003) Oxidative DNA damage and repair in skeletal muscle of humans exposed to high-altitude hypoxia. Toxicology 192:229–236
17. Applegate LA, Luscher P, Tyrrell RM (1991) Induction of heme oxygenase: a general response to oxidant stress in cultured mammalian cells. Cancer Res 51:974–978
18. Keyse SM, Applegate LA, Tromvoukis Y, Tyrrell RM (1990) Oxidant stress leads to transcriptional activation of the human heme oxygenase gene in cultured skin fibroblasts. Mol Cell Biol 10: 4967–4969
19. Ryter SW, Tyrrell RM (2000) The heme synthesis and degradation pathways: role in oxidant sensitivity. Heme oxygenase has both pro- and antioxidant properties. Free Radic Biol Med 28: 289–309
20. Phillips HJ (1973) Dye exclusion tests for cell viability. In: Tissue culture, method and applications. Academic Press, London, pp 406–408
21. Rundhaug JE, Pung A, Read CM, Bertram JS (1988) Uptake and metabolism of beta-carotene and retinal by C3H/10T1/2. Carcinogenesis 9: 1541–1545
22. Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 65:55–63
23. Singh NP, McCoy MT, Tice RR, Schneider EL (1988) A simple technique for quantitation of low levels of DNA damage in individual cells. Exp Cell Res 175:184–191
24. Olive PL, Banath JP, Durand RE (1990) Heterogeneity in radiation-induced DNA damage and repair in tumor and normal cells using the comet assay. Radiat Res 122:86–94
25. Collins AR (1992) Workshop on single cell gel electrophoresis (the 'comet assay'). Mutagenesis 7:357–358
26. Fuhrman B, Volkova N, Rosenblat M, Aviram M (2000) Lycopene synergistically inhibits LDL oxidation in combination with vitamin E, glabridin, rosmarinic acid, carnosic acid, or garlic. Antioxid Redox Signal 2:491–506
27. Ukai N, Lu Y, Etoh H, Yagi A, Ina K, Oshima S, Ojima F, Sakamoto H, Ishiguro Y (1994) Photosensitized oxygen-action of lycopene. Biosci Biotechnol Biochem 58:1718–1719
28. Sies H, Stahl W (2003) Non-nutritive bioactive constituents of plants: lycopene, lutein and zeaxanthin. Int J Vitam Nutr Res 73:95–100
29. Luy H, Frenk E, Applegate LA (1994) Ultraviolet A-induced cellular membrane damage in the photosensitivity dermatitis/actinic reticuloid syndrome. Photodermatol Photoimmunol Photomed 10:126–133