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Protective effects of tomato extract with elevated β-carotene levels on oxidative stress in ARPE-19 cells

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Epidemiological studies show that dietary products rich in carotenoids delay the progression of age-related macular degeneration. Experimental evidence from cellular studies on the antioxidant actions of carotenoids in the retinal pigment epithelium is still, however, fragmentary. The present study examined the uptake and protective potential of dietary carotenoids from tomato on the human retinal pigment epithelial cell line ARPE-19. ARPE-19 cells were incubated in medium supplemented with tomato extract containing high levels of β-carotene, lycopene and traces of lutein. The cellular uptake of carotenoids was analysed by reverse-phase HPLC. Oxidative stress was induced by treatment with 1 mM-H_2O_2. Nitrotyrosine was detected by immunocytochemistry, and oxidised proteins (protein carbonyls) were measured by a quantitative ELISA method. Lipid peroxidation was assessed by quantifying thiobarbituric acid reactive substances. ARPE-19 cells preferentially accumulated lutein and β-carotene rather than lycopene. Nitrotyrosine formation was considerably reduced in cells incubated with tomato extract compared with controls after H_2O_2 treatment. Protein carbonyls were reduced by 30 % (P=0·015), and the formation of thiobarbituric acid-reactive substances was reduced by 140 % (P=0·003) in cells incubated with tomato extract. The present study provides the experimental evidence for protective effects of dietary tomatoes rich in carotenoids on oxidative stress in the retinal pigment epithelium.

β-carotene: Oxidative stress: Retina: AMD: Retinal pigment epithelium: Tomato: Carotenoids

Several epidemiological studies have shown the beneficial effect of tomato consumption on the prevention of some major chronic diseases, such as CVD and some types of cancer (Giovannucci, 1999; Willcox *et al.* 2003). The intake of tomato and tomato-based food products contributes to the absorption of a wide range of carotenoids in human serum and tissues (Tonucci *et al.* 1995; Paetau *et al.* 1999; van het Hof *et al.* 2000).

Carotenoids are among the most important pigments occurring in living organisms, including fruits and vegetables (Gross, 1987). Epidemiological studies have shown that individuals who consume a relatively large quantity of carotenoid-rich fruits and vegetables have a decreased risk of ocular disease conditions such as age-related macular degeneration (AMD; Snodderly, 1995; Mayne, 1996). AMD is the leading cause of blindness in the elderly population of the developed world (Hawkins *et al.* 1999). The retina, especially the macula, is continuously exposed to high levels of focused radiant energy in a highly oxygenated environment (Winkler *et al.* 1999). This simultaneous presence of light and oxygen together gives the potential for oxygen free radicals and singlet oxygen to be generated (Roberts, 2001; Glickman, 2002). Oxidative processes are thought to play an important role in the pathogenesis of AMD (Beatty *et al.* 2000).

Nutritional status has been proposed as a potential risk factor in AMD. The frequency and intake of consumption of fruits and vegetables rich in carotenoids is negatively correlated with the risk of AMD (Hogg & Chakravarthy, 2004; Van Leeuwen *et al.* 2005; Seddon *et al.* 1994). In an interventional study, supplementation with β-carotene along with other antioxidants resulted in a significant reduction in the development of advanced AMD (Age-Related Eye Disease Study Group, 2001). The intake of α-carotene and β-carotene, as well as the baseline intake of non-provitamin A carotenoids, is associated with a reduced risk of large drusen (Van den Langenberg *et al.* 1998).

The protective effects of β-carotene on light-induced retinal damage have also been reported in monkeys and rats (Ham *et al.* 1984; Tso, 1989). β-Carotene and lycopene are present, along with lutein and zeaxanthin, in human retinal pigment epithelium (RPE)/choroid tissue (Bernstein *et al.* 2001). We have previously shown that RPE cells efficiently take up and metabolise β-carotene (Chichili *et al.* 2005).

Abbreviations: AMD, age-related macular degeneration; MDA, malondialdehyde; RPE, retinal pigment epithelium.

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Carotenoids are efficient singlet O quenchers (Hirayama *et al.* 1994; Krinsky, 1998; Cantrell *et al.* 2003). Although the antioxidant effects of carotenoids are well recognised, surprisingly there is little evidence that carotenoids possess this property in cells of retinal origin. In the present study, we demonstrate carotenoid uptake, and the protective potential of those carotenoids, from tomato with elevated β-carotene levels on H_2O_2-induced oxidative damage by investigating protein nitrosylation, protein oxidation and lipid peroxidation in ARPE-19 cells.

Materials and methods

Cell culture

The ARPE-19 cell line was obtained from American Type Culture Collection (Manassas, VA, USA). Cells were grown in a 1:1 (v/v) mixture of Dulbecco's Modified Eagle's Medium and HAM's F 12 (Biochrom, Berlin, Germany) supplemented with 10 % fetal calf serum and 2·5 mM-L-glutamine. The cells were seeded at high density (100 000 cells/cm^2) and maintained in culture by feeding weekly.

Preparation of tomato extract for supplementation

Tomato plants with elevated β-carotene levels were produced by manipulating the carotenoid biosynthetic pathway (Roemer *et al.* 2000). Plants were cultivated under glasshouse conditions. The fruits were quartered, the seeds were removed, and the remaining pericarp tissue was freeze-dried to complete dryness. A homogenous powder was made by homogenisation in a freezer-mill (6750) apparatus (Glen Creston Ltd, Middlesex, UK) for 1 min at 70 % full power.

The powder was transferred into a 15 ml tube, and 8 ml n-hexane was added, the mixture being shaken for 30 min. The tubes were then centrifuged at 3000 ***g*** for 1 min, and the hexane phase was transferred into a new tube. The extraction procedure was repeated three times, and n-hexane phases were pooled and stored at −80°C until use. Carotenoid concentrations in the extracts were analysed by HPLC as described for cells in the subsequent paragraph. n-Hexane tomato extract (1 ml) was transferred into a sterile glass tube and dried under the stream of nitrogen gas. The dried residues were dissolved in 1 ml absolute ethanol and used for supplementation. One-week-old confluent ARPE-19 cell cultures were maintained in medium without fetal calf serum for 48 h and then incubated with medium with tomato extract containing 1 μM-β-carotene at final concentration for 24 h. Control cells received the same amount of ethanol.

Extraction and quantification of carotenoids from cells

After incubation, the cell monolayers were washed thoroughly three times with PBS. Cells were scraped, collected in 5 ml PBS and sedimented by centrifugation. The cell pellet was dissolved in 0·5 ml PBS, and cells were disrupted by three freeze–thaw cycles in liquid nitrogen. Absolute ethanol (0·2 ml) was added to the cell suspension, mixed by vortexing and extracted with 2 ml n-hexane. The extraction with n-hexane was repeated three times, after which the n-hexane phases were pooled and evaporated under the stream of nitrogen gas. The residues were dissolved in 0·2 ml acetonitrile and analysed by HPLC.

Reverse-phase HPLC analysis was carried by an isocratic method described by Nomura *et al.* (1997) using the Spherisorb ODS 2 (3 μM) column (GROM, Rottenburg-Hailhingen, Germany; dimensions 250 mm × 4 mm). The solvent contained 82 % acetonitrile, 15 % dioxane and 3 % methanol (with 0·1 % triethylamine). Carotenoids were analysed by UV/visible-detection at a fixed wavelength of 450 nm. Chromatograms were recorded and analysed with Star Chromatography Workstation software version 5.31 (Varian, Germany). Authentic standards of all-*trans*-β-carotene, lutein, lycopene (Sigma, Tauf-kirchen, Germany) and zeaxanthin (gift from Hoffmann-la Roche, Basel, Switzerland) were included in every run for detection and determination of concentration. Carotenoid concentrations were normalised to DNA content in corresponding cell pellets, determined by diphenylamine assay (Natarajan *et al.* 1994).

Induction of oxidative stress

After incubation with tomato extract, cells were washed thoroughly three times with PBS and treated with 1 mM-H_2O_2 in PBS for 30 min at room temperature. Controls were treated with PBS for the same time.

Nitration of proteins (nitrosative stress)

ARPE-19 cells were grown on glass cover slips placed in cell culture dishes. After incubation with tomato extract, the cells were washed three times with PBS and treated with 1 mM-H_2O_2 and 1 mM-$NaNO_2$ for 30 min at room temperature. After oxidant treatment, the cover slips were taken out, washed three times with PBS and fixed in 4 % paraformaldehyde for 20 min at room temperature.

After three washes with PBS, cover slips were blocked with 1 % blocking reagent (Dako, Carpinteria, CA, USA) with (1:10 v/v) normal donkey serum for 1 h at room temperature followed by incubation with rabbit polyclonal anti-nitrotyrosine antiserum (Upstate Biotechnologies, Lake Placid, NY, USA) overnight at 4°C. After three washes with PBS, cover slips were incubated in fluorescein-isothiocyanate-labelled anti-rabbit immunoglobulin G from donkey for 1 h at room temperature, washed three times with PBS, mounted in anti-fade glycerol (Molecular Probes, Eugene, OR, USA) and photographed using a fluorescent microscope (Axiophot; Zeiss, Ober-kochen, Germany) attached to an imaging system. The specificity of staining of the nitrated proteins was verified by pre-absorption of the diluted anti-nitrotyrosine polyclonal antiserum with 10 mM-nitrotyrosine in PBS for 1 h at room temperature. This solution was used instead of the primary antibody and was expected to result in no staining.

Protein carbonylation

Protein carbonyls were measured as described by Buss *et al.* (1997) with minor modifications. Cell monolayers were scraped, collected into 5 ml PBS and sedimented by centrifugation. Lysis buffer 100 μl (0·1 M-sodium phosphate buffer, 1 % digitonin, 0·5 M-EDTA and protease inhibitors) was added to the cell pellet and homogenised using plastic

homogenisers for 1·5 ml Eppendorf tubes. The lysates were incubated at room temperature for 15 min and centrifuged at 5000 ***g*** for 10 min, and the supernatants were saved. Streptomycin sulphate (10 %, 10 μl) was added to the supernatant and incubated at room temperature for 15 min. The samples were centrifuged at 6000 ***g*** for 10 min, and the supernatants were collected.

Protein concentrations in the samples were assayed by Biorad DC Protein Assay (Biorad, Munich, Germany). Protein (60 μg) was mixed with 45 μl dinitrophenylhydrazin solution (2 mg/ml dinitrophenylhydrazin in 6 M-guanidium hydrochloride and 0·5 M-potassium dihydrogen phosphate, pH 2·5) and incubated in the dark for 45 min at room temperature. A volume of 5 μl of the mixture was added to 1 ml coating buffer (10 mM-sodium phosphate buffer and 140 mM-NaCl, pH 7·0); this was added to a microtitre plate (Nunc immunoplate Maxisorb Nunc, Wiesbaden, Germany) at 200 μl/well and incubated overnight at 4°C in the dark.

The next day, the contents of the plate were emptied and blocked with 0·1 % reduced bovine serum albumin for 90 min in the dark at room temperature. After incubation, the contents were emptied; anti-dinitrophenylhydrazin antibody was added and the mixture was incubated at 37°C for 1 h. The plate was then washed three times with PBS and incubated with peroxidase-conjugated anti-rabbit immunoglobulin G for 1 h at room temperature in the dark. The cells were washed three times with PBS and then incubated with 200 μl of a solution containing *o*-phenylenediamine (0·6 mg/ml) and H_2O_2 (stock diluted 1:2500 v/v) in 50 mM-Na_2HPO_4 and 24 mM-citric acid and left to develop colour for 30 min at 37°C before stopping the reaction with 100 μl 2·5 M-H_2SO_4. Absorbance was read with a 492 nm filter (reference filter 750 nm) using a BioKinetics Microplate reader (Reader EL 340; Bio-Tek Instruments, Winooski, VT, USA). An eight-point standard curve consisting of oxidised and reduced bovine serum albumin ranging from 0·2 to 0·8 nmol protein carbonyl/mg protein was included with each plate and used for quantification.

Lipid peroxidation

The analysis of thiobarbituric acid-reactive substances as a marker of lipid peroxidation was carried out as previously described by Jentzsch *et al.* (1996). Briefly, cells were washed three times with PBS, scraped in 5 ml PBS and pelleted by centrifugation at 2000 ***g*** for 5 min. The cell pellet was dissolved in 300 μl PBS, and an aliquot was stored at −80°C for protein determination. A volume of 200 μl cell suspension was mixed with 10 μl 227 mM-butylated hydroxy toluene, followed by the addition of 200 μl 0·2 M-H_3PO_4 and 25 μl thiobarbituric acid (16 mg/ml). The tubes were vortexed and incubated for 45 min at 90°C in a water bath. Then 500 μl n-butanol was added, mixed by vortexing and centrifuged at 7000 ***g*** for 1 min. Butanol phase (250 μl) was transferred into a microplate, and the fluorescence was read at wavelengths 530 nm (excitation) and 590 nm (emission). For calibration in the range of 0·05–2·0 μM-malondialdehyde (MDA; freshly synthesised by adding 50 μl 10 mM-tetramethoxy propane to 10 ml 0·01 M HCl and keeping at room temperature for 10 min) was used. MDA concentrations in the test samples were normalised to the total protein content determined by Dc protein assay (Biorad). The amount of lipid peroxidation was expressed in micromoles of MDA formed per milligram of protein.

Statistical analysis

The statistical significance comparing the treated conditions with the controls was determined by using a paired Student's *t* test in Microsoft Excel software (unterschleibheim, Germany). $P < 0{\cdot}05$ was considered significant.

Results

Protective effects of tomato extract against oxidative stress in ARPE-19 cells

Protein nitration. Peroxynitrite is formed in biological systems when superoxide and NO are produced together. It is a powerful oxidant exhibiting a wide array of tissue-damaging effects including lipid peroxidation, inactivation of enzymes and ion channels via protein oxidation, and nitration. Furthermore, nitrosative stress is involved in the cellular suicide mechanism.

To determine whether carotenoids from tomato protect against protein nitration, ARPE-19 cells were maintained in the medium supplemented with tomato extract and treated with of 1 mM-H_2O_2 and 1 mM-$NaNO_2$ for 30 min at room temperature. The cells were then subjected to immunocytochemistry with polyclonal anti-nitrotyrosine antibody. As judged by nitrotyrosine detection with immunocytochemistry, the treatment induced a considerable amount of nitrotyrosine formation. As can be seen in Fig. 1, very strong nitrotyrosine staining was observed in the control cells, treated with H_2O_2 and $NaNO_2$. In cells incubated with tomato extract prior to H_2O_2 treatment, nitrotyrosine formation was considerably reduced (Fig. 1 (C)). These results indicate that carotenoids from tomato extract protected RPE cells against oxidative stress-induced protein nitration.

Protein carbonylation. The introduction of carbonyl groups into the amino acid residues of proteins is a hallmark of oxidative modification. The carbonyl content of proteins is therefore an index of the amount of oxidative protein damage attributable to either direct attack from free radicals or the modification of proteins by the oxidation products of carbohydrates or PUFA. Therefore, protein carbonyl formation is used in a multitude of publications as a parameter for oxidant stress. To assess the protective effects of tomato extract on protein carbonylation, ARPE-19 cells were incubated with or without tomato extract and subjected to H_2O_2 treatment for 30 min at room temperature. Protein carbonyls were measured by quantitative ELISA in total protein that was isolated from H_2O_2 -treated cells. A significant amount of carbonyl formation was observed after H_2O_2 treatment. In cells incubated with tomato extract prior to H_2O_2 treatment, protein carbonylation was reduced by 30 % ($P = 0{\cdot}035$) compared with the control cells (Fig. 2), suggesting that tomato extract could reduce the protein carbonylation caused by oxidative stress.

Lipid peroxidation. To assess lipid peroxidation, thiobarbituric acid reactive substances were measured in ARPE-19 cells incubated with tomato extract after treatment with

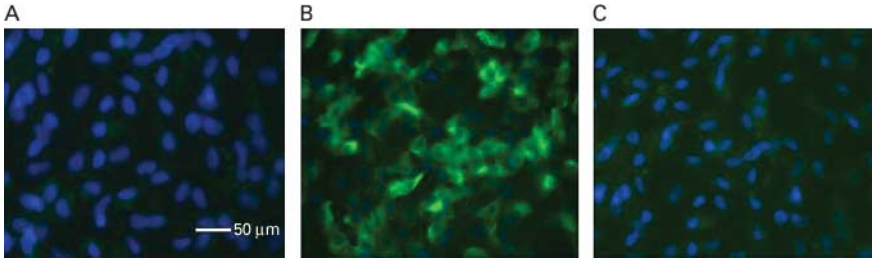

Fig. 1. Nitrotyrosine in H_2O_2- and $NaNO_2$-treated ARPE-19 cells incubated with or without tomato extract. ARPE-19 cells were grown on glass cover slips to confluence, incubated with tomato extract for 24 h and treated with 1 mM-H_2O_2 and 1 mM-$NaNO_2$ for 30 min at room temperature. The cells were immunostained for nitrotyrosine with anti-nitrotyrosine antibody. The immunofluorescence of nitrotyrosine in (A) a negative control, (B) control cells and (C) cells incubated with tomato extract prior to the H_2O_2 and $NaNO_2$ treatment is shown. The experiment was repeated three times in triplicate, and the images represent the result of a representative experiment. Blue fluorescence, nuclear staining (DAPI), green fluorescence, nitrated proteins.

H_2O_2. Freshly prepared MDA was used for the calibration. High amounts of MDA were formed in H_2O_2-treated cells. In cells incubated with tomato extract prior to H_2O_2 treatment, MDA formation was reduced by 140 % (P=0·003), indicating that carotenoids from tomato extract could successfully reduce the lipid peroxidation caused by oxidative stress (Fig. 3).

Carotenoid uptake from tomato extracts by ARPE-19 cells

The predominant fat-soluble antioxidants present in the hexane extract of tomato were carotenoids (β-carotene 1200 µg/g DW, lutein 50 µg/g DW, lycopene 750 µg/g DW, zeaxanthin not detectable). Tocopherols, principally α-tocopherol, is also present (170 µg/g DW), with trace levels of γ-tocopherols.

To determine carotenoid uptake, ARPE-19 cells were incubated in medium supplemented with tomato extract for 24 h, and total carotenoids were extracted from cells and analysed by HPLC. Tomato extract was completely soluble in ethanol at the concentration used in the present study. Carotenoid stability in the medium was determined in the experimental conditions and found to be unchanged until 24 h. The reason why only β-carotene was detectable in the control cells might be due to the β-carotene present in the fetal calf serum used for cell culture. Cell cultures were maintained in medium without serum for 48 h before using for experiments. All the supplementation experiments were performed in medium without serum.

Interestingly, ARPE-19 cells preferentially accumulated lutein and β-carotene rather than lycopene. Despite a higher lycopene content in the tomato extract, its uptake was minimal compared with that of lutein and β-carotene. The lutein concentration was less than 10 % that of lycopene and β-carotene in the tomato extract. Despite this, considerable quantities of lutein were accumulated in the cells incubated with the tomato extract (Table 1). An uptake ratio was calculated from the carotenoid content of the tomato extract and uptake by ARPE-19 cells, which showed that the lutein uptake ratio was two-fold greater than that of β-carotene and ten-fold greater than that of lycopene (Table 1). These results may indicate a preferential uptake mechanism for lutein in the RPE.

Discussion

The aim of the present work was to investigate the antioxidant potential of tomato extract and the uptake of carotenoids from tomato extract into human retinal pigment epithelial cells. Several epidemiological studies have proved the beneficial effect of tomato consumption in

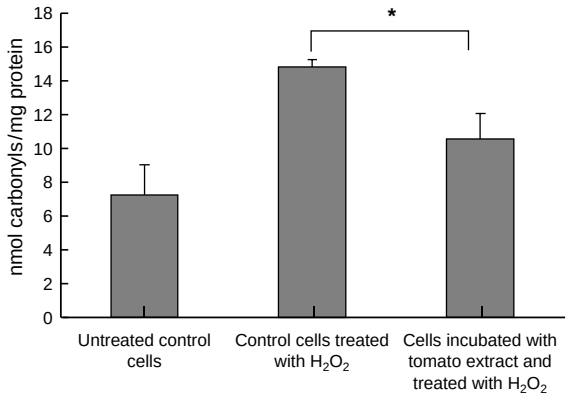

Fig. 2. Protein carbonylation in H_2O_2-treated ARPE-19 cells. Confluent cultures of ARPE-19 cells were incubated in the medium supplemented with tomato extracts for 24 h and treated with 1 mM-H_2O_2. Carbonylated proteins were assayed as described on page 644. Values were means and standard deviations of three separate experiments in triplicate. *Significantly different from the control (P=0·035).

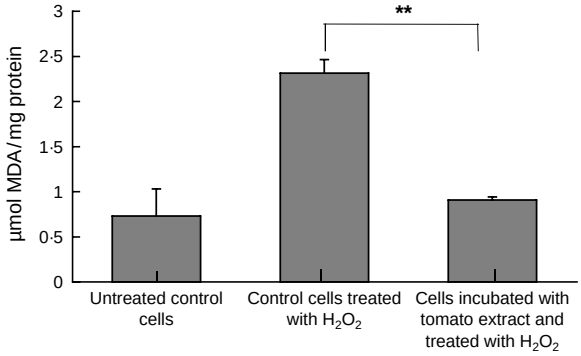

Fig. 3. Thiobarbituric acid-reactive substances (TBARS) in ARPE-19 cells treated with H_2O_2. Confluent cultures of ARPE-19 cells were incubated in medium supplemented with tomato extracts for 24 h and treated with 1 mM-H_2O_2. TBARS were assayed as described on page 644. The concentration of malondialdehyde (MDA) was normalised to protein concentration in the corresponding samples. Values were mean values and standard deviations of three separate experiments in triplicate. **Significantly different from the control (P=0·003).

Table 1. Carotenoid uptake by ARPE-19 cells from tomato extracts
(Mean values and standard deviations of three different experiments)

	Carotenoids in medium supplemented with tomato extract (ng/ml)	Endogenous carotenoids in ARPE-19 cells (ng/μg DNA)	Uptake by ARPE-19 cells from tomato extract (ng/μg DNA)		Uptake ratio (uptake by cells/concentration in the medium) × 10^{-5}
			Mean	SD	
β-Carotene	537	0·06	0·15	0·0093	27
Lutein	23	ND	0·016	0·0004	69
Lycopene	297	ND	0·021	0·0003	5

Confluent cultures of ARPE-19 cells were incubated in medium supplemented with tomato extract for 24 h, and carotenoids were extracted from the cells and analysed by HPLC as described on page 644. The carotenoid values were normalised to the DNA content present in the corresponding sample. The experiment was repeated three times in triplicate. Zeaxanthin was not detectable in the tomato extract or the cells.
ND, not detected.

preventing some major chronic diseases, such as CVD and some types of cancer (Giovannucci, 1999; Willcox *et al.* 2003). It is thought that this protective role is due to tomato antioxidants, which could contribute to the inhibition of abnormal oxidative processes (Abushita *et al.* 1997; Beecher, 1998; Raffo *et al.* 2002). Because of their high consumption rates, tomatoes and tomato oxidants can contribute significantly to the total intake of the carotenoids.

Carotenoids have long been considered as antioxidants; their ability to interact with and quench free radical reactions has been well established (Krinsky & Denecke, 1982). Although epidemiological data suggest that the consumption of diets rich in fruits and vegetables is decreasing the occurrence of certain chronic diseases such as cancer, CVD and AMD (Goldberg *et al.* 1988; Seddon *et al.* 1994), there is no good evidence from RPE cellular studies that carotenoids possess antioxidant properties.

In the present study, we used a β-carotene concentration (1 μM) that is close to the plasma concentrations in human subjects. ARPE-19 cells showed an interesting carotenoid uptake pattern by taking up more lutein and β-carotene than lycopene. In general, cellular carotenoid uptake is thought to be concentration-dependent and a passive non-regulatory process (Wamer *et al.* 1993). The preferential uptake of lutein and β-carotene observed in the present study indicates the likely presence of another uptake mechanism in RPE cells besides passive diffusion. The existence of carotenoid-binding proteins has been reported in recent years. Yemelyanov *et al.* (2001) described membrane proteins from the human retina that specifically bind to xanthophyll carotenoids. A lutein-binding protein has been reported in the midgut of the silk worm (Jouni & Wells, 1996). A carotene-binding protein that specifically binds to β-carotene has been characterised from ferret liver (Rao *et al.* 1997).

The RPE is the intermediary between the blood supply and the retina, and is responsible for the regulated exchange of nutrients and metabolites between retina and blood supply. Macular pigments, lutein and zeaxanthin have to pass through the RPE to the retina, where they are accumulated. The preferential uptake reported in this study corroborates the view that RPE might play a central role in the selective accumulation of lutein and zeaxanthin in the macula. Further investigations are necessary to understand whether the binding proteins play any role in the preferential uptake of lutein. Carotenoids are completely soluble in ethanol at the concentrations used in the present study, ruling out the possibility that the preferential uptake is occurring because of differences in solubility.

It might be possible for the observed antioxidant effects to be also partly due to other lipophilic antioxidants, such as α-tocopherol, present in tomatoes. In the tomatoes used in the present study, the predominant antioxidants were carotenoids (total carotenoids approximately 3 mg/g dry weight) and tocopherols (0·17 mg/g dry weight). We believe that the antioxidant effects observed in this study are largely due to carotenoids. Further studies are underway to investigate the individual and synergistic antioxidant actions of carotenoids in the RPE. In the tomato extract used to study the protective effects, β-carotene was the carotenoid present in the highest amounts, followed by lycopene and traces of lutein. As the intracellular accumulation of β-carotene is nearly ten-fold higher than that of lycopene and lutein, the protective effects observed might be attributed predominantly to β-carotene.

Protein carbonyl groups are introduced via an oxidation of proteins and can be used as markers for oxidatively modified proteins (Davies *et al.* 1999). β-Carotene supplementation has been shown to reduce the carbonyl content of the peripheral blood in smokers (Lee *et al.* 1998). Nitrotyrosine is one of several protein modifications that can occur as a result of oxidative stress (Beckman, 1996). Protein nitration has also been reported in light-induced retinal damage in the rat RPE (Miyagi *et al.* 2002). In the present study, nitrotyrosine and protein carbonyl formation were reduced in the cells preincubated with the tomato extract, suggesting that carotenoids from the tomato extract are protective against protein modifications caused by oxidative stress. It has been shown that β-carotene scavenges toxic NO_2, peroxynitrous acid and peroxynitrite more effectively than other antioxidants (Kikugawa *et al.* 1997), as well as promoting the degradation of nitrotyrosine and inhibiting the transformation of tyrosine to nitrotyrosine in the presence of NO_2 at low levels in solution (Kikugawa *et al.* 1999).

In the present study, treating the ARPE-19 cells with H_2O_2 resulted in the formation of the lipid peroxidation product MDA; this was reduced by the preincubation of cells with the tomato extract, suggesting that carotenoids from tomato extract are protective against peroxide-induced lipid peroxidation in RPE cells.

Oxidative stress is believed to play a major role in AMD pathogenesis. Carotenoid and mineral intake is a proven way to prevent the progression of AMD. Seddon and colleagues (1994) demonstrated a negative association between carotenoid intake and advanced AMD in a multicentre case-control study. Protective effects of β-carotene against experimental retinal damage from blue light exposure have been reported in monkeys and rats (Ham *et al.* 1984; Tso, 1986). The Beaver Dam Eye Study (Van den Langenberg *et al.* 1998) reported that a past intake of α-carotene, β-carotene and provitamin A carotenoids, as well as a baseline intake of non-provitamin A carotenoids, was associated with a reduced risk of large drusen at 5 years follow-up. In an intervention study, the Age-Related Eye Disease Study, treating AMD patients with β-carotene along with other antioxidants reduced the progression of both wet and dry forms of the disease (Age-Related Eye Disease Study Group, 2001).

In summary, we have demonstrated that tomato extract is protective against oxidative stress and nitrosative stress in RPE, and that RPE cells preferentially accumulate lutein as well as β-carotene, but less lycopene. In general, these findings might provide the experimental evidence for the view that carotenoids and carotenoid-rich tomatoes are protective against the progression of AMD.

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