A Food-Based Formulation Provides Lycopene with the Same Bioavailability to Humans as That from Tomato Paste[1,2]

Myriam Richelle,[3] Karlheinz Bortlik, Stéphanie Liardet,* Corinne Hager, Pierre Lambelet, Markus Baur, Lee A. Applegate* and Elizabeth A. Offord

*Department of Nutrition, Nestlé Research Center, Lausanne, Switzerland and *Department of Dermatology, Centre Hospitalier Universitaire Vaudois, 1011 Lausanne, Switzerland*

ABSTRACT Lycopene from fresh and unprocessed tomatoes is poorly absorbed by humans. Absorption of lycopene is higher from processed foods such as tomato paste and tomato juice heated in oil. The aim of the present study was to develop a food-grade lycopene formulation that is bioavailable in humans. A formulation of lycopene named "lactolycopene" has been designed in which lycopene is entrapped with whey proteins. Healthy subjects (n = 33; 13 men and 20 women) participated and were allocated randomly to one of the three treatment groups. After a 3-wk deprivation of dietary lycopene, subjects ingested 25 mg lycopene/d for 8 wk from lactolycopene, tomato paste (positive control) or a placebo of whey proteins while consuming their self-selected diets. Plasma lycopene concentrations reached a maximum after 2 wk of supplementation in both lycopene-treated groups and then a plateau was maintained until the end of the treatment. Increases in plasma lycopene at wk 8 were not different between supplemented groups (mean ± SEM): 0.58 ± 0.13 μmol/L with lactolycopene and 0.47 ± 0.07 μmol/L with tomato paste, although they were different from the control ($P < 0.001$). Similar time-concentration curves of lycopene incorporation were observed in buccal mucosa cells. Although lycopene was present mainly as all-*trans* isomers (>90%) in both lycopene supplements, plasma lycopene enrichment consisted of 40% as all-*trans* and 60% as *cis* isomers. The precursor of lycopene, phytofluene, was better absorbed than lycopene itself. The lactolycopene formulation and tomato paste exhibited similar lycopene bioavailability in plasma and buccal mucosa cells in humans. J. Nutr. 132: 404–408, 2002.

KEY WORDS: • *lycopene* • *phytofluene* • *bioavailability* • *isomers* • *humans*

Lycopene is a natural red pigment synthesized exclusively by plants and microorganisms. In the pericarp tissue of ripe tomato fruits, lycopene is localized in cellular compartments, the chromoplasts, where the crystals are associated with membrane structures (1,2).

Absorption of carotenoids is a complex issue involving release from the food microstructure matrix, dissolution into mixed micelles, intestinal uptake, incorporation into chylomicrons, distribution to the tissues, uptake by liver and resecretion into VLDL, which are progressively transformed into LDL. Lycopene absorption from food sources is widely documented. The best food sources providing lycopene in a bioavailable form are tomato paste and tomato sauce, whereas lycopene from other sources such as fresh tomatoes and unheated tomato juice is poorly absorbed (3–8).

The key issue is to have a bioavailable source of lycopene. Tomato extracts in the form of oleoresin or beadlets are commercially available but the bioavailability of lycopene from these sources is rather limited in humans (8–10). The bioavailability of lycopene from tomato products is greatly enhanced after mechanical texture disruption and thermal processing (4,5,7). Such treatment may increased the accessibility of the lycopene but could also help to disperse the liposoluble tomato constituents including lycopene in the food matrix. In concentrated tomato extracts, the poorly soluble lycopene is predominantly crystallized. However, the crystalline form of carotenoids has been found to be one of the primary factors that reduce their bioavailability (11). One working hypothesis is that if the particle size of lycopene is minimized, its bioavailability will be enhanced. To test this hypothesis, a formulation of lycopene was designed consisting of lycopene from tomato oleoresin embedded in a whey protein matrix; this formulation is termed "lactolycopene" throughout the rest of this text.

Another aspect influencing the bioavailability of lycopene is its stereochemistry. Lycopene is a highly unsaturated molecule containing 13 double bonds, 11 of which are conjugated. The all-*trans* isomer of lycopene is the most predominant geometrical isomer found in fresh tomatoes. However, lycopene can undergo *trans* to *cis* isomerization during tomato processing and storage (12,13). *Cis*-isomers display a lower tendency to aggregate and therefore to form crystals (14). In humans, lycopene exists in both tissues and serum as all-*trans* and *cis*-isomers (3,12,15). However, it is not yet clear whether

[1] Supported in part by the Swiss League against Cancer (grant KFS- 95–7-1998) and the Erwin Brown Foundation.
[2] The first two authors contributed equally to this work.
[3] To whom correspondence should be addressed.
E-mail: Myriam.Richelle@rdls.nestle.com.

Manuscript received 17 September 2001. Initial review completed 18 October 2001. Revision accepted 6 December 2001.

TABLE 1

Composition of the lycopene supplements[1,2]

	Tomato paste	Lactolycopene
	mg/g	
γ-Tocopherol	0.014 ± 0.002	0.098 ± 0.005
α-Tocopherol	0.099 ± 0.005	0.483 ± 0.020
β-Carotene	0.025 ± 0.002	0.131 ± 0.005
Lycopene	0.751 ± 0.009	1.972 ± 0.020
Phytofluene	0.060 ± 0.005	0.130 ± 0.004
Phytoene	0.110 ± 0.007	0.250 ± 0.006

[1] Values are means ± SD, based on analysis of duplicate samples of supplement (n = 3).

isomerization occurs in vivo, or if there is a preferential uptake of the *cis* or *trans* form.

The first objective of the present study was to compare, in plasma and buccal mucosa cells (BMC) of humans, the bioavailability of lycopene from a lycopene formulation "lactolycopene" with that from a tomato paste used as positive control. The second objective was to characterize the profile of lycopene isomers in the enrichment of plasma lycopene.

SUBJECTS AND METHODS

Subjects. Healthy subjects (n = 36; 13 men and 23 women) aged 21–58 y were enrolled in this study and divided into three groups of 12 subjects. Subjects were nonsmokers and were not supplemented with vitamin or minerals during the study. They were apparently healthy as assessed by a medical questionnaire, took no medication except for oral contraceptive and did not report gastrointestinal disturbances.

The protocol was approved by the ethical committee of the Center Hospitalier Universitaire Vaudois, Lausanne, Switzerland. Subjects received information on the background and design of the study and gave their informed consent before participation. They could withdraw from the study at any time point if desired.

Among the 36 subjects enrolled in the study, three did not complete the study, one due to pregnancy and two for personal reasons. Eleven subjects completed the study with the placebo, ten subjects with the lactolycopene formulation and twelve subjects with the tomato paste. The weight of volunteers remained constant over the 11 wk of the study. The data were analyzed only for the 33 subjects who completed the study. The number of women/men present was 7/4 in placebo group, 6/4 in lactolycopene group and 7/5 in tomato paste group. The mean age (±SD) of subjects was 30.8 ± 8.7, 33.1 ± 12.2 and 32.8 ± 8.0 y in placebo, lactolycopene and tomato paste groups, respectively.

Study design. The study was a randomized design with three treatments in parallel. The subjects were allocated to one of the three treatments with age and sex as stratification factors for the randomization.

Throughout the 11 wk of the study, subjects ate self-selected diets while avoiding foods containing lycopene (tomatoes and tomato-derived foods). After 3 wk of lycopene restriction, subjects were supplemented daily with 25 mg lycopene or the placebo. Subjects mixed their supplement with 250 mL apple juice and consumed it within 30 min during their main meal (either lunch or dinner). To monitor their supplement intake, subjects were asked to complete a record, which was checked weekly by the investigator.

Dietary supplements and lactolycopene formulation. Lycopene (Lyc-o-mato 6%, Beer-Sheva, LycoRed, Israël) was prepared with whey proteins as carriers (16). This process results in a reduction of crystal size from 5 μm in oleoresin to <500 nm in lactolycopene, as evaluated by light and transmission electron microscopy. This formulation is named "lactolycopene." The final concentration of lycopene was 2 mg/g powder.

The supplement provided 25 mg lycopene present either in the lactolycopene (12.5 g of powder) or in the tomato paste (33 g, Thomy, Vevey, Switzerland). The placebo consisted of whey proteins (12.5 g). The daily doses of lactolycopene and placebo were packed separately as aliquots in light-protected containers, sealed under nitrogen and stored at room temperature. Tomato paste was a commercially available product, packed in a 300-g tube. The amount of tomato paste was dosed by filling a graduated container. Subjects were asked to use one tube of tomato paste for 1 wk and then discard it. The concentrations of tocopherols, carotenoids, phytofluene and phytoene in the supplements were determined before the initiation of the study (**Table 1**) and again at the middle and at the end of the study. The lycopene concentration was stable in the lactolycopene, indicating that the storage conditions were adequate.

Collection and analysis of blood samples. A fasting blood sample was drawn from the arm via venipuncture into evacuated tubes containing potassium EDTA at the start of the study (wk −3), after the 3 wk of tomato restriction (wk 0) and again after 1, 2, 4 and 8 wk of supplementation. Blood samples were immediately placed on ice, protected from light and then centrifuged (10 min, 4°C, 3000 × *g*) to separate the plasma, which was stored at −80°C until analysis.

BMC were collected at the same time as plasma by scraping the inside of the cheeks and collecting the cells by centrifugation (3000 × *g*, 4°C, 10 min); they were then stored in brown Eppendorf tubes at −80°C as described by Paetau et al. (10).

For carotenoid analysis, all manipulations were performed in brown Pyrex tubes to protect the sample from light exposure. Carotenoid extractions from BMC were performed as previously described by Paetau et al. (10). Plasma carotenoids were extracted by the method of Hess et al. (17).

Carotenoid concentrations in plasma and buccal mucosa cells were determined by HPLC on a C18 precolumn (ODS Hypersil, 5 μm, 20 × 4 mm; Hewlett Packard, Geneva, Switzerland) and a C18 column (Nova pak, 3.9 μm i.d. × 300 mm length, Millipore, Volketswil, Switzerland). The separation was achieved at room temperature under isocratic conditions with a mobile phase consisting of acetonitrile/tetrahydrofuran/methanol/ammonium acetate 1% (533.5:193.6:53.7:28, wt/wt/wt/wt). The mobile phase flow rate was 1.5 mL/min. All solvents were HPLC grade and were used without purification. UV-visible (vis) detection was used to monitor concentration of lycopene at 472 nm, α- and β-carotene at 450 nm, and α- and γ-tocopherol at 297 nm.

The separation of plasma lycopene isomers, phytoene and phytofluene was performed by HPLC following the method described by Schierle et al. (12). The separation was performed on three Nucleosil 300–5 columns (4 μm i.d. × 250 mm length, Macherey-Nagel, Oensingen, Switzerland) in series. The separation was achieved at room temperature under isocratic conditions with a mobile phase consisting of hexane with 0.15% *N*-ethyldiisopropylamine. The mobile phase flow rate was 1.0 mL/min. All solvents were HPLC grade

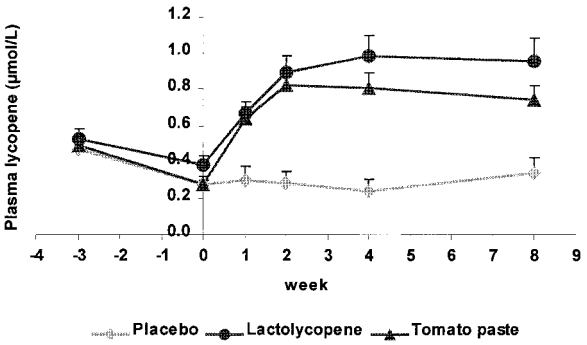

FIGURE 1 Changes in concentration of lycopene in human plasma throughout the course of the study representing a 3-wk deprivation period of dietary lycopene followed by 8 wk daily supplementation of 25 mg lycopene from tomato paste or the lactolycopene formulation or no lycopene from the placebo. Results are expressed as means ± SEM, n = 11 (placebo), n = 10 (lactolycopene) and n = 12 (tomato paste).

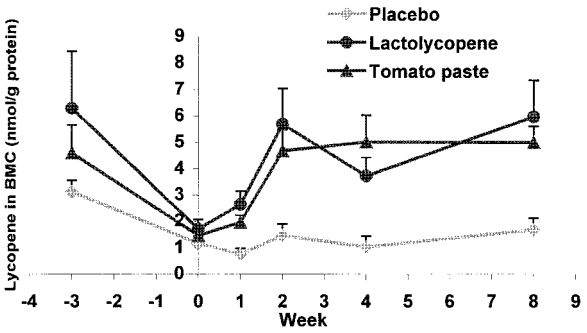

FIGURE 2 Changes in concentration of lycopene in the human buccal mucosa cells (BMC) throughout the course of the study representing a 3-wk deprivation period of dietary lycopene followed by 8 wk daily supplementation of 25 mg lycopene from tomato paste or the lactolycopene formulation or no lycopene from the placebo. Results are expressed as mean ± SEM, n = 11 (placebo), n = 10 (lactolycopene) and n = 12 (tomato paste).

and were used without purification. UV-vis detection was acquired for *trans*-lycopene at 470 nm, for *cis* isomers of lycopene at 464 nm, for phytofluene at 346 nm and for phytoene at 294 nm.

The BMC protein content was determined with a commercial kit (BCA, Pierce, Rockford, IL) using bovine serum albumin as standard.

Statistical analysis. All results are presented as mean values ± SEM. Differences among the three treatments for lycopene concentration were tested for significance by one-way ANOVA (SAS for Windows, version 6.12; SAS Institute, Cary, NC). A significant difference was accepted if $P < 0.05$; Turkey's test was used to make pairwise comparisons. Correlations between the different type of samples used the Pearson product moment correlation coefficient.

RESULTS

Lycopene response in plasma and buccal mucosa cells after 8 wk supplementation. At the beginning of the study, the plasma lycopene concentrations of the three groups of subjects did not differ. In response to the 3 wk of dietary restriction from tomatoes and tomato-derived products, plasma lycopene decreased from 0.47 ± 0.07 to 0.28 ± 0.04 μmol/L for the placebo group; from 0.52 ± 0.06 to 0.38 ± 0.05 μmol/L for the lactolycopene group and from 0.49 ± 0.05 to 0.28 ± 0.02 μmol/L for the tomato paste group (**Fig. 1**), indicating that subjects effectively followed this dietary restriction. The placebo treatment, consisting of the daily ingestion of 12.5 g whey proteins had no further effect on plasma lycopene concentration, confirming that subjects did follow this dietary restriction throughout the succeeding 8 wk of the study. Daily consumption of tomato paste providing 25 mg lycopene increased plasma lycopene concentration by 0.47 ± 0.07 μmol/L. Plasma lycopene concentration reached a maximum concentration after 2 wk of supplementation and remained stable thereafter. Lycopene from lactolycopene was as bioavailable as tomato paste, inducing a similar increase of plasma lycopene concentration, 0.58 ± 0.13 μmol/L. During the 8-wk period of treatment, the increase in plasma lycopene concentration was greater in both lycopene groups than in the placebo group ($P < 0.001$).

Three weeks of dietary tomato restriction markedly decreased the lycopene concentration in BMC (**Fig. 2**). The SEM also decreased, indicating a homogeneity of the subjects. The placebo treatment did not further modify the BMC concentration of lycopene, whereas both lycopene treatments markedly increased the concentration of lycopene in BMC ($P < 0.01$). The profile of the BMC lycopene curve was similar to that observed in plasma, suggesting that a maximum concentration was reached after 2 wk of supplementation. The concentration reached a plateau over the next 6 wk of supplementation. The increases in plasma and BMC lycopene concentration after the 8 wk of treatment were correlated ($r = 0.64$, $P < 0.001$).

Lycopene isomer profile in plasma. The pattern of plasma lycopene isomers was analyzed at the beginning and end of the study. The lycopene supplements contained >90% all-*trans* isomer, which is characteristic of fresh tomatoes. The increase of plasma total lycopene at the end of the supplementation period did not affect the relative proportion of *cis*:*trans* isomers, which was maintained as 36% all-*trans* isomer and 64% *cis*-isomers with a predominance of 5-*cis* (26%) followed by 13-*cis* (13%) then 15-*cis* (6%) and 9-*cis* (5%) (**Table 2**).

Plasma phytofluene response. Tomato products contain not only lycopene but also its precursor phytofluene. The lycopene supplements provided 1.6 and 1.98 mg phytofluene/d with lactolycopene and tomato paste, respectively (**Table 3**). Daily ingestion of these supplements over an 8-wk period caused a plasma phytofluene increase of 0.23 ± 0.05 μmol/L with lactolycopene and of 0.33 ± 0.08 μmol/L with tomato paste ($P < 0.001$).

TABLE 2

Profile of plasma lycopene isomer of supplements and of plasma of subjects before and after 8 wk daily supplementation of 25 mg lycopene from either lactolycopene formulation or tomato paste[1]

	Lactolycopene			Tomato paste		
		Plasma			Plasma	
	Product	wk 0	wk 8	Product	wk 0	wk 8
			% total lycopene			
all-*trans*	92	38 ± 2	36 ± 1	95	33 ± 1	39 ± 1
5-*cis*	8	28 ± 1	26 ± 1	5	28 ± 1	22 ± 1
9-*cis*		5 ± 0	7 ± 0		5 ± 0	4 ± 0
13-*cis*		11 ± 1	12 ± 0		14 ± 1	15 ± 1
15-*cis*		5 ± 1	5 ± 0		7 ± 1	6 ± 0
Unidentified-*cis*		13 ± 1	14 ± 1		13 ± 1	15 ± 1

[1] Values are means ± SEM, n = 10 (lactolycopene) or n = 12 (tomato paste).

TABLE 3

Plasma concentration of phytofluene and lycopene in the daily dose of both supplements and in the plasma before and after 8 wk daily supplementation of 25 mg lycopene from either lactolycopene formulation or tomato paste or no lycopene from the placebo[1]

	Phytofluene					Lycopene				
		Plasma					Plasma			
	Product[2]	wk 0	wk 8	Change[3]	*P*[4]	Product	wk 0	wk 8	Change[3]	*P*[4]
	mg	*μmol/L*				*mg*	*μmol/L*			
Placebo	0	0.18 ± 0.02	0.18 ± 0.04	0.00 ± 0.03	NS	0	0.28 ± 0.04	0.34 ± 0.09	0.06 ± 0.06	NS
Lactolycopene	1.6	0.18 ± 0.03	0.41 ± 0.06	0.23 ± 0.05	<0.001	25	0.38 ± 0.05	0.96 ± 0.13	0.58 ± 0.13	0.02
Tomato paste	1.98	0.16 ± 0.03	0.49 ± 0.08	0.33 ± 0.08	<0.001	25	0.28 ± 0.02	0.75 ± 0.07	0.47 ± 0.07	<0.001

[1] Plasma values are expressed as means ± SEM; $n = 11$ (placebo group), $n = 10$ (lactolycopene) or $n = 12$ (tomato paste).
[2] Phytofluene and lycopene content (mg) in the daily dose of each supplement.
[3] Change of plasma concentration between week 8 and week 0.
[4] Change significantly different from 0. NS, $P \geq 0.05$.

DISCUSSION

Lycopene bioavailability has been studied in humans after chronic ingestion of tomato-based products. The experimental design of these clinical studies varies greatly with respect to the daily lycopene dose as well as the duration of the supplementation. However, the data summarized in **Table 4** show the increase of plasma lycopene concentration in response to these treatments to be quite consistent with little difference among subjects enrolled in these studies. The main conclusion is that lycopene from fresh tomatoes or tomato juice is poorly absorbed, whereas lycopene from processed tomatoes such as tomato paste is better absorbed. In the present study, we used tomato paste as a positive control, and the observed increase of plasma lycopene is in agreement with the other studies reported in the literature (7,9). In addition, on the basis of measurements of lycopene in human plasma and in BMC, we demonstrate, for the first time, that lycopene in a food-based formulation (lactolycopene) is as bioavailable as lycopene from tomato paste. The results presented here, therefore, confirm that the characteristics of a lycopene-based formulation markedly affect the bioavailability of lycopene, including complexation with proteins, crystal size or environment of lycopene. However, future studies will be required to understand the underlying mechanism.

To produce a biological effect, lycopene must first be absorbed by the gut and thereafter reach the tissue of interest. Plasma analysis provides information on what crosses the intestinal barrier but gives no information on how intake affects tissue concentration. However, this important issue is very difficult to assess because tissue biopsies are very invasive and rarely available. An alternative is BMC, which can be collected noninvasively and analyzed for carotenoid content

TABLE 4

Comparison of the human plasma lycopene response after 8 wk of daily consumption of lactolycopene to plasma lycopene response after daily consumption of other lycopene matrices as described by different investigators in the literature[1]

				Increase of plasma lycopene				
Reference	*n*[2]	Intake[3]	Duration[4]	Fresh tomatoes	Tomato juice	Oleoresin	Tomato paste	Lactolycopene
		mg	*wk*	*μmol/L*				
20	5	12	6		No effect			
9	20	50	1		0.30 ± 0.06			
8	8	5	6	No effect	0.10 ± 0.07	0.15 ± 0.07		
21	15	75	4		0.17 ± 0.05	0.24 ± 0.06		
9	20	75	1			0.33 ± 0.08		
9	20	150	1			0.45 ± 0.08		
7	5	16.5	1	0.3 ± 0.1			0.45 ± 0.1	
9	20	21	1				0.45 ± 0.08	
9	20	39	1				0.48 ± 0.08	
Present study	12	25	8				0.47 ± 0.07	0.58 ± 0.13

[1] Values represent the plasma lycopene concentration at the end of the supplementation minus the baseline plasma lycopene concentration. The increase of plasma lycopene concentration is expressed as means ± SEM.
[2] *n* represents the number of subjects participating to the study.
[3] Intake (mg) is the amount of lycopene daily consumed by the subject.
[4] Duration (week) of the supplementation.

(10). In this study, we showed for the first time the kinetics of uptake of lycopene into BMC, which paralleled the plasma enrichment. Our results emphasize that BMC may represent a good, noninvasive, surrogate tissue for measuring lycopene absorption.

Lycopene contains 13 double bonds, which could be in either the *trans* or *cis* configuration. In tomatoes, lycopene is present mainly as the all-*trans* isomer. Heat processing of tomato-based foods could induce a conversion of all-*trans* lycopene into *cis* isomers. In humans, *cis* and all-*trans* isomers of lycopene are present in plasma and also in tissues (3,12,15). Moreover, some tissues such as prostate and testis are particularly rich in *cis* isomers, comprising >80% (15). Several studies have shown that *cis* lycopene is more bioavailable than the *trans* isomer, for example, in ferrets (18) and in humans (3). This observation is supported by the higher solubility of the *cis* isomers of lycopene in lipophilic phases, which could promote their incorporation into mixed micelles in the intestine. In the present study, chronic ingestion of lycopene mainly (>90%) as the all-*trans* isomer resulted in an increase of plasma lycopene of ~40% all-*trans* isomer and 60% *cis* isomers, emphasizing the importance of the *cis* lycopene in vivo. This marked increase of plasma *cis* isomers could be due to a preferential absorption of the *cis* isomers present in the supplement and/or to an in vivo isomerization of all-*trans* lycopene (19).

In tomatoes, lycopene is synthesized from mevalonate through phytoene and phytofluene precursors. Both supplements used in this study contained low amounts of phytofluene, and chronic absorption results in a significant elevation of its plasma concentration. The relative plasma bioavailability of phytofluene and lycopene can be compared through the ratio of the plasma concentration to the daily dose ingested. Phytofluene was better absorbed than lycopene (see Table 3). This interesting observation confirms the previous report of Boileau et al. (18). Additional investigations will be required to understand the biological activity of phytofluene in vivo.

Until now, tomato paste has been demonstrated to be the most valuable food source of bioavailable lycopene in humans. This study clearly shows that embedding lycopene into whey proteins enhances its bioavailability so that it is equal to that from tomato paste. Whether it is the crystal size of lycopene, the whey protein matrix or another factor that is responsible for the enhanced bioavailability of lycopene in lactolycopene is not yet clear but could be addressed by comparing structure characteristics and composition of tomato paste and lactolycopene.

In conclusion, this lycopene formulation could be used in several food applications to deliver lycopene in a bioavailable and concentrated form.

ACKNOWLEDGMENTS

We are grateful to Didier Gumy, Angiolino Delvecchio and Maurizio Beggio for the scaled-up preparation of the lactolycopene formulation. We thank Armand Malnoe and Ernesto Dalan for their contribution in optimizing the organoleptic properties of the lycopene supplements. The expertise of Eric Koloziejczyk in electronic microscopy is greatly appreciated. We thank Isabelle Tavazzi, Françoise Saucy, Corinne Scaletta, Corinne Charbonnet, Eliane Duruz and Marie-Rose Fernandez-Graf for excellent technical assistance. We also thank Marcel Baumgartner for his statistical advice during the setting of the protocol. The authors thank B. Halliwell for his critical comments during the preparation of the manuscript. Our thanks extend to the subjects without whom this study would not have been possible.

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