ANTIOXIDANTS & REDOX SIGNALING
Volume 2, Number 3, 2000
Mary Ann Liebert, Inc.

Original Research Communication

Lycopene Synergistically Inhibits LDL Oxidation in Combination with Vitamin E, Glabridin, Rosmarinic Acid, Carnosic Acid, or Garlic*

BIANCA FUHRMAN, NINA VOLKOVA, MIRA ROSENBLAT, and MICHAEL AVIRAM

ABSTRACT

Several lines of evidence suggest that oxidatively modified low-density lipoprotein (LDL) is atherogenic, and that atherosclerosis can be attenuated by natural antioxidants, which inhibit LDL oxidation. This study was conducted to determine the effect of tomato lycopene alone, or in combination with other natural antioxidants, on LDL oxidation. LDL (100 μg of protein/ml) was incubated with increasing concentrations of lycopene or of tomato oleoresin (lipid extract of tomatoes containing 6% lycopene, 0.1% β-carotene, 1% vitamin E, and polyphenols), after which it was oxidized by the addition of 5 μmol/liter of $CuSO_4$. Tomato oleoresin exhibited superior capacity to inhibit LDL oxidation in comparison to pure lycopene, by up to five-fold [97% vs. 22% inhibition of thiobarbituric acid reactive substances (TBARS) formation, and 93% vs. 27% inhibition of lipid peroxides formation, respectively]. Because tomato oleoresin also contains, in addition to lycopene, vitamin E, flavonoids, and phenolics, a possible cooperative interaction between lycopene and such natural antioxidants was studied. A combination of lycopene (5 μmol/liter) with vitamin E (α-tocopherol) in the concentration range of 1–10 μmol/liter resulted in an inhibition of copper ion-induced LDL oxidation that was significantly greater than the expected additive individual inhibitions. The synergistic antioxidative effect of lycopene with vitamin E was not shared by γ-tocotrienol. The polyphenols glabridin (derived from licorice), rosmarinic acid or carnosic acid (derived from rosemary), as well as garlic (which contains a mixture of natural antioxidants) inhibited LDL oxidation in a dose-dependent manner. When lycopene (5 μmol/liter) was added to LDL in combination with glabridin, rosmarinic acid, carnosic acid, or garlic, synergistic antioxidative effects were obtained against LDL oxidation induced either by copper ions or by the radical generator AAPH. Similar interactive effects seen with lycopene were also observed with β-carotene, but, however, to a lesser extent of synergism. Because natural antioxidants exist in nature in combination, the *in vivo* relevance of lycopene in combination with other natural antioxidants was studied. Four healthy subjects were administered a fatty meal containing 30 mg of lycopene in the form of tomato oleoresin. The lycopene concentration in postprandial plasma was elevated by 70% in comparison to plasma obtained before meal consumption. Postprandial LDL isolated 5 hr after meal consumption exhibited a significant ($p < 0.01$) reduced susceptibility to oxidation by 21%. We conclude that lycopene acts synergistically, as an effective antioxidant against LDL oxidation, with several natural antioxidants such as vitamin E, the flavonoid glabridin, the phenolics rosmarinic acid and carnosic acid, and garlic. These observations suggest a superior antiatherogenic characteristic to a combination of different natural antioxidants over that of an individual one. Antiox. Redox Signal. 2, 491–506.

Lipid Research Laboratory, Technion Faculty of Medicine, The Rappaport Family Institute for Research in the Medical Sciences and Rambam Medical Center, Haifa, Israel.

*This work was recognized as the best scientific presentation in the 1999 Israeli National Meeting of the Israel Society for Oxygen and Free Radical Research.

INTRODUCTION

OXIDATIVE MODIFICATION of low-density lipoprotein (LDL) is thought to play a key role during early atherogenesis (Witztum and Steinberg, 1991; Aviram, 1993a,b). LDL, which has undergone lipid peroxidation, either by metal ions or by cells of the arterial wall, was shown to be taken up by macrophages at enhanced rate via the scavenger receptors (Parthasarathy *et al.*, 1986), leading to foam cell formation, the hallmark of the early atherosclerotic lesion (Brown and Goldstein, 1983). Thus, prevention of LDL oxidation by antioxidants may arrest the progression of atherosclerosis (Aviram, 1996; Aviram and Fuhrman, 1998a,b). Recently, an effort has been made to identify natural dietary antioxidants, which can offer protection against LDL oxidation.

Carotenoids, tocopherols, flavonoids, and phenolics are associated with beneficial health effects, which are attributed to the consumption of fruits and vegetables (Ziegler, 1991; Hertog *et al.*, 1993; Halliwell, 1994; Mayne, 1996; Muldoon and Kritchevsky, 1996; Clinton, 1998; Nagourney, 1998), and are related at least in part, to their antioxidant activity.

α-Tocopherol has been proposed to be the most important lipid-soluble radical-scavenging antioxidant in membranes and in plasma (Burton *et al.*, 1983). An increased intake of vitamin E has been shown to correlate negatively with the risk of heart disease in some (Rimm *et al.*, 1993; Stampfer *et al.*, 1993), although not all (The Alpha-Tocopherol Beta-Carotene Cancer Prevention Study Group, 1994) epidemiological studies. Tocotrienols have an unsaturated isoprenoid side-chain rather than the saturated side-chain found in tocopherols. Tocotrienols were shown to exhibit antioxidative effects in patients with hyperlipidemia and carotid stenosis (Tomeo *et al.*, 1995). It was also demonstrated that dietary tocotrienols become incorporated into circulating lipoproteins, where they react with peroxyl radicals as efficiently as their corresponding tocopherol isomers (Suarna *et al.*, 1993).

Phenolics compose the major class of plant-derived antioxidants, and, among them, the flavonoids constitute probably the most important group. We and others have shown that dietary supplementation with nutrients rich in flavonoids such as olive oil (Aviram and Eias, 1993; Visioli *et al.*, 1998), which contains hydroxytyrosol and oleuropein, red wine (Fuhrman *et al.*, 1995; Aviram *et al.*, 1997; Hayek *et al.*, 1997), which contains catechin and quercetin, or the extract of the root of *Glycyrrhiza glabra* (licorice), a plant originating from Asia (Fuhrman *et al.*, 1997a; Belinky *et al.*, 1998), which contains glabridin, resulted in an *ex vivo* reduced susceptibility of LDL to oxidation along with a reduction in the development of atherosclerotic lesions (Fuhrman *et al.*, 1997a).

The commonly used spice and flavoring agent rosemary, derived from the leaves of the plant *Rosmarinus officinalis L,* also possesses antioxidant properties (Wu *et al.*, 1982; Offord *et al.*, 1995). Its most active antioxidant constituents include the phenolics rosmarinic acid and carnosic acid, which account for 90% of the antioxidant activity (Aruoma *et al.*, 1992). Carnosic acid was shown to inhibit superoxide anion production in the xanthine/xanthine oxidase system, thus being effective in protecting biological systems against oxidative stress (Haraguchi *et al.*, 1995). Carnosic acid was also shown to act as a powerful inhibitor of lipid peroxidation in microsomal and liposomal systems, and to scavenge peroxyl radicals and hydrogen peroxide (Haraguchi *et al.*, 1995).

Garlic is perhaps the most widely quoted herb with medicinal potentials known in the literature, and its therapeutic actions has been widely reported (Lawson, 1983; Nagourney, 1998). Experimental and clinical data have demonstrated antiatherosclerotic effects of garlic (Orekhov *et al.*, 1995; Orekhov and Grunwald, 1997), which have been attributed to its hypocholesterolemic (Larner, 1995; Simons *et al.*, 1995), or antioxidative properties (Phelps and Harris, 1993; Orekhov *et al.*, 1996). The antioxidative capacity of garlic can be attributed to its major biologically active component allicin, (Rabinkov *et al.*, 1998), as well as to its flavonoids (Carotenuto *et al.*, 1996).

Carotenoids are natural pigments synthesized by plants, and dietary carotenoid consumption was shown in epidemiological studies to be associated with reduced cardiovascular mortality (Verlangieri *et al.*, 1985; Kohlmeier

and Hasting, 1995). β-Carotene and lycopene are the major carotenoids in human plasma, and their antiatherogenic activity may be related to their hypocholesterolemic (Fuhrman *et al.*, 1997b) or antioxidant (Burton, 1989) activity. Lycopene, the open-chain analog of β-carotene, is an acyclic carotenoid that contains 11 conjugated double bonds arranged linearly in the all-*trans* form, and is the major carotenoid in tomatoes. It has been suggested that lycopene consumption may be antiatherogenic secondary to its antioxidant properties (Stahl and Sies, 1996; Gerster, 1997; Sies and Stahl, 1998). However, natural antioxidants exist in nature in combination, and a combination of different antioxidants may act additively and even synergistically. Administration of licorice extract or of its isolated flavonoid glabridin, at equivalent glabridin concentrations, to the atherosclerotic, apolipoprotein E-deficient (E^0) mice resulted in a significant reduction in the susceptibility of their LDL to oxidation. However, the effect obtained after administration of pure glabridin was four-fold lower than that of whole licorice extract, suggesting a possible cooperative interaction between glabridin and other antioxidants present in licorice extract in the inhibition of LDL oxidation (Fuhrman *et al.*, 1997a).

Recently, we have demonstrated that protection of LDL against oxidative modification by tomato lycopene or by β-carotene was potentiated when these carotenoids were present in combination with vitamin E (Fuhrman *et al.*, 1997b). Similarly, a mixture of carotenoids with vitamin E analyzed for their antioxidant activity in multilamellar liposomes were more effective than the single compounds (Stahl *et al.*, 1998). This synergistic effect was most pronounced when lycopene or lutein was present in combination with vitamin E (Stahl *et al.*, 1998). Furthermore, we have recently shown that dietary supplementation for a period of 6 weeks to E^0 mice of tomato oleoresin, which contains 6% lycopene, 0.1% β-carotene, 1% vitamin E, and also flavonoids and phenolics, was more effective in protecting their LDL from oxidation than dietary supplementation of lycopene or β-carotene alone (Fuhrman *et al.*, 1997a).

β-Carotene and vitamin E can act synergistically in a membrane system of rat liver microsomes (Palozza and Krinsky, 1992), and β-carotene was shown to enhance vitamin E antioxidant efficiency (Bohm *et al.*, 1997). There is no data available to date on the cooperative interactions of lycopene with various natural antioxidants, in the protection of LDL against oxidation. Because natural antioxidants exist in nature in combination, the present study was carried out to compare the effect of lycopene alone or in association with various antioxidants such as vitamin E, γ-tocotrienol, glabridin, rosmarininc acid, carnosic acid, or garlic, on copper ion or free radicals-induced LDL oxidation.

MATERIALS AND METHODS

Materials

Lycopene (cystalline), 99% pure β-carotene, and tomato oleoresin were supplied by LycoRed Natural Products Industries Ltd. (Beer Sheva, Israel). The tomato oleoresin consists of crystalline 6% lycopene in the lipid phase of the tomato, which contains 1% vitamin E, 0.1% β-carotene and less than 0.05% of polyphenols (Beecher, 1998). Vitamin E (α-tocopherol) and tetrahydrofuran (THF) free of BHT high-performance liquid chromatography (HPLC) grade were purchased from Sigma Chemicals (St. Louis, MO). γ-Tocotrienol was supplied by Eastman Chemical Company, and it consisted of a mixture of naturally occurring tocotrienols. Carnosic acid was obtained as a rosemary extract dispersed in vegetable oil and contains 5% carnosic acid. Rosmarinic acid was purchased from Indofine, Chemical Company Inc., (NY). Glabridin was a generous gift from Dr. Jacob Vaya (see Belinky *et al.*, 1998). Garlic powder was purchased from Pure-Gar and it contains 10,000 mg/kg of the active ingredient allicin. 2′,2′-Azobis 2-amidino propane hydrochloride (AAPH) was purchased from Wako Pure Chemical Industries (Osaka, Japan).

Compounds preparation

Stock solutions of lycopene, tomato oleoresin, and β-carotene were prepared in THF at a concentration of 20 mmol/liter lycopene or β-carotene, respectively. Stock solution of vitamin E and tocotrienols were prepared in THF

at a concentration of 10 mmol/liter. Carnosic acid and glabridin were prepared in THF at a concentration of 10 mmol/liter. Rosmarinic acid, which is a water-soluble compound, was prepared in water at a concentration of 10 mmol/liter. Garlic powder (1 gram) was extracted in 9 ml of water, evaporated to dryness, and resolved in 10 ml of water to get a stock solution of 100 mg/ml.

Subjects

Four normolipidemic subjects aged 30–45, nonsmokers, and under no medication or vitamins supplements, were included in the *in vivo* study. The study procedure was approved by the Helsinki Committee of Rambam Medical Center, Israel Ministry of Health (No. 911).

The subjects consumed a fatty meal of 1,200 calories (18% protein, 45% fat, 37% carbohydrates) including 30 mg of lycopene that was administered as tomato oleoresin. Blood samples were collected before meal consumption in the fasting state, and in the postprandial state, 3 and 5 hr after meal consumption.

Lycopene determination in plasma

Carotenoids were extracted from 0.5 ml of plasma in hexane: isopropanol (3:2, vol/vol), and the upper hexane phase was evaporated to dryness under a gentle stream of nitrogen. The residue was reconstituted in 0.1 ml of HPLC injection solvent (60% acetonitrile, 10% methanol, 15% methylene chloride, 15% hexane) vortexed and centrifuged for 2 min at 1,200 $\times$ *g* at room temperature to force particulate matter to the bottom of the vial. A total of 70 μl was injected onto the HPLC system. The HPLC system consisted of Varian 9012 solvent delivery system and Varian 9050 variable wavelength UV-Visible detector, using RP C-18 column (15 cm $\times$ 4.6 mm, 5-μm particle size). The mobile phase was methanol: acetonitril: hexane: dimethyl chloride (475:475:25:25, vol/vol/vol/vol). All procedures were performed under dim light to inhibit lycopene oxidation.

Human LDL isolation

LDL was isolated from plasma by discontinuous density gradient ultracentrifugation (Aviram, 1983). The LDLs were dialyzed against saline with Na_2 EDTA (1 mmol/liter). Prior to oxidation, LDL was diluted in phosphate-buffered saline (PBS) to 100 μg of protein/ml and dialyzed against PBS at 4°C to remove the EDTA. LDL protein concentration was determined with the Folin phenol reagent assay (Lowry *et al.*, 1951).

LDL oxidation

Oxidation of LDL was carried out in a shaking water bath at 37°C under air in plastic tubes 1 cm in diameter. LDL (100 μg of protein/ml) was preincubated with the antioxidants, as specified in each experiment, for 30 min at 37°C, after which it was further incubated for 3 hr at 37°C with freshly prepared $CuSO_4$ (5 μmol/liter), or with freshly prepared AAPH (5 mmol/liter). LDL oxidation was terminated by refrigeration at 4°C, and the oxidation rate was immediately determined by measuring the amount of thiobarbituric acid reactive substances (TBARS) (Buege and Aust, 1978).

LDL oxidation was also analyzed by the lipid peroxides assay (El-Saadani *et al.*, 1989). This assay is based on the oxidative activity of lipid peroxides that convert iodide to iodine and is measured spectrophotometrically at 365 nm. The antioxidants alone (without LDL), which were run as controls in both assays, did not interfere with the analytical methods.

Calculation of synergism between antioxidants

The synergistic effects of a combination of lycopene with other antioxidants on copper ions-induced LDL oxidation were calculated as the ratio between the obtained inhibition (I_F) and the calculated expected inhibition (I_E). The expected inhibition was calculated as follows:

$$(A + B) - (A \times B/100) = \text{Expected inhibition } (I_E)$$

where A is inhibition by antioxidant alone (% of control LDL oxidation with no added antioxidants, nor lycopene) and B is inhibition by lycopene alone (% of control). Synergism is proven if the ratio I_F/I_E is greater than 1.

Statistics

Student's *t*-test was performed for the statistical analyses of the *in vivo* study. Results are given as mean ±SD for the *in vitro* studies and as mean ±SEM for the *in vivo* studies. The computer software program STATEASE (version 1.00; Data Plus Systems Inc., New York) was used for computation.

RESULTS

LDL (100 μg of protein/ml) incubated with increasing concentrations of pure lycopene or tomato oleoresin, at comparable lycopene concentrations, was oxidized by the addition of 5 μmol/liter of $CuSO_4$. Addition of increasing concentrations of lycopene inhibited copper ion-induced LDL oxidation moderately in a dose-dependent manner (Fig. 1). A maximal 22% and 27% inhibition in TBARS (Fig. 1A) and in lipid peroxides (Fig. 1B) formation, respectively, was obtained on using 50 μmol/liter of lycopene. However, addition of tomato oleoresin to the lipoprotein inhibited LDL oxidation to a much greater extent (about 90% inhibition), with an IC_{50} (the concentration needed to inhibit LDL oxidation by 50%) of 8.0 μmol/liter and 8.4 μmol/liter of lycopene equivalents required for the inhibition of TBARS (Fig. 1A) and lipid peroxides (Fig. 1B) formation, respectively. These results suggest that the antioxidant efficiency of lycopene when present together with other antioxidants, such as in tomato oleoresin, is substantially superior to that of lycopene alone. We next analyzed possible cooperative interactions of lycopene with other natural antioxidants such as vitamin E, γ-tocotrienol, the licorice-derived flavonoid glabridin, the phenolics carnosic acid and rosmarinic acid, and also with garlic. The chemical structure of the above compounds is shown in Fig. 2. Vitamin E alone inhibited copper ion-induced LDL oxidation in a dose dependent manner, reaching a 94% and 91% inhibition in TBARS (Fig. 3A) and lipid peroxides (Fig. 3C) formation, respectively, when using 10 μmol/liter of vitamin E.

Addition of lycopene (5 μmol/liter) alone inhibited LDL oxidation, measured as TBARS or as lipid peroxides formation, by only 5% or 9%, respectively. However, when the lycopene was added together with increasing concentrations of vitamin E, ranging from 1 to 10 μmol/liter, the inhibition of LDL oxidation exceeded the expected additive contribution of the individual antioxidants, by 10%, 25%, and 45% for the TBARS formation (Figs. 3B), and by 8%, 23%, and 53% for the lipid peroxides formation (Fig. 3D), at 1, 2.5, and 5 μmol/liter of vitamin E, respectively, suggesting a synergistic effect be-

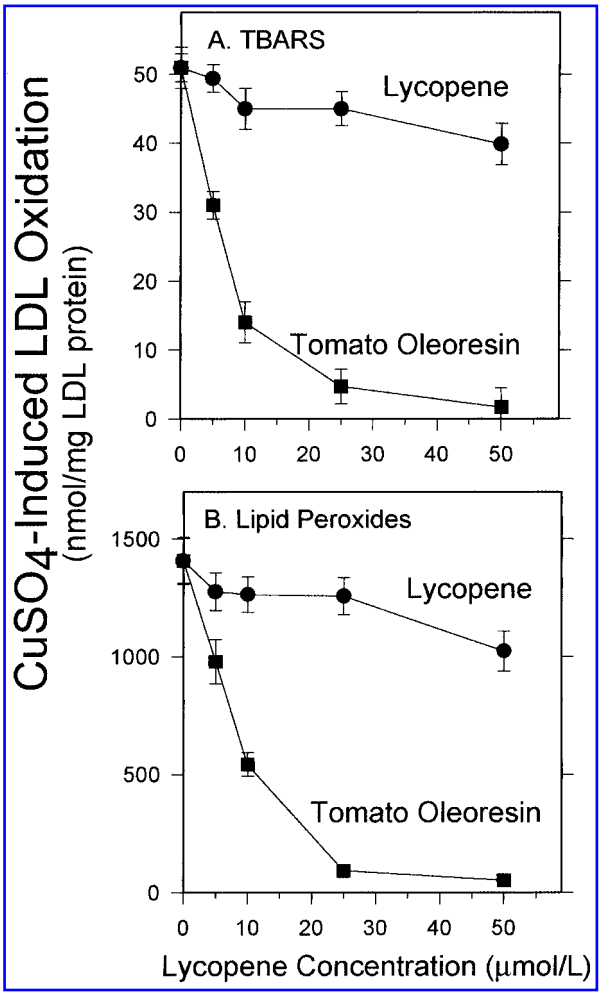

FIG. 1. Effect of crystalline lycopene and of tomato oleoresin on copper ion-induced LDL oxidation: concentration study. LDL (100 μg of protein/ml) was preincubated for 30 min at 37°C with increasing concentrations of crystalline pure lycopene, or tomato oleoresin (at equivalent lycopene concentrations), followed by a further incubation for 2 hr at 37°C in the presence of 5 μmol/liter of $CuSO_4$. LDL oxidation was measured as TBARS **(A)** or lipid peroxides **(B)** formation. Results are given as mean ± SD (n = 3).

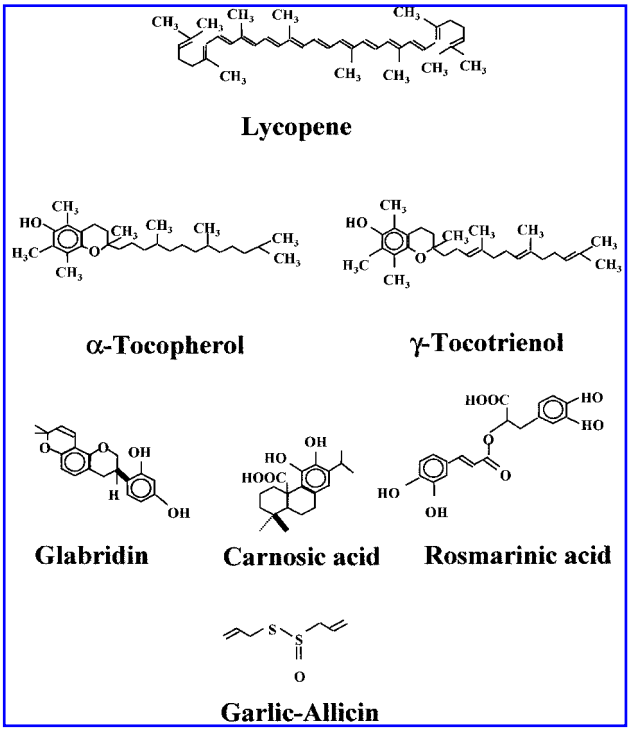

FIG. 2. Structural formulas of the antioxidant compounds used.

tween vitamin E and lycopene. However, at higher concentration (10 μmol/liter) of vitamin E alone, a 92% inhibition of LDL oxidation was obtained, and this remarkable effect was not further extended by lycopene addition. The calculated degree of synergism between 5 μmol/liter of lycopene and vitamin E in the inhibition of LDL oxidation, measured as TBARS (Fig. 3B) or as lipid peroxides (Fig. 3D) formation, was vitamin E dose-dependent between 1 and 5 μmol/liter of vitamin E, and it reached its maximum value of 2.0 for TBARS and a value of 2.4 for lipid peroxides, at 5 μmol/liter of vitamin E.

Because vitamin E shares similar chemical structure with tocotrienol, except that tocotrienol contains an unsaturated isoprenoid side-chain rather than the saturated side-chain present in tocopherols, we questioned whether lycopene also potentiates the antioxidative effect of tocotrienol.

γ-Tocotrienol alone inhibited LDL oxidation in a dose-dependent manner (a 18%, 37%, 44%, and a maximal 50% inhibition by 1, 2, 5, and 10 μmol/liter of tocotrienols, respectively). Because 5 μmol/liter of tocotrienols inhibited LDL oxidation to a similar extent exhibited by 5 μmol/liter of vitamin E (Fig. 4A), we have used this concentration in the combination study. In contrast to the synergism observed between vitamin E and lycopene, the combination of lycopene with tocotrienol showed no synergistic effect (Fig. 4A). Furthermore, the obtained inhibition in LDL oxidation by tocotrienol in combination with lycopene was far below the expected additive inhibition (Fig. 4A). This antagonistic effect of tocotrienol in combination with lycopene was evidenced by the calculated degree of synergism (Fig. 4B), which is only 0.3 (a value of 1.0 represents an additive effect of both antioxidants).

As active antioxidant ingredients in tomato oleoresin include polyphenols and phenolics (Beecher, 1998), we further questioned the capacity of lycopene to potentiate the antioxidative effect against LDL oxidation of several natural polyphenolic flavonoids and phenolics with different chemical structure. These included licorice-derived glabridin, which is an isoflavan, and rosemary-derived rosmarinic acid and carnosic acid, which are phenolics. Figure 5 demonstrates that copper ion-induced LDL oxidation measured as TBARS formation was inhibited by glabridin, or by rosmarinic acid, or by carnosic acid, in a dose-dependent manner, with an IC_{50} of 1.8, 87, and 105 μmol/liter, respectively. A maximal inhibition of 94%, 93%, and 99% was achieved by the addition of 6.75 μmol/liter of glabridin or 225 μmol/liter of rosmarinic acid or carnosic acid (Fig. 5). The minimal concentration of each antioxidant, which exhibited potency to inhibit LDL oxidation, was chosen from the data presented in Fig. 5, and has been used in the combination study. Lycopene alone inhibited LDL oxidation by 9%. The inhibition of LDL oxidation by glabridin (1 μmol/liter), rosmarinic acid (25 μmol/liter), or carnosic acid (25 μmol/liter), in combination with 5 μmol/liter of lycopene, exceeded the calculated additive effects by 32%, 32%, and 15%, respectively (Fig. 6A). The calculated degree of synergism in the inhibition of LDL oxidation between lycopene and the above phenolics reached values of 1.95, 2.40, and 1.77 for the combination of lycopene with glabridin, rosmarinic acid, and carnosic acid, respectively (Fig. 6B).

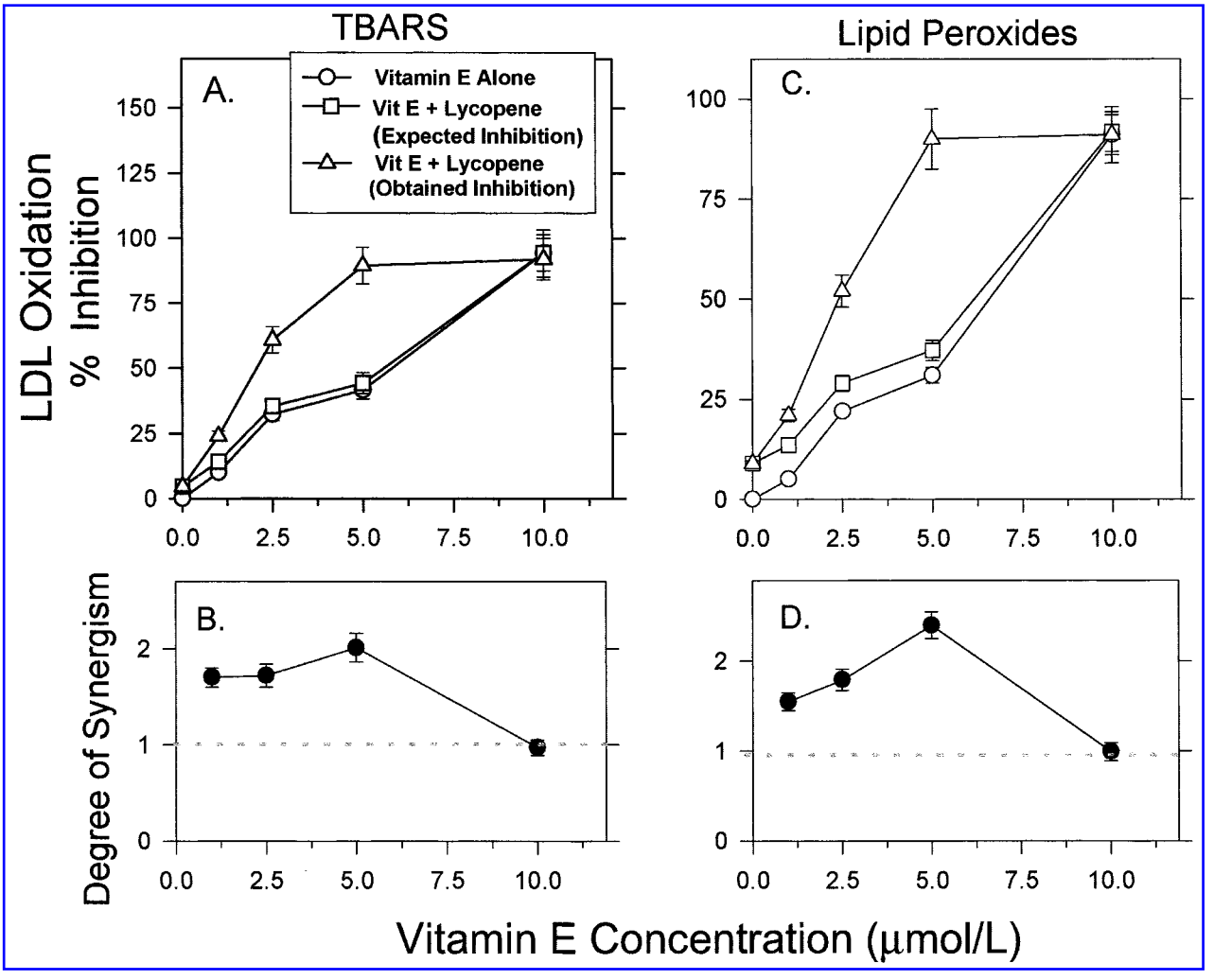

FIG. 3. (A and C) Effect of lycopene in combination with vitamin E on LDL oxidation induced by copper ions. (B and D) Calculated degree of synergism between lycopene and vitamin E as a function of vitamin E concentration. LDL (100 μg of protein/ml) was preincubated for 30 min at 37°C with increasing concentrations of vitamin E alone (circles), or with a combination of the indicated concentrations of vitamin E with lycopene (5 μmol/liter), followed by a further incubation for 2 hr at 37°C in the presence of 5 μmol/liter of $CuSO_4$. The expected additive and the obtained values for inhibition of LDL oxidation are presented as squares and triangles, respectively. LDL oxidation was measured as TBARS **(A,B)** or as lipid peroxides **(C,D)** formation. Data are expressed as percentage of inhibition obtained in the presence of antioxidants out of oxidation rate of control LDL that was incubated with no added antioxidants. Results are given as mean ± SD (n = 3).

Because the exact mechanism responsible for LDL oxidation *in vivo* is not known yet, we have also examined the capacity of lycopene to potentiate the antioxidative effect of the above natural antioxidants against LDL oxidation induced by the radical generator AAPH. Table 1 demonstrates that lycopene (5 μmol/liter) alone inhibited AAPH-induced LDL oxidation by 9%, whereas the inhibition of LDL oxidation by lycopene together with vitamin E (5 μmol/liter), glabridin (1 μmol/liter), carnosic acid (25 μmol/liter), rosmarinic acid (25 μmol/liter), or with garlic (250 μg/ml), exceeded the expected additive inhibition by 79%, 72%, 20%, 17%, and 33%, respectively (Fig. 7A). However, the combination of lycopene with tocotrienol (5 μmol/liter) showed no synergistic effect (Table 1). These results clearly show that lycopene potentiate the antioxidative effect of several polyphenols against either copper ion or AAPH-induced LDL oxidation, and the extent of synergism depends on the compounds chemical structure.

Similar interactive effects seen with lycopene

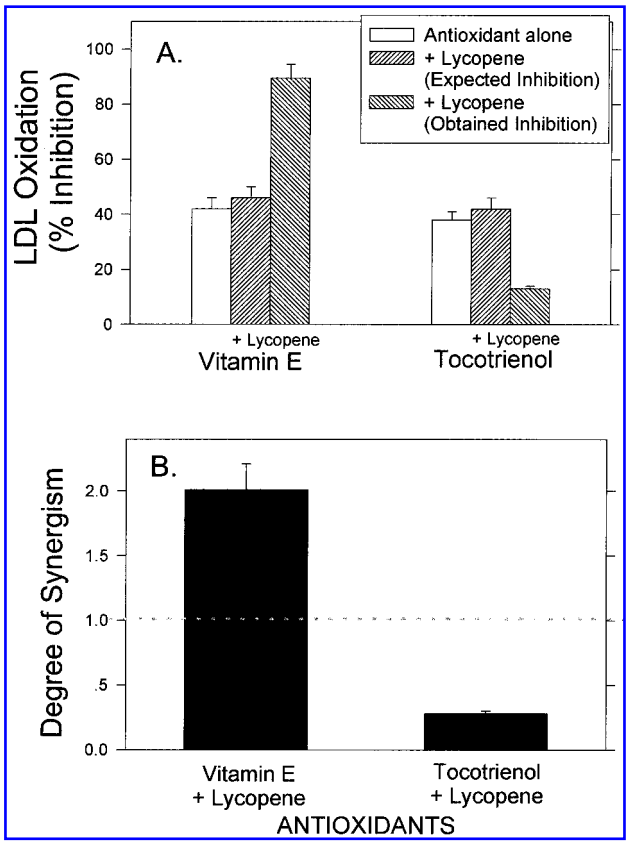

FIG. 4. (A) Effect of lycopene in combination with vitamin E or with tocotrienols on LDL oxidation induced by copper ions. (B) Calculated degree of synergism between lycopene and vitamin E or tocotrienol. LDL (100 μg of protein/ml) was preincubated for 30 min at 37°C with 5 μmol/liter of vitamin E, or with 5 μmol/liter of tocotrienol, alone (open bars), or in combination with lycopene (5 μmol/liter) (hatched bars). Then, the LDL was further incubated for 2 hr at 37°C with 5 μmol/liter of $CuSO_4$. The expected additive and the obtained values for the inhibition of LDL oxidation are presented as left diagonals bars and right diagonal bars, respectively. LDL oxidation was measured as TBARS formation. Data are expressed as percentage of inhibition obtained in presence of antioxidants out of oxidation rate of control LDL incubated with no added antioxidants. Results are given as mean ± SD (n = 3).

were also observed with β-carotene. β-Carotene (5 μmol/liter) alone inhibited copper ion-induced LDL oxidation by 4.5%, whereas the inhibition of LDL oxidation by β-carotene together with vitamin E (5 μmol/liter), glabridin (1 μmol/liter), carnosic acid (25 μmol/liter), rosmarinic acid (25 μmol/liter), or with garlic (250 μg/l), exceeded the expected additive inhibition by 6%, 9%, 15%, 15%, and 4%, respectively (Fig. 7A). However, similarly to lycopene, the combination of β-carotene with tocotrienol (5 μmol/liter) also showed no synergistic effect (Fig. 7). The calculated degree of synergism in the inhibition of LDL oxidation by β-carotene in combination with vitamin E, glabridin, carnosic acid, rosmarinic acid, or with garlic, reached values of 1.24, 1.16, 1.46, 1.27, and 1.11, respectively (Fig. 7B).

Next, we questioned whether lycopene can potentiate the antioxidative effect of a mixture of natural antioxidants, such as garlic, because garlic was demonstrated to possess antiatherogenic and antioxidant effects (Phelps and Harris, 1993; Orekhov *et al.*, 1995). Thus, we have tested the capability of garlic in combination with lycopene to protect LDL against copper ion-induced lipid peroxidation. LDL oxidation was inhibited by garlic alone in a dose-dependent manner, with an IC_{50} of 190 μg/ml and 120 μg/ml for the inhibition of TBARS (Fig. 8A) or lipid peroxides (Fig. 8C) formation, respectively. Lycopene alone (5 μmol/liter) inhibited LDL oxidation measured as TBARS or as lipid peroxides formation, by 7% and 9%, respec-

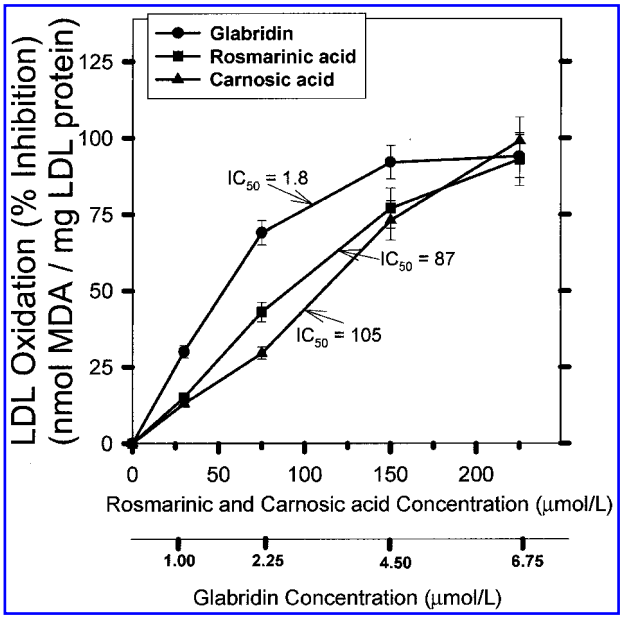

FIG. 5. Effect of glabridin, rosmarinic acid, and carnosic acid on copper ion-induced LDL oxidation: concentration study. LDL (100 μg of protein/ml) was preincubated for 30 min at 37°C with increasing concentrations of glabridin, rosmarinic acid, or carnosic acid, followed by a further incubation for 2 hr at 37°C in the presence of 5 μmol/liter of $CuSO_4$. LDL oxidation was measured as TBARS formation. Results are expressed as mean ± SD (n = 3).

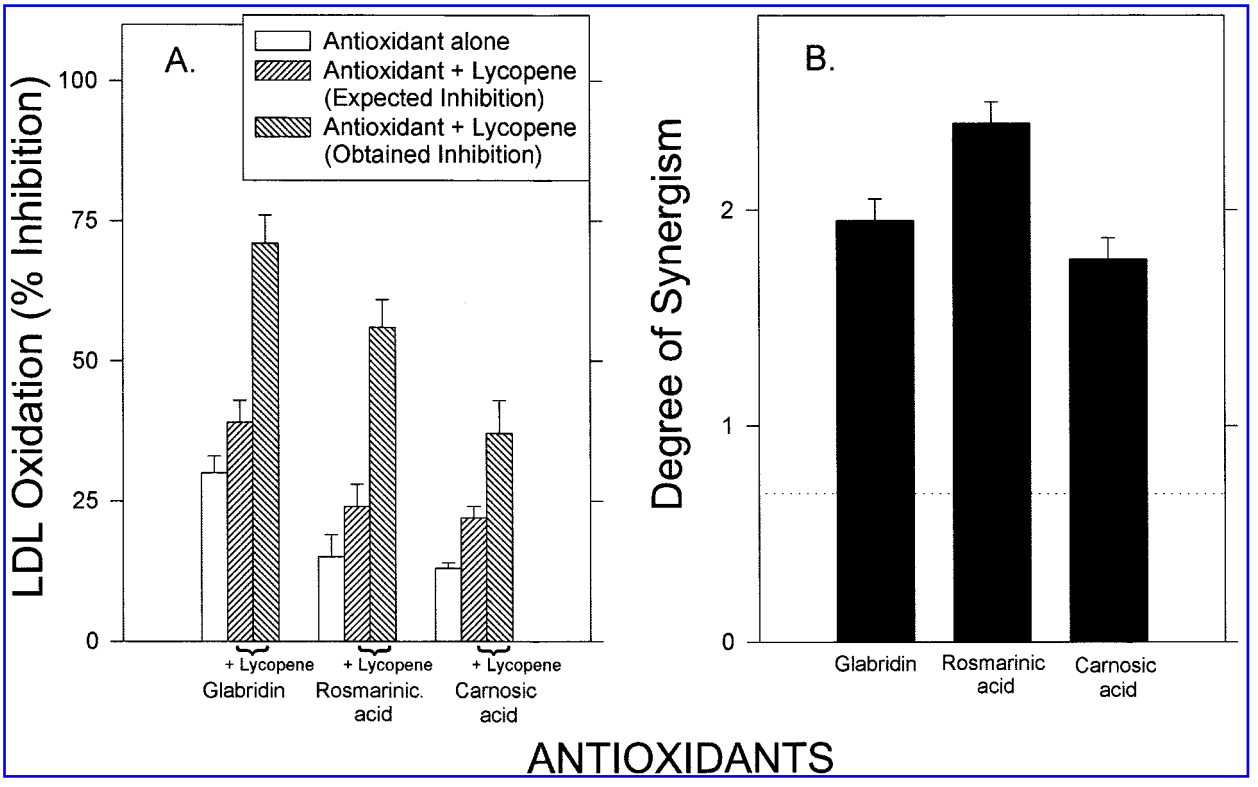

FIG. 6. (A) Effect of lycopene in combination with glabridin, rosmarinic acid, or carnosic acid on LDL oxidation induced by copper ions. (B) Calculated degree of synergism between lycopene and glabridin, rosmarinic acid, or carnosic acid. LDL (100 μg of protein/ml) was preincubated for 30 min at 37°C with glabridin (1 μmol/liter), or with rosmarinic acid (25 μmol/liter) or with carnosic acid (25 μmol/liter) alone (open bars), or in combination with lycopene (5 μmol/liter) (hatched bars). Then, LDL was further incubated for 2 hr at 37°C with 5 μmol/liter of $CuSO_4$. The expected additive and the obtained values for inhibition of LDL oxidation are represented as left diagonal hatched bars and right diagonal hatched bars, respectively. LDL oxidation was measured as TBARS formation. Data are expressed as percentage of inhibition obtained in the presence of antioxidants out of oxidation rate of control LDL incubated with no added antioxidants. Lycopene alone inhibited LDL oxidation by 9%. Results are given as mean ± SD (n = 3).

tively. Addition of increasing concentrations of garlic (ranging from 100 μg/ml to 500 μg/ml), together with a constant lycopene concentration (5 μmol/liter), inhibited LDL oxidation, as measured by the TBARS (Fig. 8A) or lipid peroxide (Fig. 8C) assays, in a synergistic manner. Figure 8, B and D, shows that on using garlic concentration of 250 μg/ml a degree of synergism of 2.18 and 2.3 was obtained for the TBARS and lipid peroxides assays, respectively (Fig. 8B,D). However, at garlic concentrations as high as 500 μg/ml only the expected additive effect of both antioxidants was obtained.

The *in vivo* relevance of the effect of lycopene in combination with other natural antioxidants on the susceptibility of LDL to oxidation was then studied. We administered a fatty meal containing 30 mg of lycopene in the form of tomato oleoresin to 4 healthy volunteers. Consumption of a fatty meal containing 30 mg of lycopene in tomato oleoresin (that contains 6% lycopene, 1% vitamin E, 0.1% β-carotene, and also phenolics) by the volunteers resulted in a significant gradual increase in plasma lycopene concentrations (Fig. 9A) by 16% and by 70% after 3 and 5 hr of the meal consumption, respectively. LDL that was isolated from these subjects before meal consumption and in the postprandial state 3 and 5 hr after the meal, was exposed to copper ion-induced lipid peroxidation. LDL, which was isolated 3 hr after ingestion of the fatty meal containing tomato oleoresin, demonstrated a nonsignificant increment in copper ion-induced formation of TBARS (Fig. 9B), whereas LDL, which was isolated 5 hr after meal consumption, exhibited a signifi-

Table 1. Effect of Lycopene in Combination with Vitamin E, Tocotrienol, Glabridin, Rosmarinic Acid, Carnosic Acid, or Garlic on LDL Oxidation Induced by AAPH

	AAPH-induced LDL oxidation			
	Antioxidant alone	*Antioxidant + lycopene*		
	Percent of inhibition	*Percent expected additive inhibition*	*Percent obtained inhibition*	*Degree of synergism*
Lycopene (5 μmol/liter)	9.0 ± 0.5			
Vitamin E (5 μmol/liter)	10.0 ± 0.6	19.0 ± 0.8	34.0 ± 1.1	1.78 ± 0.15
Tocotrienol (5 μmol/liter)	8.0 ± 0.5	17.0 ± 1.1	11.3 ± 0.9	0.66 ± 0.06
Glabridin (1 μmol/liter)	14.6 ± 0.7	23.6 ± 0.9	40.7 ± 1.5	1.72 ± 0.07
Carnosic acid (25 μmol/liter)	22.6 ± 0.9	31.6 ± 1.6	38.0 ± 1.2	1.20 ± 0.06
Rosmarinic acid (25 μmol/liter)	40.8 ± 1.8	49.8 ± 1.5	58.4 ± 2.1	1.17 ± 0.05
Garlic (250 mg/ml)	19.5 ± 0.9	28.5 ± 1.3	37.8 ± 2.2	1.32 ± 0.8

LDL (100 of μg protein/ml) was preincubated for 30 min at 37°C with: vitamin E (5 μmol/liter), tocotrienol (5 μmol/liter), glabridin (1 μmol/liter), carnosic acid (25 μmol/liter), rosmarinic acid (25 μmol/liter), or garlic (250 μmol/liter), alone, or in combination with lycopene (5 μmol/liter). Then, LDL was further incubated for 2 h at 37°C with 5 mmol/liter of AAPH. LDL oxidation was measured as TBARS formation. Data are expressed as percentage of inhibition obtained in the presence of antioxidants out of oxidation rate of control LDL incubated with no added antioxidants. Vitamin E refers to tocopherol. Results are given as mean ± SD ($n = 3$).

cant ($p < 0.01$) reduced susceptibility to oxidation by 21%, in comparison to values obtained before meal consumption (Fig. 9B). These results suggest that lycopene acts as an antioxidant *in vivo* when administered together with natural antioxidants, such as vitamin E, flavonoids, and phenolics.

DISCUSSION

This study presents the first evidence that lycopene acts synergistically with several natural antioxidants as an effective antioxidant against LDL oxidation. This effect was demonstrated in two different model systems of oxidation: one induced by copper ions and the other induced by free radicals. Among the antioxidants studied, lycopene acted synergistically with vitamin E, with the isoflavan glabridin and with the phenolics rosmarinic and carnosic acid, but not with tocotrienols. Furthermore, lycopene potentiated the antioxidant activity of garlic, which is by itself a natural product containing a mixture of antioxidants. The ability of lycopene in combination with these diverse antioxidants to protect against lipoprotein oxidation was tested in a system using isolated LDL exposed to copper ions, as well as to free radicals.

Oxidation of LDL is now commonly implicated as an initiator of atherosclerosis, and thus antioxidants that can protect LDL against oxidation are potential antiatherogenic compounds. This experimental system makes the assumption that direct protection of LDL from oxidation is the mode by which antioxidants might be, at least in part, conferring the protection against atherosclerosis, as noted in several epidemiological studies. Antioxidants can also alter cellular oxidative state, hence affecting the ability of the cells to initiate LDL oxidation.

The concentration of lycopene used in these *in vitro* experiments was 5 μmol/liter, which is relatively high concentration, in comparison to plasma lycopene levels, or to those measured after dietary supplementation of 30 mg of ly-

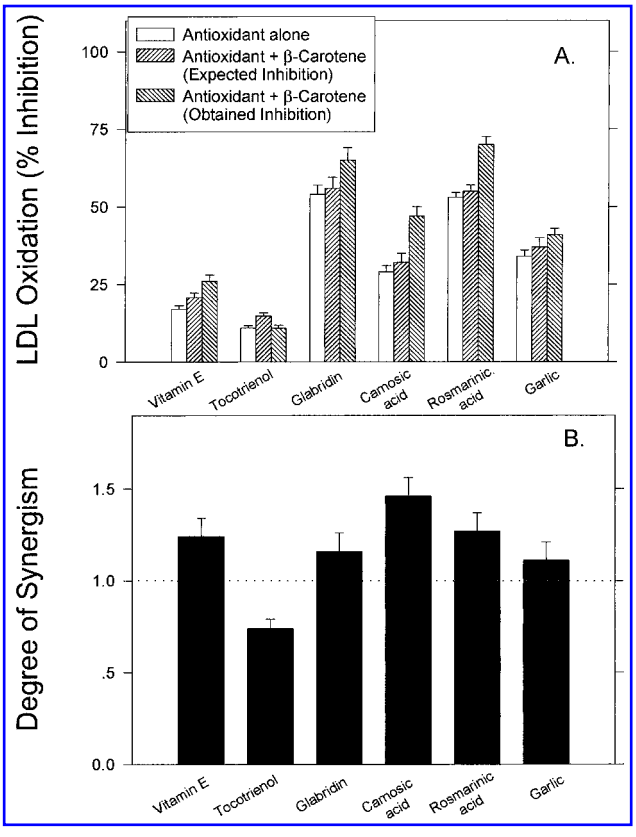

FIG. 7. (A) Effect of β-carotene in combination with vitamin E, tocotrienol, glabridin, rosmarinic acid, carnosic acid, or garlic on LDL oxidation induced by copper ions. (B) Calculated degree of synergism between β-carotene and vitamin E, tocotrienol, glabridin, rosmarinic acid, carnosic acid, or garlic. LDL (100 μg protein/ml) was preincubated for 30 min at 37°C with: vitamin E (5 μmol/liter), tocotrienol (5 μmol/liter), glabridin (1 μmol/liter), carnosic acid (25 μmol/liter), rosmarinic acid (25 μmol/liter), or garlic (250 μmol/liter), alone (open bars) or in combination with β-carotene (5 μmol/liter) (hatched bars). Then, LDL was further incubated for 2 hr at 37°C with 5 μmol/liter of $CuSO_4$. The expected additive and the obtained values for inhibition of LDL oxidation are represented as left diagonal hatched bars and right diagonal hatched bars, respectively. LDL oxidation was measured as TBARS formation. Data are expressed as percentage of inhibition obtained in the presence of antioxidants out of oxidation rate of control LDL incubated with no added antioxidants. β-Carotene alone inhibited LDL oxidation by 4.5%. Results are given as mean ± SD ($n = 3$).

copene. However, we have previously shown (Lavy *et al.*, 1993) that only about 5% of the added carotenoid remains associated with the LDL. Thus, it may be estimated that the LDL in the *in vitro* studies was enriched with approximately 250 nmol lycopene/liter, which is in the range of the values obtained in the subjects' plasma, following dietary supplementation of tomato oleoresin. Similar levels of plasma β-carotene were achieved following dietary supplementation of 50–100 mg of β-carotene/day for a period of 30 days in healthy subjects (Gaziano *et al.*, 1995).

Recently, we have reported that enrichment of LDL with lycopene can increase its resistance to metal ion-induced oxidation, similarly to the effect of β-carotene in some but not all LDLs that were studied (Fuhrman *et al.*, 1997c). However, when lycopene was supplemented as tomato oleoresin, which besides lycopene contains several other micronutrient antioxidants, the inhibition of LDL oxidation was significantly greater in comparison to the inhibitory effect of lycopene alone. Because tomatoes were shown to contain, in addition to lycopene, vitamin E, flavonoids, and phenolics (Beecher, 1998), we have analyzed possible cooperation between lycopene and diverse antioxidants with different chemical structure. The data obtained in this study show that lycopene acts synergistically in combination with vitamin E (α-tocopherol) but not with tocotrienol. The beneficial effects of combined natural antioxidants may be related to different physicochemical properties of the different antioxidants. Lycopene is an acyclic carotenoid with 11 linearly arranged conjugated double bonds, which accounts for its hydrophobicity. On the other hand, vitamin E is the most abundant lipophilic antioxidant *in vivo*. It is a chain-breaking antioxidant that protects lipids by scavenging peroxyl radicals without reacting in further chain-propagating steps. The reactivity of vitamin E with peroxyl radicals is associated with the redox properties of the chromone ring (Fig. 2), and accounts for its antioxidant activity.

The location of the antioxidants within the LDL is an additional determinant factor for the interaction between them. Vitamin E is known to be located at or near the membrane surface (Gomez-Fernandez *et al.*, 1989), and it has been shown that the efficiency of radical scavenging by vitamin E decreases as the radical goes deeper into the interior (Takahashi *et al.*, 1989). Lycopene, which lacks hydrophilic substituents, is extremely hydrophobic, is located within the hydrophobic core of LDL, and, thus, the free radical-scavenging ability of lycopene

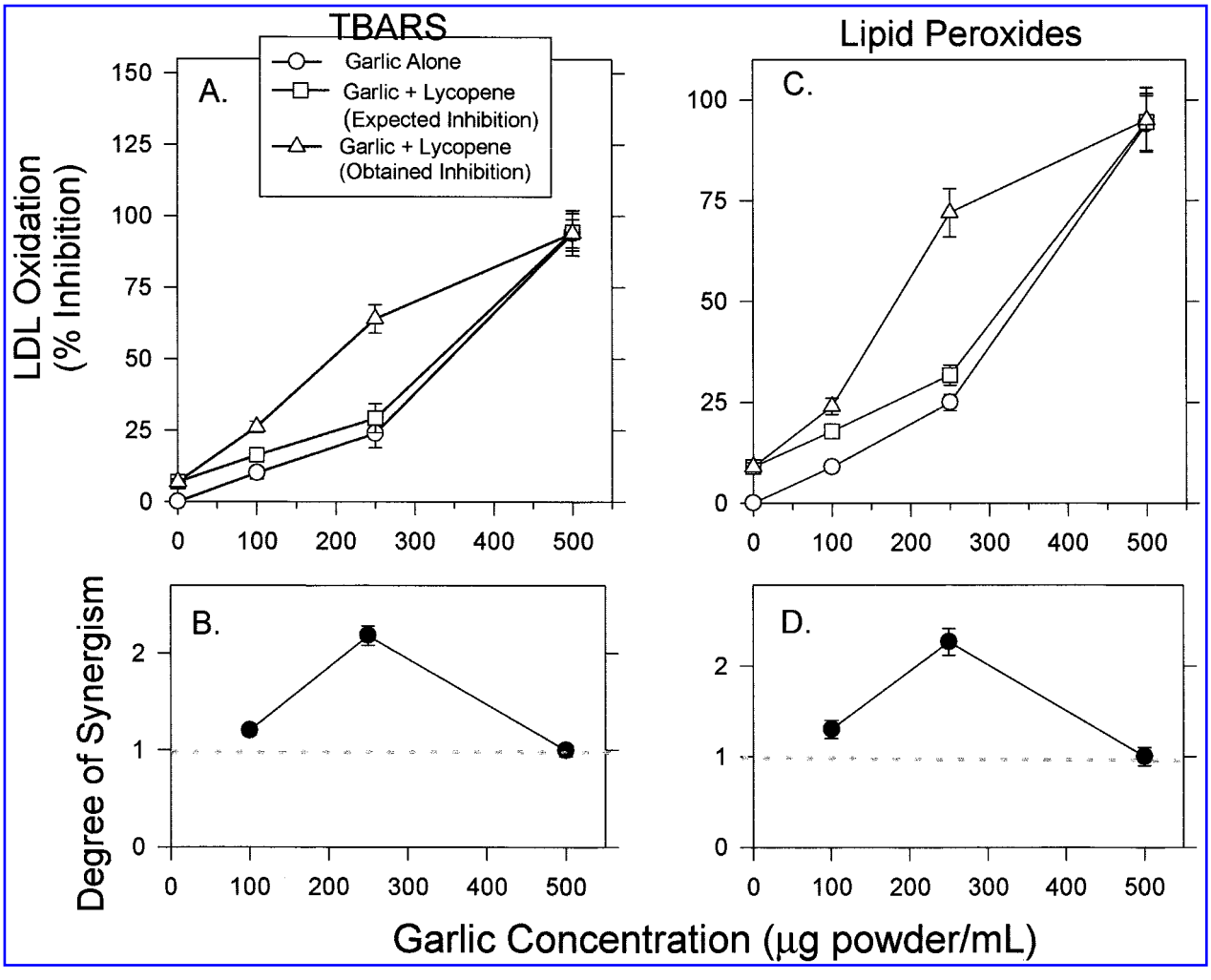

FIG. 8. (A and C) Effect of lycopene in combination with garlic on LDL oxidation induced by copper ions. (B and D) Calculated degree of synergism between lycopene and garlic as a function of garlic concentration. LDL (100 μg of protein/ml) was preincubated for 30 min at 37°C with increasing concentrations of garlic alone (circles), or with the indicated concentrations of garlic in combination with lycopene (5 μmol/liter). Then, LDL was further incubated for 2 hr at 37°C with 5 μmol/liter of $CuSO_4$. The expected additive and the obtained values for inhibition of LDL oxidation are presented as squares and triangles, respectively. LDL oxidation was measured as TBARS **(A,B)** or as lipid peroxides **(C,D)** formation. Data are expressed as percentage of inhibition obtained in the presence of antioxidants out of oxidation rate of control LDL incubated with no added antioxidants. Lycopene alone inhibited TBARS or lipid peroxide formation in LDL by 7% and 9%, respectively. Values are given as mean ± SD (n = 3).

is limited to the interior of the lipoprotein. Lycopene, in combination with vitamin E, may exert a synergistic effect by acting at different portions of the LDL particle, *i.e.*, vitamin E at the surface and lycopene in the interior. Antioxidants, which act by different mechanisms, may inhibit LDL oxidation additively or even synergistically. Chemically, lycopene is less reactive toward free radicals than is vitamin E. Vitamin E is a scavenger of the peroxyl radical by hydrogen atom donation, whereas lycopene can prevent lipid peroxidation via singlet oxygen quenching (DiMascio *et al.*, 1989), or via scavenging of peroxyl radicals (Miller *et al.*, 1996). However, lycopene scavenges free radicals by an addition to the double bond to give a conjugated polyene carbon-centered radical, which is resonance stabilized. The lycopene–peroxyl radical formed by an interaction with oxygen molecule is not stable, and is capable of inducing oxidation. Synergism between vitamin E and lycopene thus may be explained by the scavenging of the lycopene-derived peroxyl radicals by vitamin E.

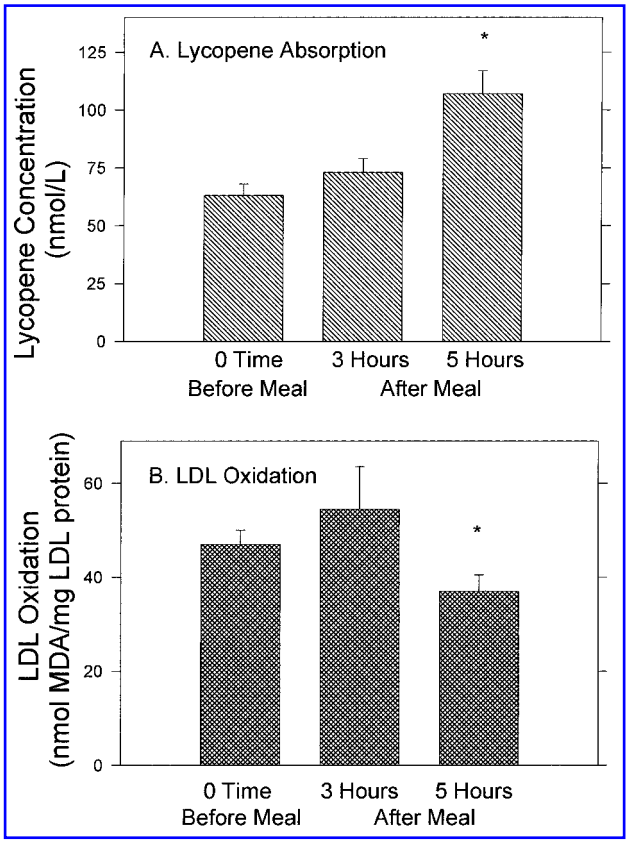

FIG. 9. Effect of tomato oleoresin consumption on the *ex vivo* susceptibility of LDL to oxidation. (A) Lycopene concentrations in plasma derived from healthy volunteers before or after 3 or 5 hr of a fatty meal consumption, which contained 30 mg of lycopene in tomato oleoresin. **(B)** Plasma LDL derived before or after 3 or 5 hr of meal consumption was incubated for 2 hr at 37°C with 5 μmol/liter of $CuSO_4$. LDL oxidation was measured as TBARS formation. Results are expressed as mean ± SD ($n = 3$). $*p < 0.01$.

Similar synergistic effect was demonstrated between β-carotene and vitamin E by Palozza and Krinksy (1992). However, these authors suggest that vitamin E protects β-carotene from oxidation, whereas Bohm *et al.* (1997) provide evidence for the opposite effect.

In contrast to the synergism between lycopene and vitamin E, the combination of lycopene with tocotrienols did not synergistically inhibit LDL oxidation. On the contrary, addition of lycopene to LDL incubated with tocotrienol antagonized the inhibitory effect exerted by tocotrienol alone. Tocotrienols are a form of vitamin E having an unsaturated side-chain, rather than the saturated side-chain of vitamin E. Tocotrienol may be localized in a different compartment within the lipoprotein, in comparison to vitamin E, and this location may result in a pro-oxidative effect of lycopene toward tocotrienols.

Our results demonstrate that lycopene also potentiates the antioxidative effect of several polyphenols, and the extent of synergism depends on the compound chemical structure. The polyphenol antioxidants constitute a large class of compounds, containing a number of phenolic hydroxyl groups attached to ring structures, conferring their antioxidant activity (Rice-Evans *et al.*, 1996; Van Acker *et al.*, 1996). Polyphenols are multifunctional and can act as reducing agents, as hydrogen-donating antioxidants, and as singlet oxygen quenchers. In some cases, polyphenols exhibit also metal ion chelation properties.

In our present study, we have examined interactions between lycopene and three chemically different polyphenols. Glabridin is an isoflavan with hydrophobic characteristics that direct its position in LDL toward the lipid compartment where lycopene is also localized. The phenolics carnosic acid and rosmarinic acid are less hydrophobic in comparison to glabridin, and thus their location in LDL may be more toward the LDL surface. Our data demonstrated a most remarkable synergistic effect between lycopene and glabridin, as well as with rosmarinic acid and carnosic acid. However, the differences in antioxidant capacities could be the result of the different positioning in the LDL, as well as different antioxidant activities of these polyphenols, as determined by the number of hydroxyl groups in the molecule. Lycopene acts synergistically not only with isolated antioxidants, but also with a mixture of different antioxidants, such as those found in garlic. Garlic contains flavonoids and phenolics in addition to allicin, which is its major active component (Carotenuto *et al.*, 1996).

The interactive effects seen with lycopene were also observed with β-carotene, but to a lesser degree of synergism, probably due to the structural differences between lycopene and β-carotene.

Taken together, our results clearly indicate that a combination of different antioxidants is superior to the action of an individual antioxidant in protecting LDL against oxidation. Indeed, a combination of lycopene with other natural antioxidants present in tomato oleo-

resin possesses a remarkable antioxidant activity. Dietary administration of tomato oleoresin, where lycopene is present together with diverse natural antioxidants, including vitamin E, flavonoids, and phenolics, resulted in reduced susceptibility to oxidation of LDL that was isolated at the postprandial state. In accordance with these results, we have previously demonstrated that dietary administration of lycopene as tomato oleoresin to the atherosclerotic apolipoprotein E knockout mice results in a superior inhibition of their LDL oxidation, in comparison to dietary administration of lycopene alone (Fuhrman *et al.*, 1997c). These effects can be attributed to absorption of lycopene, as demonstrated by a 70% elevation in lycopene levels in plasma derived after consumption of a fatty meal containing 30 mg of lycopene in tomato oleoresin. Similar results were recently reported (Crawford *et al.*, 1998), showing that one portion of both raw or processed tomatoes, corresponding to 0.56 μmol lycopene/kg body weight, was able to increase the plasma lycopene concentration significantly. Furthermore, it was demonstrated that plasma lycopene concentration increased significantly after ingestion of tomato juice, tomato oleoresin, and lycopene beadlets. Combined supplemented dietary antioxidants (vitamin E, β-carotene, and vitamin C) also inhibited both LDL oxidation and atherogenesis in animals with elevated LDL (Crawford *et al.*, 1998). However, in other studies, similar antioxidant combinations administered to healthy human volunteers were not found to be superior to a high dose of vitamin E alone in inhibiting LDL oxidation (Jialal and Grundy, 1993; Reaven *et al.*, 1993).

In conclusion, the present study is the first one to report that lycopene can potentiate the activity of several other natural antioxidants against LDL oxidation. Because LDL oxidation is thought to play a key role in early atherogenesis, the use of lycopene in combination with vitamin E or several polyphenols may be proven beneficial for attenuation of atherosclerosis.

ACKNOWLEDGMENT

The dietary assistance of Mrs. Vera Cohan-Levental is very much appreciated.

ABBREVIATIONS

AAPH, 2,2′-Azobis (2-amidinopropane) dihydrochloride; BHT, butylated hydroxytoluene; EDTA, ethylenediamine tetraacetic acid; HPLC, high-performance liquid chromatography; LDL, low-density lipoprotein; PBS, phosphate-buffered saline; TBARS, thiobarbituric acid reactive substances; THF, tetrahydrofuran; Vitamin E, tocopherol.

REFERENCES

ARUOMA, O.I., HALLIWELL, B., AESCHBACH, R., and LOLIGERS, J. (1992). Antioxidant and pro-oxidant properties of active rosemary constitutents: carnosol and carnosic acid. Xenobiotica **22,** 257–268.

AVIRAM, M. (1983). Plasma lipoprotein separation by discontinuous density gradient ultracentrifugation in hyperlipoproteinemic patients. Biochem Med. **30,** 111–118.

AVIRAM, M. (1993a). Beyond cholesterol: Modifications of lipoproteins and increased atherogenicity. In: *Atherosclerosis, Inflammation and Thrombosis.* G.G. Neri Serneri, G.F. Gensini, R. Abbate, and D. Prisco, eds. (Scientific Press-Florence, Italy) pp. 15–36.

AVIRAM, M. (1993b). Modified forms of low density lipoprotein and atherosclerosis. Atherosclerosis **98,** 1–9.

AVIRAM, M. (1996). Interaction of oxidized low density lipoprotein with macrophages in atherosclerosis and the antiatherogenicity of antioxidants. Eur. J. Clin. Chem. Clin. Biochem. **34,** 599–608.

AVIRAM, M., and FUHRMAN, B. (1998a). LDL oxidation by arterial wall macrophages depends on the oxidative status in the lipoprotein and in the cells: role of prooxidants vs. antioxidants. Mol. Cell. Biochem. **188,** 149–159.

AVIRAM, M., and FUHRMAN, B. (1998b). Polyphenolic flavonoids inhibit macrophage-mediated oxidation of LDL and attenuate atherogenesis. Atherosclerosis **137** (Suppl.), S45–S50.

AVIRAM, M., and EIAS, K. (1993). Dietary olive oil reduces low-density lipoprotein uptake by macrophages and decreases the susceptibility of the lipoprotein to undergo lipid peroxidation. Ann. Nutr. Metab. **37,** 75–84.

AVIRAM, M., HAYEK, T., and FUHRMAN, B. (1997). Red wine consumption inhibits LDL oxidation and aggregation in humans and in atherosclerotic mice. Biofactors **6,** 415–419.

BELINKY, P.A., AVIRAM, M., FUHRMAN, B., ROSENBLAT, M., and VAYA, J. (1998). The antioxidative effects of the isoflavan glabridin on endogenous constituents of LDL during its oxidation. Atherosclerosis **137,** 49–61.

BEECHER, G.R. (1998). Nutrient content of tomatoes and tomato products. Proc. Soc. Exp. Biol. Med. **218,** 98–100.

BOHM, F., EDGE, R., LANK, E.J., McGARVEY, D.J., and

TRUSCOTT, T.G. (1997). Carotenoids enhance vitamin E antioxidant efficiency. J. Am. Chem. Soc. **119,** 621–622.

BROWN, M.S., and GOLDSTEIN, J.L. (1983). Lipoprotein metabolism in the macrophage: implications for cholesterol deposition in atherosclerosis. Annu. Rev. Biochem. **52,** 223–261.

BUEGE, J.A., and AUST, S.D. (1978). Microsomal lipid peroxidation. Methods Enzymol. **52,** 302–310.

BURTON, G.W. (1989). Antioxidant action of carotenoids. J. Nutr. **119,** 109–111.

BURTON, G.W., JOYCE, A., and INGOLD, K.U. (1983). Is vitamin E the only lipid-soluble, chain-breaking antioxidant in human blood plasma and erythrocyte membranes? Arch. Biochem. Biophys. **221,** 281–290.

CAROTENUTO, A., DE FEO, V., FATTORUSSO, E., LANZOTTI, V., MAGNO, S., and CICALA, C. (1996). The flavonoids of Allium ursinum. Phytochemistry **41,** 531–536.

CLINTON, S.K. (1998). Lycopene: chemistry, biology, and implications for human health and disease. Nutr. Rev. **56,** 35–51.

CRAWFORD, R.C., KIRK, E.A., ROSENFELD, M.E., LEBOEUF, R.C., and CHAIT, A. (1998). Dietary antioxidants inhibit development of fatty streak lesions in the LDL receptor-deficient mouse. Arterioscler. Thromb. Vasc. Biol. **18,** 1506–1513.

DI MASCIO, P., KAISER, S., and SIES, H. (1989). Lycopene as the most efficient biological carotenoid singlet oxygen quencher. Arch. Biochem. Biophys. **274,** 532–538.

EL-SAADANI, M., ESTERBAUER, H., EL-SAYED, M., GOHER, M., NASSAR, A.Y., and JURGENS, G. (1989). A spectrophotometric assay for lipid peroxides in serum lipoproteins using a commercially available reagent. J. Lipid Res. **30,** 627–630.

FUHRMAN, B., LAVY, A., and AVIRAM, M. (1995). Consumption of red wine with meals reduces the susceptibility of human plasma and low-density lipoprotein to lipid peroxidation. Am. J. Clin. Nutr. **61,** 549–554.

FUHRMAN, B., BUCH, S., VAYA, J., BELINKY, P.A., COLEMAN, R., HAYEK, T., and AVIRAM, M. (1997a). Licorice extract and its major polyphenol glabridin protect low-density lipoprotein against lipid peroxidation; in vitro and ex vivo studies in humans and in atherosclerotic apolipoprotein E-deficient mice. Am. J. Clin. Nutr. **66,** 267–275.

FUHRMAN, B., ELIS, A., and AVIRAM, M. (1997b). Hypocholesterolemic effect of lycopene and beta-carotene is related to suppression of cholesterol synthesis and augmentation of LDL receptor activity in macrophages. Biochem. Biophys. Res. Commun. **233,** 658–662.

FUHRMAN, B., BEN-YAISH, L., ATTIAS, J., HAYEK, T., and AVIRAM, M. (1997c). Tomato lycopene and β-carotene inhibit low density lipoprotein oxidation and this effect depends on the lipoprotein vitamin E content. Nutr. Metab. Cardiovasc. Dis. **7,** 433–443.

GAZIANO, J.M., HATTA, A., FLYNN, M., JOHNSON, E.J., KRINSKY, N.I., RIDKER, P.M., HENNEKES, C.H., and FREI, B. (1995). Supplementation with β-carotene in vivo and in vitro does not inhibit low density lipoprotein oxidation. Atherosclerosis **112,** 187–195.

GERSTER, H. (1997). The potential role of lycopene for human health. J. Am. Coll. Nutr. **16,** 109–126.

GOMEZ-FERNANDEZ, J.C., VILLALAIN, J., ARANDA, F.J., ORTIZ, A., MICOL., V., COUTINHO, A., BERBERAN-SANTOS, M.N., and PRIETO, M.J. (1989). Localization of α-tocopherol in membranes. Ann. N.Y. Acad. Sci. **570,** 109–120.

HALLIWELL, B. (1994). Free radicals, antioxidants and human disease: curiosity, cause, or consequence. Lancet **344,** 721–724.

HARAGUCHI, H., SAITO, T., OKAMURA, N., and YAGI, A. (1995). Inhibition of lipid peroxidation and superoxide generation by diterpenoids from Rosmarinus officinalis. Planta Med. **61,** 333–336.

HAYEK, T., FUHRMAN, B., VAYA, J., ROSENBLAT, M., BELINKY, P., COLEMAN, R., ELIS, A., and AVIRAM, M. (1997). Reduced progression of atherosclerosis in apolipoprotein E-deficient mice following consumption of red wine, or its polyphenols quercetin or catechin, is associated with reduced susceptibility of LDL to oxidation and aggregation. Arterioscler. Thromb. Vasc. Biol. **17,** 2744–2752.

HERTOG, M.G., FESKENS, E.J., HOLLMAN, P.C., KATAN, M.B., and KROMHOUT, D. (1993). Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. Lancet **342,** 1007–1011.

JIALAL, I., and GRUNDY, S.M. (1993). Effect of combined supplementation with α-tocopherol, ascorbate, and beta carotene on low density lipoprotein oxidation. Circulation **88,** 2780–2786.

KOHLMEIER, L., and HASTING, S.B. (1995). Epidemiologic evidence of a role of carotenoids in cardiovascular disease prevention. Am. J. Clin. Nutr. **62,** 137S–146S.

LARNER, A.J. (1995). How does garlic exert its hypocholesterolaemic action? The tellurium hypothesis. Med. Hypotheses **44,** 295–297.

LAVY, A., BEN-AMOTZ, A., and AVIRAM, M. (1993). Preferential inhibition of LDL oxidation by the all-*trans* isomer of β-carotene in comparison with 9-*cis* β-carotene. Eur. J. Clin. Chem. Clin. Biochem. **31,** 83–90.

LAWSON, L.D. (1983). Bioactive organsulfur compounds of garlic and their role in reducing blood lipids. In: *Human Medicinal Agents from Plants.* A.D. Kinghom and M.F. Balandrin, eds. (American Chemical Society Books, Washington, D.C.) pp. 308–330.

LOWRY, O.H., ROSENBROUGH, N.J., FARR, A.L., and RANDAL, R.J. (1951). Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193,** 265–275.

MAYNE, S.T. (1996). Beta-carotene, carotenoids, and disease prevention in humans. FASEB J. **10,** 690–710.

MILLER, N.J., SAMPSON, J., CANDEIAS, L.P., BRAMLEY, P.M., and RICE-EVANS, C.A. (1996). Antioxidant activities of carotenoids and xanthophylls. FEBS Lett. **384,** 240–242.

MULDOON, M.F., and KRITCHEVSKY, S.B. (1996). Flavonoids and heart disease. Br. Med. J. **312,** 458–459.

NAGOURNEY, R.A. (1998). Garlic: medicinal food or nutritious medicine. J. Med. Food **1,** 13–27.

OFFORD, E.A., MACE, K., BUFFIEUX, C., MALNOE, A., and PFEIFER, A.M.A. (1995). Rosemary components inhibit benzo(a)pyrene-induced genotoxicity in human bronchial cells. Carcinogenesis **16,** 2057–2062.

OREKHOV, A.N., and GRUNWALD, A.J. (1997). Effects of garlic on atherosclerosis. Nutrition **13,** 656–663.

OREKHOV, A.N., TERTOV, V.V., SOBENIN, I.A., and PIVOVAROVA, E.M. (1995). Direct anti-atherosclerosis-related effects of garlic. Ann. Med. **27,** 63–65.

OREKHOV, A., PIVOVAROVA, E.M., and TERTOV, V.V. (1996). Garlic powder tablets reduce atherogenicity of low density lipoprotein. A placebo-controlled double-blind study. Nutr. Metab. Cardiovasc. Dis. **6,** 21–31.

PALOZZA, P., and KRINSKY, N.I. (1992). β-Carotene and a-tocopherol are synergistic antioxidants. Arch. Biochem. Biophys. **297,** 184–187.

PARTHASARATHY, S., PRINTZ, D.J., BOYD, D., JOY, L., and STEINBERG, D. (1986). Macrophage oxidation of low-density lipoprotein generates a modified form recognized by the scavenger receptor. Arteriosclerosis **6,** 505–510.

PHELPS, S., and HARRIS, W.S. (1993). Harris Garlic supplementation and lipoprotein oxidation susceptibility. Lipids **28,** 475–477.

RABINKOV, A., MIRON, T., KONSTANTIONOVSKI, L., WILCHEK, M., MIRELMAN, D., and WEINER, L. (1998). The mode of action of allicin: trapping of radicals and interaction with thiol containing proteins. Biochim. Biophys. Acta **1379,** 233–244.

REAVEN, P.D., KHOUW, A., BELTZ, W.F., PARTHASARATHY, S., and WITZTUM, J.L. (1993). Effect of dietary antioxidant combinations in humans: protection of LDL by vitasmin E but not by β-carotene. Aterioscler. Thromb. **13,** 590–600.

RICE-EVANS, C.A., MILLER, N.J., and PAGANGA, G. (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic. Biol. Med. **20,** 933–956.

RIMM, E.B., STAMPFER, M.J., ASCHERIO, A., GIOVANNUCCI, E., COLDITZ, G.A., and WILLETT, W.C. (1993). Vitamin E consumption and the risk of coronary heart disease in men. N. Engl. J. Med. **328,** 1450–1456.

SIES, H., and STAHL, W. (1998). Lycopene: antioxidant and biological effects and its bioavailability in the human. Proc. Soc. Exp. Biol. Med. **218,** 121–124.

SIMONS, L.A., BALASUBRAMANIAN, S., VON KONIGSMARK, M., PARFITT, A., SIMONS, J., and PETERS, W. (1995). On the effect of garlic on plasma lipids and lipoproteins in mild hypercholesterolemia. Atherosclerosis **113,** 219–225.

STAHL, W., and SIES, H. (1996). Lycopene: A biologically important carotenoid for humans? Arch. Biochem. Biophys. **336,** 1–9.

STAHL, W., JUNGHANS, A., DE BOER, B., DRIOMINA, E.S., BRIVIBA, K., and SIES, H. (1998). Carotenoid mixtures protect multilamellar liposomes against oxidative damage: synergistic effects of lycopene and lutein. FEBS Lett. **427,** 305–308.

STAMPFER, M.J., HENNEKENS, C.H., MANSON, J.E., COLDITZ, G.A., ROSNER, B., and WILLETT, W.C. (1993). Vitamin E consumption and the risk of coronary disease in women. N. Engl. J. Med. **328,** 1444–1449.

SUARNA, C., HOOD, R.L., DEAN, R.T., and STOCKER, R. (1993). Comparative antioxidant activity of tocotrienols and other natural lipid-soluble antioxidants in a homogeneous system, and in rat and human lipoproteins. Biochim. Biophys. Acta **1166,** 163–170.

TAKAHSHI, M., TSUCHIA, J., and NIKI, E. (1989). Scavenging of radicals by vitamin E in the membranes as studied by spin labeling. J. Am. Chem. Soc., **111,** 6350–6353.

THE ALPHA-TOCOPHEROL BETA-CAROTENE CANCER PREVENTION STUDY GROUP. (1994). The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. N. Engl. J. Med. **330,** 1029–1035.

TOMEO, A.C., GELLER, M., WATKINS, T.R., GAPOR, A., and BIERENBAUM, M.L. (1995). Antioxidant effects of tocotrienols in patients with hyperlipidemia and carotid stenosis. Lipids **30,** 1179–1183.

VAN ACKER, S.A., VAN DEN BERG, D.J., TROMP, M.N., GRIFFIOEN, D.H., VAN BENNEKOM, W.P., VAN DER VIJGH, W.J., and BAST, A. (1996). Structural aspects of antioxidant activity of flavonoids. Free Radic. Biol. Med. **20,** 331–342.

VERLANGIERI, A.J., KAPEGHIAN, J.C., EL-DEAN, S., and BUSH, M. (1985). Fruit and vegetable consumption and cardiovascular mortality. Med. Hypotheses **16,** 7–15.

VISIOLI, F., BELLOMO, G., and BALLI, C. (1998). Free radical-scavenging properties of olive oil polyphenols. Biochem. Biophys. Res. Commun. **247,** 60–64.

WITZTUM, J.L., and STEINBERG, D. (1991). Role of oxidized low density lipoprotein in atherogenesis. J. Clin. Invest. **88,** 1785–1792.

WU, J.W., LEE, M.H., HO, C.T., and CHANG, S.S. (1982). Elucidation of the chemical structures of natural antioxidants isolated from rosemary. J. Am. Oil Chem. Soc. **59,** 339–345.

ZIEGLER, R.G. (1991). Vegetables, fruits, and carotenoids and the risk of cancer. Am. J. Clin. Nutr. **53** (1 Suppl), 251S–259S.

Address reprint requests to:
Dr. Michael Aviram
The Lipid Research Laboratory
Rambam Medical Center
Haifa, Israel

E-mail: aviram@tx.technion.ac.il

Received for publication February 7, 2000; accepted May 9, 2000.

This article has been cited by:

1. Nurit Hadad, Rachel Levy. 2012. The synergistic anti-inflammatory effects of lycopene, lutein, #-carotene, and carnosic acid combinations via redox-based inhibition of NF-#B signaling. *Free Radical Biology and Medicine* **53**:7, 1381-1391. [CrossRef]
2. Yu-Yun Gao, Qing-Mei Xie, Jing-Yun Ma, Xiang-Bin Zhang, Ji-Mei Zhu, Ding-Ming Shu, Bao-Li Sun, Ling Jin, Ying-Zuo Bi. 2012. Supplementation of xanthophylls increased antioxidant capacity and decreased lipid peroxidation in hens and chicks. *British Journal of Nutrition* 1-7. [CrossRef]
3. Graziano Riccioni, Lorenza Speranza, Mirko Pesce, Salvatore Cusenza, Nicolantonio D'Orazio, Michael J. Glade. 2012. Novel phytonutrient contributors to antioxidant protection against cardiovascular disease. *Nutrition* **28**:6, 605-610. [CrossRef]
4. In Young Bae, Bo-Yeon Kwak, Hyeon Gyu Lee. 2012. Synergistic antiradical action of natural antioxidants and herbal mixture for preventing dioxin toxicity. *Food Science and Biotechnology* **21**:2, 491-496. [CrossRef]
5. Merel Hazewindus, Guido R.M.M. Haenen, Antje R. Weseler, Aalt Bast. 2011. The anti-inflammatory effect of lycopene complements the antioxidant action of ascorbic acid and #-tocopherol. *Food Chemistry* . [CrossRef]
6. Helen Macpherson, Kathryn A. Ellis, Avni Sali, Andrew Pipingas. 2011. Memory improvements in elderly women following 16 weeks treatment with a combined multivitamin, mineral and herbal supplement. *Psychopharmacology* . [CrossRef]
7. Zora Kotíková, Jaromír Lachman, Alena Hejtmánková, Kate#ina Hejtmánková. 2011. Determination of antioxidant activity and antioxidant content in tomato varieties and evaluation of mutual interactions between antioxidants. *LWT - Food Science and Technology* **44**:8, 1703-1710. [CrossRef]
8. Baoru Yang, Markku Ahotupa, Petri Määttä, Heikki Kallio. 2011. Composition and antioxidative activities of supercritical CO2-extracted oils from seeds and soft parts of northern berries. *Food Research International* **44**:7, 2009-2017. [CrossRef]
9. Emmanouil H. Papaioannou, Nikolaos G. Stoforos, Maria Liakopoulou-Kyriakides. 2011. Substrate contribution on free radical scavenging capacity of carotenoid extracts produced from Blakeslea trispora cultures. *World Journal of Microbiology and Biotechnology* **27**:4, 851-858. [CrossRef]
10. Alvin Ibarra , Julien Cases , Antoine Bily , Kan He , Naisheng Bai , Marc Roller , Aurélie Coussaert , Christophe Ripoll . 2010. Importance of Extract Standardization and In Vitro/Ex Vivo Assay Selection for the Evaluation of Antioxidant Activity of Botanicals: A Case Study on Three Rosmarinus officinalis L. Extracts. *Journal of Medicinal Food* **13**:5, 1167-1175. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
11. Mohsen Meydani, Eunhee Kong, Ashley KnightLDL Oxidation as a Biomarker of Antioxidant Status 51-64. [CrossRef]
12. Paola Palozza, Nadia Parrone, Rossella E. Simone, Assunta Catalano. 2010. Lycopene in atherosclerosis prevention: An integrated scheme of the potential mechanisms of action from cell culture studies. *Archives of Biochemistry and Biophysics* . [CrossRef]
13. Mohammad Ali Ghaffari, Mehrnoosh Shanaki. 2010. In vitro inhibition of low density lipoprotein carbamylation by vitamins, as an ameliorating atherosclerotic risk in uremic patients. *Scandinavian Journal of Clinical & Laboratory Investigation* **70**:2, 122-127. [CrossRef]
14. Kanithaporn Puangsombat, J. Scott Smith. 2010. Inhibition of Heterocyclic Amine Formation in Beef Patties by Ethanolic Extracts of Rosemary. *Journal of Food Science* **75**:2, T40-T47. [CrossRef]
15. Assunta Zanfini, Gianfranco Corbini, Caterina La Rosa, Elena Dreassi. 2010. Antioxidant activity of tomato lipophilic extracts and interactions between carotenoids and #-tocopherol in synthetic mixtures. *LWT - Food Science and Technology* **43**:1, 67-72. [CrossRef]

16. Franziska Müller-Waldeck, Judith Sitzmann, Wilfried H. Schnitzler, Johanna Graßmann. 2010. Determination of toxic perilla ketone, secondary plant metabolites and antioxidative capacity in five Perilla frutescens L. varieties. *Food and Chemical Toxicology* **48**:1, 264-270. [CrossRef]
17. J. CHEN, J. SHI, L. MACNAUGHTON, Y. KAKUDA, S.J. XUE, Y. MA, M. ZHANG, Y. JIANG. 2009. THE SCAVENGING CAPACITY OF COMBINATIONS OF LYCOPENE, #-CAROTENE, VITAMIN E, AND VITAMIN C ON THE FREE RADICAL 2,2-DIPHENYL-1-PICRYLHYDRAZYL (DPPH). *Journal of Food Biochemistry* **33**:2, 232-245. [CrossRef]
18. Dilip Ghosh, Arjan Scheepens. 2009. Vascular action of polyphenols. *Molecular Nutrition & Food Research* **53**:3, 322-331. [CrossRef]
19. Ravit Hait-Darshan, Shlomo Grossman, Margalit Bergman, Mordehai Deutsch, Naomi Zurgil. 2009. Synergistic activity between a spinach-derived natural antioxidant (NAO) and commercial antioxidants in a variety of oxidation systems. *Food Research International* **42**:2, 246-253. [CrossRef]
20. Hoda Salama Ibrahim , Lamiaa Ali Ahmed , Maha Mohamed Essam El-din . 2008. The Functional Role of Some Tomato Products on Lipid Profile and Liver Function in Adult Rats. *Journal of Medicinal Food* **11**:3, 551-559. [Abstract] [Full Text PDF] [Full Text PDF with Links]
21. D LIU, J SHI, A COLINAIBARRA, Y KAKUDA, S JUNXUE. 2008. The scavenging capacity and synergistic effects of lycopene, vitamin E, vitamin C, and #-carotene mixtures on the DPPH free radical. *LWT - Food Science and Technology* **41**:7, 1344-1349. [CrossRef]
22. Giuseppina Tommonaro, Barbara Nicolaus, Rocco Prisco, Alfonso Giulio, Giuseppe Strazzullo, Annarita PoliAntioxidant Compound Studies in Different Tomato Cultivars 333-343. [CrossRef]
23. Pantelis Aronis , Smaragdi Antonopoulou , Haralabos C. Karantonis , Costas Phenekos , Demokritos C. Tsoukatos . 2007. Effect of Fast-Food Mediterranean-Type Diet on Human Plasma Oxidation. *Journal of Medicinal Food* **10**:3, 511-520. [Abstract] [Full Text PDF] [Full Text PDF with Links]
24. Jens Milde, Erich F. Elstner, Johanna Graßmann. 2007. Synergistic effects of phenolics and carotenoids on human low-density lipoprotein oxidation. *Molecular Nutrition & Food Research* **51**:8, 956-961. [CrossRef]
25. S QUEK, N CHOK, P SWEDLUND. 2007. The physicochemical properties of spray-dried watermelon powders. *Chemical Engineering and Processing* **46**:5, 386-392. [CrossRef]
26. A Basu, V Imrhan. 2007. Tomatoes versus lycopene in oxidative stress and carcinogenesis: conclusions from clinical trials. *European Journal of Clinical Nutrition* **61**:3, 295-303. [CrossRef]
27. W PESCHEL, F SANCHEZRABANEDA, W DIEKMANN, A PLESCHER, I GARTZIA, D JIMENEZ, R LAMUELARAVENTOS, S BUXADERAS, C CODINA. 2006. An industrial approach in the search of natural antioxidants from vegetable and fruit wastes. *Food Chemistry* **97**:1, 137-150. [CrossRef]
28. James Duke, Mary Jo Bogenschutz-Godwin, Kevin SpelmanThe Synergy Principle at Work with Plants, Pathogens, Insects, Herbivores, and Humans 475-501. [CrossRef]
29. B. Velmurugan , S. Nagini . 2005. Combination Chemoprevention of Experimental Gastric Carcinogenesis by S-Allylcysteine and Lycopene: Modulatory Effects on Glutathione Redox Cycle Antioxidants. *Journal of Medicinal Food* **8**:4, 494-501. [Abstract] [Full Text PDF] [Full Text PDF with Links]
30. Norman I. Krinsky, Elizabeth J. Johnson. 2005. Carotenoid actions and their relation to health and disease. *Molecular Aspects of Medicine* **26**:6, 459-516. [CrossRef]
31. S.-L. Yeh, M.-L. Hu, C.-S. Huang. 2005. Lycopene enhances UVA–induced DNA damage and expression of heme oxygenase–1 in cultured mouse embryo fibroblasts. *European Journal of Nutrition* **44**:6, 365-370. [CrossRef]
32. J YANEZ, N DAVIES. 2005. Stereospecific high-performance liquid chromatographic analysis of naringenin in urine. *Journal of Pharmaceutical and Biomedical Analysis* **39**:1-2, 164-169. [CrossRef]

33. David KittsAntioxidant Phytochemicals and Potential Synergistic Activities at Reducing Risk of Cardiovascular Disease 27-62. [CrossRef]
34. B Velmurugan, A Mani, S Nagini. 2005. Combination of S-allylcysteine and lycopene induces apoptosis by modulating Bcl-2, Bax, Bim and caspases during experimental gastric carcinogenesis. *European Journal of Cancer Prevention* **14**:4, 387-393. [CrossRef]
35. Lauren Petr, John ErdmanLycopene and Risk of Cardiovascular Disease . [CrossRef]
36. R. Kumaraguruparan, K.V.P. Chandra Mohan, S.K. Abraham, S. Nagini. 2005. Attenuation of N-methyl-N#-nitro-N-nitrosoguanidine induced genotoxicity and oxidative stress by tomato and garlic combination. *Life Sciences* **76**:19, 2247-2255. [CrossRef]
37. John Shi, Qin Qu, Yukio Kakuda, David Yeung, Yueming Jiang. 2005. Stability and Synergistic Effect of Antioxidative Properties of Lycopene and Other Active Components. *Critical Reviews in Food Science and Nutrition* **44**:7, 559-573. [CrossRef]
38. Kevin Spelman. 2005. Philosophy in Phytopharmacology. *Journal of Herbal Pharmacotherapy* **5**:2, 31-47. [CrossRef]
39. J. GraßmannTerpenoids as Plant Antioxidants **72**, 505-535. [CrossRef]
40. Nelson Wood, Roger B. Johnson. 2004. The relationship between tomato intake and congestive heart failure risk in periodontitis subjects. *Journal of Clinical Periodontology* **31**:7, 574-580. [CrossRef]
41. S Baba. 2004. Orally administered rosmarinic acid is present as the conjugated and/or methylated forms in plasma, and is degraded and metabolized to conjugated forms of caffeic acid, ferulic acid and m-coumaric acid. *Life Sciences* **75**:2, 165-178. [CrossRef]
42. M.L Balestrieri, R De Prisco, B Nicolaus, P Pari, V.Schiano Moriello, G Strazzullo, E.L Iorio, L Servillo, C Balestrieri. 2004. Lycopene in association with #-tocopherol or tomato lipophilic extracts enhances acyl-platelet-activating factor biosynthesis in endothelial cells during oxidative stress. *Free Radical Biology and Medicine* **36**:8, 1058-1067. [CrossRef]
43. Maike Wolters, Andreas Hahn. 2004. LDL Susceptibility to Copper-Induced Oxidation after Administration of a Single Dose of Free or Esterified Beta-Cryptoxanthin. *Annals of Nutrition and Metabolism* **48**:3, 163-168. [CrossRef]
44. John Shi, Yukio Kakuda, David Yeung. 2004. Antioxidative properties of lycopene and other carotenoids from tomatoes: Synergistic effects. *BioFactors* **21**:1-4, 203-210. [CrossRef]
45. Hirohito Tsuboi, Kayoko Shimoi, Naohide Kinae, Itaro Oguni, Reiko Hori, Fumio Kobayashi. 2004. Depressive symptoms are independently correlated with lipid peroxidation in a female population. *Journal of Psychosomatic Research* **56**:1, 53-58. [CrossRef]
46. Zsuzsanna Fazekas, Dayuan Gao, Rao Saladi, Yuhun Lu, Mark Lebwohl, Huachen Wei. 2003. Protective Effects of Lycopene Against Ultraviolet B-Induced Photodamage. *Nutrition and Cancer* **47**:2, 181-187. [CrossRef]
47. Armida S#nchez-Escalante, Gast#n Torrescano, Djamel Djenane, Jos# Antonio Beltr#n, Pedro Roncal#s. 2003. Stabilisation of colour and odour of beef patties by using lycopene-rich tomato and peppers as a source of antioxidants. *Journal of the Science of Food and Agriculture* **83**:3, 187-194. [CrossRef]
48. Mira Rosenblat, Raymond Coleman, Michael Aviram. 2002. Increased macrophage glutathione content reduces cell-mediated oxidation of LDL and atherosclerosis in apolipoprotein E-deficient mice. *Atherosclerosis* **163**:1, 17-28. [CrossRef]
49. Michiyo Matsuno, Akito Nagatsu, Yukio Ogihara, Brian E Ellis, Hajime Mizukami. 2002. CYP98A6 from Lithospermum erythrorhizon encodes 4-coumaroyl-4#-hydroxyphenyllactic acid 3-hydroxylase involved in rosmarinic acid biosynthesis. *FEBS Letters* **514**:2-3, 219-224. [CrossRef]
50. Biana Fuhrman, Michael AviramPolyphenols and Flavonoids Protect LDL Against Atherogenic Modifications . [CrossRef]

51. Bianca Fuhrman, Michael Aviram. 2001. Flavonoids protect LDL from oxidation and attenuate atherosclerosis. *Current Opinion in Lipidology* **12**:1, 41-48. [CrossRef]

52. Michiyo MATSUNO, Akito NAGATSU, Yukio OGIHARA, Hajime MIZUKAMI. 2001. Synthesis of 2-O-(4-Coumaroyl)-3-(4-hydroxyphenyl)lactic Acid, an Important Intermediate of Rosmarinic Acid Biosynthesis. *CHEMICAL & PHARMACEUTICAL BULLETIN* **49**:12, 1644-1646. [CrossRef]