

## Effects of Lycopene on the Susceptibility of Low-Density Lipoproteins to Oxidative Modification

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### Abstract

The intake of antioxidants intake has been reported to be inversely associated with the incidence of coronary artery disease. To clarify the possible role of lipophilic antioxidants in prevention of atherosclerosis, we investigated the effects of lycopene on the susceptibility of low-density lipoprotein (LDL) to oxidative modification.

In this study, "lycopene" was added to plasma and incubated for 3 h at 37°C. Then, the LDL fraction was separated by ultracentrifugation. The oxidizability of LDL was estimated by measuring conjugated dienes (CD), lipid peroxides and thiobarbituric acid-reactive substances (TBARS) after addition of cupric sulfate solution.

We showed that lycopene significantly ( $P < 0.01$  by ANOVA) and dose-dependently prolonged the lag time before the initiation of oxidation reaction. Also, it suppressed the formation of lipid peroxides and TBARS more markedly than the control.

LDL exposed to lycopene in vitro reduced oxidizability. These findings raise the possibility that lycopene has a role in ameliorating atherosclerosis.

**Keywords:** Atherosclerosis; Low-density lipoprotein; Lycopene; Oxidation.

### Introduction

Oxidation of low-density lipoproteins (LDL) has been suggested as a causal factor in human atherosclerosis (1). Oxidatively modified LDL is a potent ligand for scavenger receptors on macrophages and thus contributes to the generation of macrophage-derived foam cells, the hall-mark of early atherosclerotic fatty streak lesions (2). Many additional mechanisms by which oxidized LDL may contribute to atherosclerosis have been identified (3). The oxidative modification hypothesis of atherosclerosis is supported by numerous in vivo findings, e. g., the presence of epitopes

of oxidatively modified LDL in atherosclerotic lesions and elevated titers of circulating auto-antibodies against oxidized LDL in patients with carotid atherosclerosis (4). Antioxidant compounds provide resistance to this process and have been suggested to lower atherogenicity (5). Considerable epidemiologic (6, 7), biochemical (8), and clinical (9) evidence has accumulated in support of this hypothesis.

LDL contains different lipophilic antioxidants, the most abundant being  $\alpha$ -tocopherol that is the main form of vitamin E. All the other antioxidants ( $\gamma$ -tocopherol, carotenoids, ubiquinol-10 and lycopene) are found in much lower quantities in LDL. Lycopene is thought to be the major nonenzymatic antioxidant present in the lipid structures of cells and lipoproteins. It is a donor antioxidant (reductant), which increases the LDL

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resistance against the oxidative modification. In lipid solutions and dispersions, it inhibits radical formation linearly with time until consumed in the process (10).

Recently, it has been suggested that lycopene can protect human LDL against lipid peroxidation more efficiently than other carotenoids, even though it is present in LDL in much lower concentrations (11).

In this study, we investigated the effects of pre-incubation of plasma with lycopene on the susceptibility of LDL to oxidative modification.

## Experimental

### Materials

Lycopene and other reagents were purchased from Sigma Chemical Company (Deisenhofen, Germany) or Merck (Darmstadt, Germany).

### Methods

#### *In vitro addition of lycopene to plasma*

Blood from normolipidemic overnight fasting volunteers (n=25, age 30±5 year, men, non smokers, non diabetics, not taking any drug since at least 2 weeks) was collected into syringes containing ethylenediaminetetraacetic acid EDTA (1 g EDTA/L blood). Plasma was separated by low-speed centrifugation at 1000 g at 4 °C for 15 min. The cholesterol, triglyceride, LDL-cholesterol and HDL-cholesterol were determined using the Pars Azmon kit. To enrich LDL with lycopene, the compound was dissolved in 10% dimethyl sulfoxide (DMSO) in phosphate buffered saline (PBS, pH=7.4) and added to plasma at a ratio of 20 ml 10% DMSO/L of plasma to have final concentrations of 0, 50, 100 and 200 µmol lycopene/L of plasma. The solution was then incubated at 37 °C for 3h.

#### *Isolation of LDL*

LDL (density 1.019-1.063 g/ml) was isolated by a rapid isolation technique as reported by Chung et al (12), and dialyzed for 12h at 4°C with four 1-L changes in EDTA-free PBS that had been degassed by using a vacuum procedure. Also, the cholesterol, triglyceride, LDL-cholesterol and HDL-cholesterol of LDL

fraction were determined using the Pars Azmon kit. Control LDL was prepared by the same technique after adding only vehicle (20 min 10% DMSO/L plasma) to the plasma and incubating for 3 h at 37°C. In a preliminary study, we confirmed that DMSO at this concentration did not affect the oxidizability of LDL. The LDL suspension was then stored at 4°C under argon in the dark.

#### *Measurement of oxidizability of LDL*

The oxidizability of LDL was estimated by measuring three indices including: conjugated dienes (CD), lipid peroxides, and thiobarbituric acid-reactive substances (TBARS). Freshly prepared LDL (50 mg protein/L) was incubated with cupric sulphate solution (final concentration 10 µmol/L) in PBS buffer at 37°C in a Hitachi spectrophotometer (Hitachi Sangyo Co, Tokyo) fitted with a peltier heater. Absorbance at 234 nm was automatically recorded at 10 min intervals. Lag time before the initiation of oxidation and the propagation rate were determined according to the methods of Esterbauer et al (13) with some modification. After incubation for 90 or 180 min with 10 µmol CuSO<sub>4</sub>/L, the oxidation reaction was stopped by adding EDTA (100 µmol/L final concentration).

The content of lipid peroxides in LDL was measured colorimetrically with a commercially available kit. For measuring the content of lipid peroxides in LDL, we used an assay based on the oxidative activity of LOOH, which convert iodide to iodine. Iodine is further converted to triiodide ion, which absorbs light at 365 nm. We used the microtiter plate procedure propose by an available kit (CHOD-iodide, Merck, Darmstadt, Germany). The LDL oxidation was carried out in polystyrene microtiter the plates. 190 µL of room-tempered CHOD iodide reagent was added to plates and incubated for 60 min at 37°C. The absorbance at 365 nm was measured in a microplate reader (Labsystems, Muluskan MCC/340, Uppsala, Sweden). The concentration of LOOH was calculated from the molar absorption coefficient of 246,000 mol/cm for the triiodide ion and a path length in of 1 cm and a final volume of 330 µL.

The concentration of TBARS was also measured by using the method described by

**Table 1.** Effect of lycopene (0-200  $\mu\text{mol/L}$ ) on susceptibility of plasma low-density lipoprotein to copper-induced oxidation\*.

	Lag time ( min )			
	0 $\mu\text{mol/L}$ (control)	50 $\mu\text{mol/L}$	100 $\mu\text{mol/L}$	200 $\mu\text{mol/L}$
Lycopene	58.1 $\pm$ 3.0	59.1 $\pm$ 6.6	90.0 $\pm$ 8.8 <sup>a</sup>	131.9 $\pm$ 14.0 <sup>b</sup>

\* Mean  $\pm$  SD of three separate experiments.  $P < 0.01$  (ANOVA).

<sup>a,b</sup> Significantly different from control ( Fisher's test): <sup>a</sup>  $P < 0.05$  & <sup>b</sup>  $P < 0.001$ .

Buege and Aust (14). The concentration of malondialdehyde (MDA) was calculated by using the extinction coefficient of 165000 mol/cm for MDA.

#### Statistical analysis

All results were presented as mean $\pm$ standard deviation (n=5). Data were compared by analysis of variance (ANOVA). Fisher's test was used whenever a statistically significant difference between the two groups was shown by ANOVA.

### Results and Discussion

Addition of lycopene to plasma for 3 h at 37°C resulted in lipoprotein antioxidant enrichment. Of course, efficiency of the enrichment was dependant on compound concentration of the compound in the medium. Incubation of copper ions with the LDL suspension caused extensive oxidation of the lipoprotein as judged by an accumulation of CD, lipid peroxides and TBARS in the LDL sample. Enriching LDL with lycopene made it more resistant to copper-induced oxidation in comparison with the natural LDL. This effect was demonstrated with all the indices of oxidation used, and appeared to be most pronounced within the first hours of oxidation. Table 1 shows the effects of lycopene on

the susceptibility of LDL to copper-induced oxidation. Lycopene significantly increased the lag time before the onset of CD formation ( $P < 0.01$  by ANOVA). Prolonging of the lag time by this component was dose-dependent.

Suppression of TBARS and lipid peroxides formation were also observed in the LDL samples to which lycopene had been added. Table 2 and 3 show the results. The effects were proportional to the dose of the compound.

In this study we showed that the copper-catalyzed oxidation of LDL (isolated from plasma and preincubated with lycopene) was significantly inhibited in a dose-dependent manner as assessed by the lag time before initiation of oxidation and formation of TBARS and lipid peroxides. To add this compound to LDL in vitro, we incubated EDTA-containing plasma at 37°C for 3h with lycopene at the concentrations of 50, 100 and 200  $\mu\text{mol/L}$ . LDL was then separated and dialyzed against PBS before the induction of oxidation, therefore, lycopene not associated with LDL particles was removed by dialysis. Thus, the lycopene that was on the surface of or within LDL particles was considered to be responsible for inhibiting LDL oxidation. The concentrations of lycopene chosen for this study were obtained from our previous dose-response studies and published data, and were equivalent to values in the upper end of the reference range (15, 16).

**Table 2.** Effect of lycopene (0-200  $\mu\text{mol/L}$ ) on susceptibility of low-density lipoprotein to Cu-induced oxidation as measured by lipid peroxides\*.

	Lipid peroxides (nmol/mg protein)			
	0 $\mu\text{mol/L}$ (control)	50 $\mu\text{mol/L}$	100 $\mu\text{mol/L}$	200 $\mu\text{mol/L}$
Lycopene	214.0 $\pm$ 2.1	191.5 $\pm$ 4.8 <sup>a</sup>	129.4 $\pm$ 14.5 <sup>b</sup>	59.8 $\pm$ 10.2 <sup>b</sup>

\* Mean  $\pm$  SD of three separate experiments.  $P < 0.01$  (ANOVA).

<sup>a,b</sup> Significantly different from control ( Fisher's test): <sup>a</sup>  $P < 0.05$  & <sup>b</sup>  $P < 0.001$ .

**Table 3.** Effect of lycopene (0-200  $\mu\text{mol/L}$ ) on susceptibility of plasma low-density lipoprotein to Cu-induced oxidation as measured by TBARS\*.

	TBARS (nmol MDA/mg protein)			
	0 $\mu\text{mol/L}$ (control)	50 $\mu\text{mol/L}$	100 $\mu\text{mol/L}$	200 $\mu\text{mol/L}$
Lycopene	15.60 $\pm$ 1.60	9.51 $\pm$ 0.88 <sup>a</sup>	8.39 $\pm$ 0.47 <sup>a</sup>	4.89 $\pm$ 1.68 <sup>b</sup>

\* Mean  $\pm$  SD; n=4.

TBARS, thiobarbituric acid-reactive substances; MDA, malondialdehyde.

TBARS differ significantly among groups, P&lt;0.01 (ANOVA).

<sup>a,b</sup> Significantly different from control: <sup>a</sup> P<0.05 & <sup>b</sup> P<0.0001.

Lycopene is known to be a highly efficient lipid-soluble antioxidant in the protection of lipids in a variety of biological and model systems including LDL and liposomes. It is generally assumed that it may exhibit its protective effects by preventing the formation of lipid free radicals and/or eliminating them. On the other hand, the possibility that the inhibition of LDL modification such as oxidation or glycation by lycopene is related to its interactions on the lipid structure of the LDL particles needs further investigation (17). Lycopene contains shielding methyl (-CH<sub>3</sub>) groups and is optimally positioned in LDL particles by its phytyl side-chain. In addition, it protects LDL against peroxidative modification and maintains its ability to act as a ligand for LDL receptors (18). The inhibition of photosensitized oxidants of lipids and lipoproteins by lycopene mediated by singlet oxygen has been demonstrated (19).

Incubation of lycopene with plasma over a long period of time may enrich the LDL particles sufficiently to make them less susceptible to oxidative reactions.

In conclusion, we clearly demonstrated that the incubation of plasma with lycopene protects LDL from copper-induced oxidation reactions. This compound significantly decreases the susceptibility of LDL to oxidative modification; therefore, it may have favorable effects in ameliorating atherosclerosis.

### References

- (1) Stocker R and Keaney JF. Role of oxidative modifications in atherosclerosis. *Physiol. Rev.* (2004) 84: 1381-1388
- (2) Heinecke JW. Oxidative stress: new approaches to diagnosis and prognosis in atherosclerosis. *Am. J. Cardiol.* (2003) 91: 12A-16A
- (3) Galle J. Atherosclerosis and arthritis: implications for therapy of cardiovascular disease. *Herz.* (2004) 29: 4-11
- (4) Palade F, Alexa ID and Azoicai D. Oxidative stress in atherosclerosis. *Rev. Med. Chir. Soc. Med. Nat.* (2003) 107: 502-511
- (5) Niki E. Antioxidants and atherosclerosis. *Biochem. Soc. Trans.* (2004) 32: 156-159
- (6) Violi F, Loffredo L and Marcocchia A. Antioxidants in peripheral arterial disease. *Curr. Drug Targets Cardiovasc. Haematol. Disord.* (2004) 4: 289-294
- (7) Vivekananthan DP, Penn MS and Sapp SK. Use of antioxidant vitamins for the prevention of cardiovascular disease: meta-analysis of randomised trials. *Lancet* (2003) 361: 2017-2023
- (8) Todd J, Anderson L, Meredith I, Alan C, Yeung MD and Frei B. The effect of cholesterol-lowering and antioxidant therapy on endothelium-dependent coronary vasomotion. *New Engl. J. Med.* (1995) 332: 488-492
- (9) Norata GD, Pirillo A and Catapano AL. Statins and oxidative stress during atherogenesis. *J. Cardiovasc. Risk* (2003) 10: 181-189
- (10) Rissanen T, Voutilainen S, Nyssoonen K, Salonen R and Salonen JT. Low plasma lycopene concentration is associated with increased intima-media thickness of the carotid artery wall. *Arterioscler. Thromb. Vasc. Biol.* (2000) 20: 2677-2681
- (11) Esterbauer H, Ramos P. Chemistry and pathophysiology of oxidation of low density lipoprotein. *Rev. Physiol. Biochem. Pharmacol.* (1995) 127: 31-64
- (12) Chung BH, Segrest JP and Ray MJ. Single vertical spin gradient ultracentrifugation. *Meth. Enzymol.* (1986) 128: 181-209
- (13) Esterbauer H, Striegl H and Puhl H. Continuous monitoring of *in vitro* oxidation of human low density lipoprotein. *Free Rad. Res. Commun.* (1989) 6: 67-75
- (14) Buege JA and Aust SD. Microsomal lipid peroxidation. *Meth. Enzymol.* (1978) 52: 302-310
- (15) Safari MR. Effects of vitamin E and volatile oils on the susceptibility of LDL to oxidative modification. *Iranian Biomed. J.* (2003) 7: 79-84
- (16) Safari MR and Sheikh N. The effect of flavonoids on the susceptibility of LDL to oxidative modification. *Prostaglan. Leukotrienes Essential Fatty Acids* (2003) 69: 73-77

- (17) Mayne ST, Cartmel B, Silva F, Kim CS and Fallon BG. Plasma lycopene concentrations in humans are determined by lycopene intake, plasma cholesterol concentration and selected demographic factors. *J. Nutr.* (1999) 129: 849-854
- (18) Arab L and Steck S. Lycopene and cardiovascular disease. *Am. J. Clin. Nutr.* (2000) 71: 1691-1695
- (19) Sies H and Stahl W. Lycopene, antioxidant and biological effects and its bioavailability in human. *Proc. Soc. Exp. Biol. Med.* (1998) 218: 121-124

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