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Enhanced oxidizability of ubiquinol and α-tocopherol during lovastatin treatment

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Abstract A double-blinded, placebo-controlled cross-over trial was carried out with 27 hypercholesterolemic men with coronary heart disease. During the 6-week treatment period lovastatin (60 mg/day) decreased fasting serum LDL cholesterol by 45%, LDL phosphorus by 38% and apoB by 33%. Ubiquinol content diminished by 13% as measured per LDL phosphorus. When LDL was oxidized ex vivo with AMVN both LDL ubiquinol and α-tocopherol were exhausted faster after lovastatin treatment compared to placebo, by 24% ($P < 0.005$) and 36% ($P < 0.0001$), respectively. Lag time in copper-induced oxidation of LDL decreased by 7% ($P < 0.01$). This suggests diminished antioxidant-dependent resistance of LDL to the early phase of oxidative stress.

Key words: Antioxidant; LDL cholesterol; Lovastatin; Oxidation; Ubiquinol; Vitamin E

1. Introduction

The 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase) inhibitors or statins are widely used drugs in hypercholesterolemia. They affect the early key enzyme of the mevalonate pathway. Doing so, the statins do not only inhibit the synthesis of cholesterol but also other non-sterol end products, such as coenzyme Q_{10} or ubiquinone (CoQ) [1]. In most human tissues it appears mainly in its reduced form, ubiquinol (CoQOH or ubiquinol-10) which acts as a powerful antioxidant even in low concentrations [2].

The plasma LDL cholesterol to ubiquinone ratio has been a more remarkable risk factor of coronary heart disease (CHD) than the more familiar total cholesterol to HDL cholesterol ratio [3]. After the initiation of a peroxidative insult against LDL the production of lipid hydroperoxides does not begin until most of ubiquinol has been oxidized [4]. It protects α-tocopherol from oxidation, possibly through efficient reduction of the tocopheroxyl radical [5,6]. Some lipid peroxidation occurs already during the measured lag phase when conjugated diene formation is not yet fully propagated. In a method using parinaric acid as a probe, its oxidation occurs immediately upon consumption of ubiquinol and α-tocopherol [7]. Hence, the depletion of these lipid-soluble antioxidants makes the initiation of oxidative modification of LDL possible [4,6,7].

There are no publications on the rate of antioxidant consumption during radical attack to LDL during statin treatment. We studied if lovastatin treatment diminishes LDL ubiquinol or α-tocopherol contents, or affects the consumption of those antioxidants in oxidative stress indicating a change in the LDL antioxidative capacity.

2. Materials and methods

2.1. Study design

A randomized, double-blinded cross-over study, where the effects of lovastatin treatment were compared to those of placebo, was carried out in the cardiology outpatient clinic at Kanta-Häme Central Hospital, Hämeenlinna, Finland. The study was carried out according to the recommendations of the Declaration of Helsinki and monitored according to the Good Clinical Trial Practice [8]. It was approved by the Ethical Committee of Kanta-Häme Central Hospital and the National Agency for Medicines. Every patient gave a written informed consent before entering the study.

On the pre-study visit the inclusion criteria were checked and a wash-out of at least 6 weeks begun where any treatment affecting lipid metabolism was prohibited. The HMG CoA reductase inhibitor used was Lovacol (20 mg lovastatin; Orion, Finland under license from MSD). The patients took daily one lovastatin 20 mg or an identical placebo tablet during the 1st week, two tablets/day on the 2nd week and three tablets daily on weeks 3–6. No concomitant lipid lowering agents or vitamins were allowed. All other medications were kept constant during the whole study. Compliance was checked by tablet counting. Assessment of the diet was made on each visit. Safety of the therapy was evaluated by frequent clinical visits, ECG and analyzing serum enzymes indicating the function of the muscles, liver and kidneys.

2.2. Subjects

The patients with verified CHD and primary hyperlipidemia were included in the study. The definitions of CHD were either an angiographically determined occlusion/obliteration in coronary vessels (19 patients) or a previous heart infarction (9 patients). The patients had not had a myocardial infarction within 6 months before the study. The inclusion criteria for fasting serum lipids were as follows: LDL-cholesterol > 4.0 mmol/l or total/HDL-cholesterol ratio > 5.5. All the patients were on cholesterol-lowering diet. Exclusion criteria were concomitant steroid therapy, diabetes, alcoholism or misuse of narcotics, overt hypertriglyceridemia (> 5 mmol/l), liver, renal or endocrine disease, malignant tumor or chronic terminal disease.

28 men with the age of 56 ± 8 years (mean ± S.D., range 41–69) were selected in the study. They were well-informed and co-operative. 12 patients had undergone previous coronary artery bypass grafting and 3 had had coronary angioplasty. 16 patients had apolipoprotein E (apoE) phenotype E3/3, 10 had E3/4 and 2 had E4/4. Three of the men were current smokers. 25 of the patients were on β-blocking agent therapy and 26 on acetosalisylic acid. Two patients used also an ACE-inhibitor, 9 a long-acting nitrate and 8 had a calcium channel blocking agent. Additional patient characteristics are compiled in Table 1.

2.3. Blood samples

Venous blood samples were drawn between 08:00 and 09:00 h on the study visits, before and after the 6-week treatment periods. The patients were advised not to eat, take any medication, drink coffee or other beverages, or smoke 12 h before the venipuncture. Alcohol was

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[1]Deceased during the study.

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prohibited for 36 h before sampling. Blood samples were drawn in sitting position after a rest of at least 15 min. Plasma was separated by centrifugation immediately after cooling (5 min) the sample in ice in the dark. After separation, the samples were frozen and stored at −80°C until analyzed.

2.4. Oxidation of LDL with AMVN

LDL was precipitated from 2 ml EDTA-plasma with 75 μl heparin (5000 IU/ml, Noparin, Novo Nordisk) and 7.5 ml trisodiumcitrate (64 mmol/l pH 5.0) in acid-washed Kimax glass tubes [9]. The supernatant was very carefully removed after centrifugation under nitrogen atmosphere and the precipitated LDL fraction was dissolved in 2 ml of chloroform : methanol (1 : 1). After shaking, 2*100 μl samples were taken for the measurement of phosphorus, lipid-soluble α-tocopherol and ubiquinol, and 1 ml was used in the LDL oxidation experiment.

Peroxyl radicals were produced at a constant rate by the thermal decomposition of 2,2-azobis(2,4-dimethylvaleronitrile) (AMVN, Polysciences, Warrington, PA) [10] dissolved in benzene. The test tube was placed in a temperature-controlled incubator (37°C), and the reaction was initiated by mixing 94 μl AMVN solution (final concentration 2.1 mmol/l) with 1 ml chloroform : methanol (1 : 1) containing LDL. 100 μl samples were taken every 3 min during half an hour for the determination of α-tocopherol and ubiquinol. Oxidation was stopped by deep-freezing the samples in liquid nitrogen where they were stored until HPLC-analyses.

2.5. Determination of reduced ubiquinol and α-tocopherol

Ubiquinol and α-tocopherol were determined with an HPLC method modified from a previously published procedure [11]. The equipment consisted of a pump (LKB 2150, Bromma, Sweden), a reversed-phase column (Super Pac Spherisorb ODS 2.5 μm, Pharmacia, Uppsala, Sweden) and a redox-sensitive electrochemical detector (Antec, Leiden, The Netherlands). The mobile phase was methanol : ethanol : isopropanol in the ratio 65 : 33 : 2 containing lithium perchlorate 20 mmol/l, and its flow rate was 1 ml/min at 22°C. The internal standard for ubiquinol was coenzyme Q_9 with nine isoprenoid units (Sigma, St. Louis, MO) and that for α-tocopherol was tocol (Roche, Basel, Switzerland). The retention times of tocol, α-tocopherol, Q_9 and ubiquinol were 3.3, 4.3, 9.3 and 12.6 min, respectively. All plasma samples of each individual patient from all periods were analyzed in the same run with known calibration samples prepared using d-α-tocopherol (Kodak, Rochester, NY) and ubidecarone (coenzyme Q_{10}, Pharma Nord, Denmark). The lowest calibration concentration used was 1.67 μmol/l and the highest 33.4 μmol/l for α-tocopherol, and 76 nmol/l and 1530 nmol/l for ubiquinol. The detection limit was defined as 1 μmol/l for α-tocopherol and 50 nmol/l for ubiquinol. The mean value of the signal/noise-ratio at the specified detection limits was ≈4. Because the recovery is variable due to manual extraction of LDL, the results are standardized with LDL phosphorus (inorganic) and expressed as mmol of α-tocopherol or ubiquinol per mol of LDL phosphorus.

2.6. Oxidation of LDL with $CuSO_4$

LDL was isolated with a single-step ultracentrifugation (Beckman TL-100, Palo Alto, CA) using TLV-100 rotor. 0.57 ml plasma density adjusted to 1.21 g/ml by adding 0.4664 KBr mg per 1 ml plasma was added carefully on 1.43 ml of NaCl solution with density of 1.006 g/ml. After ultracentrifugation at 10°C for 30 min at 338 000 G sample of LDL was taken with a needle punctured horizontally through the vial wall. LDL was first desalted and made free of EDTA by gel filtration with an Econo-Pac 10 DG column (Bio-Rad Laboratories, CA) using phosphate buffer (PBS pH 7.4) as eluent. A 50 μl sample was taken for protein determination [12]. 1-ml sample of LDL solution standardized to 0.05 mg protein/ml with PBS was oxidized at 37°C as described previously [13]. The final concentration of $CuSO_4$ in the mixture was 1.67 μmol/l. UV-absorbance at 234 nm was monitored every 1 min during 300 min using Perkin-Elmer Lambda Bio 10 (Überlingen, Germany) spectrometer. Lag time to the start of the propagation phase of diene formation was defined as the crossing of the tangents of the initial phase (first 5 min) and the maximal propagation. The maximal concentration of conjugated dienes is presented as a proportion to protein content in LDL fraction. All the samples of an individual patient were analyzed in one run.

2.7. Determination of lipids, apolipoproteins and apoE phenotype

Total cholesterol, HDL-cholesterol and triglycerides were analyzed immediately after the separation of serum samples. LDL-cholesterol was calculated using Friedewald's formula. Apolipoprotein A-I and B (apoB) were measured by a nephelometric method using highly specific antisera (rabbit, OUED 14/15 for apoA-I, OSAN 14/15 for apoB, Behring; Behring Laser Nephelometer, Behringswerke, Marburg, Germany). LDL phosphorus (Pi) concentration was determined using an inorganic ammonium molybdate reaction with colorimetric detection at 799 nm [14]. 4-Hydroxy-*m*-phenylenediammonium chloride was used as the reducing agent [15]. The lowest calibration concentration was 161 μmol/l. ApoE phenotypes were determined from delipidated fasting plasma after isoelectric focusing and cysteamine treatment using an immunoblotting technique [16].

2.8. Numerical analyses

Rates of individual consumption of ubiquinol and α-tocopherol were calculated using linear regression analysis. In case of non-normal distribution (D'Agostino's test: $P<0.05$), a logarithmic transformation was carried out for the comparison with a parametric test. The effects of the lovastatin and placebo interventions were compared using the two-way analysis of variance with equal replications, and with the factors treatment and treatment order. Results of two-sided tests with P values of <0.05 are regarded as statistically significant. Mean ± S.E.M values are presented if not otherwise cited.

3. Results

The clinical phase of the study was carried out between August and December. No severe adverse effect was reported or observed during the whole trial. Patient compliance was good as measured by the counts of consumed tablets and protocol adherence. One patient was excluded after randomization due to the concomitant administration of a possibly antioxidative agent. No clinically significant changes were observed in the weight, diet, living habits, serum enzymes, hematological parameters or clinical status of the patients (Table 1). All the presented variables were distributed normally, except lag time in Cu-induced oxidation, where logarithmic transformation was carried out before comparison.

Serum fasting lipid values are presented in Table 2. Com-

Table 1
Demographic and metabolic parameters in fasting serum sample before the study and after 6-week treatment periods

Parameter	Before treatments and after wash-out	After placebo	After 60 mg lovastatin	Between treatment P
Body mass index kg/m^2	27.1 ± 0.5	27.1 ± 0.6	27.2 ± 0.7	N.S.
Systolic sitting blood pressure mm Hg	147 ± 4	148 ± 3	146 ± 3	N.S.
Diastolic sitting blood pressure mm Hg	88 ± 2	86 ± 1	86 ± 2	N.S.
Heart rate beat/min	60 ± 2	60 ± 2	60 ± 2	N.S.
Ethanol consumption g/week	142 ± 28	119 ± 29	120 ± 28	N.S.
S-creatinine μmol/l	93.9 ± 1.8	96.0 ± 1.8	96.4 ± 1.7	N.S.
S-urate μmol/l	359 ± 13	381 ± 15	363 ± 13	N.S.
S-alanine aminotransferase U/l	27.0 ± 2.4	31.5 ± 3.2	38.8 ± 4.7	0.033
S-creatine kinase U/l	109 ± 10	106 ± 10	109 ± 11	N.S.

Mean ± S.E.M. ($n=27$). ANOVA with equal replication and with the factors treatment and treatment order. N.S.: $P>0.05$.

Table 2
Fasting serum lipid levels before the study and after 6-week treatment periods

Parameter	Before treatments and after wash-out	After placebo	After 60 mg lovastatin	Between treatment P
fS-cholesterol mmol/l	7.08 ± 0.23	6.73 ± 0.25	4.56 ± 0.17	< 0.0001
fS-LDL-cholesterol mmol/l	4.93 ± 0.20	4.79 ± 0.21	2.65 ± 0.11	< 0.0001
fS-HDL-cholesterol mmol/l	0.91 ± 0.04	0.93 ± 0.04	1.05 ± 0.05	0.002
LDL/HDL ratio	5.81 ± 0.47	5.70 ± 0.66	2.76 ± 0.22	< 0.0001
fS-triglycerides mmol/l	2.73 ± 0.31	2.27 ± 0.21	1.89 ± 0.18	0.006
fS-apolipoprotein AI g/l	1.21 ± 0.04	1.24 ± 0.04	1.32 ± 0.05	0.003
fS-apolipoprotein B g/l	1.37 ± 0.05	1.32 ± 0.06	0.89 ± 0.04	< 0.0001
LDL-Chol/Apo B mmol/g	3.66 ± 0.13	3.67 ± 0.11	3.02 ± 0.10	< 0.0001
LDL-phophorus mmol/LDL/l plasma	0.97 ± 0.05	0.99 ± 0.05	0.62 ± 0.04	< 0.0001

Mean ± S.E.M. ($n = 27$). ANOVA with equal replication and with the factors treatment and treatment order. N.S.: $P > 0.05$.

pared to the placebo period, lovastatin treatment decreased total cholesterol by 32%, LDL cholesterol by 45%, triglycerides by 17% as well as the LDL/HDL cholesterol ratio by 52% and increased HDL cholesterol by 13%. All these changes were statistically significant (Table 2). The apolipoprotein A-I concentration was enhanced slightly (7%). Apolipoprotein B decreased less than LDL cholesterol during lovastatin treatment. It diminished on average by 33%, and LDL cholesterol/Apo B ratio reduced by 18%. The mean plasma phospholipid content in LDL decreased during lovastatin treatment by 38% compared to that during placebo, as can be seen in Table 2.

LDL ubiquinol in plasma decreased by 47% during lovastatin treatment ($P < 0.0001$, Table 3). The reduction in LDL α-tocopherol concentration in plasma, 42%, was almost the same as decrease in LDL cholesterol. When adjusted to LDL phosphorus, there was no significant difference in α-tocopherol content in LDL after the lovastatin period compared to that after placebo. However, there was a slight but significant decrease of 13% in LDL ubiquinol content after lovastatin treatment as measured per phosphorus in LDL (Table 3).

Consumption of LDL antioxidants during ex vivo peroxyl radical attack was well-fitted in a linear function. The mean correlation coefficient was 0.96 in ubiquinol fits and 0.99 in α-tocopherol fits. An individual example is presented in Fig. 1. Fig. 2 shows the mean curves of ubiquinol and α-tocopherol consumption after both lovastatin and placebo treatments. The slope measured from α-tocopherol consumption curve was statistically significantly ($P < 0.001$) steeper on lovastatin treatment than that during placebo (Table 3). The difference between the treatments in the consumption rate of ubiquinol did not reach statistical significance (Table 3). When calculating the depletion time using the rate of consumption, both ubiquinol and α-tocopherol were exhausted significantly faster after lovastatin treatment compared to that after placebo treatment, by 24 and 36%, respectively (Table 3).

Lag time in Cu-induced oxidation of LDL decreased statistically significantly during statin therapy, by 7% ($P = 0.0077$). Protein-corrected maximal absorbance at 247 nm decreased by 8% ($P = 0.0001$). The slope of the absorbance–time curve in Cu-induced oxidation describing the maximal oxidation rate, did not change during the different treatment periods (Table 4).

4. Discussion

Recent studies have shown that the treatment of hypercholesterolemic CHD patients with HMG-CoA reductase inhibitors does not only result in improved angiographic measurements and fewer cardiovascular events [17] but also decreases both CHD and overall mortality [18]. The positive prognostic effects have been confirmed both in primary [19] and secondary prevention of CHD [18,20]. Lovastatin decreases LDL cholesterol and increases HDL cholesterol in serum [21], but the other qualitative effects are scarcely explored. Statins reduce the plasma level of ubiquinone, which can be preserved by exogenous ubiquinone administration [22]. However, the effects of statins on LDL cholesterol to ubiquinol ratio have been contradictory [23–25]. We found that LDL ubiquinol was diminished during lovastatin treatment compared to that on placebo. A question has arisen, if the lowered CoQ_{10} concentration after statin treatment can provide sufficient antioxidative potential [26].

Table 3
Kinetic parameters of LDL-antioxidants before the study and after 6-week treatment periods

Parameter	Before treatments and after wash-out	After placebo	After 60 mg lovastatin	Between treatment P
LDL ubiquinol				
Observed value before oxidation nmol/l plasma	693 ± 89	662 ± 77	354 ± 46	< 0.0001
Observed value before oxidation mmol/mol P_i	0.696 ± 0.063	0.641 ± 0.052	0.558 ± 0.052	0.037
Estimated level at start of oxidation mmol/mol P_i	0.648 ± 0.052	0.601 ± 0.047	0.505 ± 0.047	0.015
Rate of consumption μmol/mol P_i/min	58.1 ± 5.3	52.3 ± 4.9	59.2 ± 5.8	N.S.
Calculated total depletion time min	11.7 ± 0.62	12.1 ± 0.52	9.1 ± 0.69	0.0022
LDL tocopherol				
Observed value before oxidation μmol/l plasma	13.6 ± 1.1	13.2 ± 1.1	7.7 ± 0.7	< 0.0001
Observed value before oxidation mmol/mol P_i	13.8 ± 0.8	13.0 ± 0.7	12.5 ± 1.0	N.S.
Estimated level at start of oxidation mmol/mol P_i	13.8 ± 0.8	12.8 ± 0.7	12.1 ± 0.9	N.S.
Rate of consumption mmol/mol P_i/min	1.48 ± 0.16	1.39 ± 0.13	2.12 ± 0.20	0.0009
Calculated total depletion time min	10.9 ± 0.9	10.9 ± 0.8	6.8 ± 0.6	< 0.0001

Mean ± S.E.M. ($n = 27$). ANOVA with equal replication and with the factors treatment and treatment order. N.S.: $P > 0.05$.

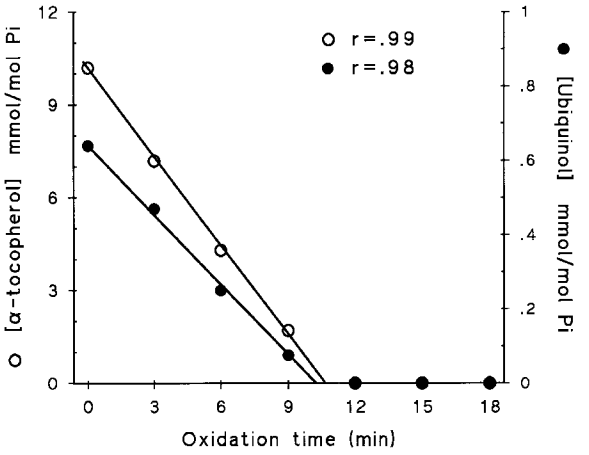

Fig. 1. An example of consumption of α-tocopherol and ubiquinol in LDL during oxidation with AMVN. Concentrations are corrected with LDL-phophorus. Consumption rate (linear regression coefficients): −0.95 mmol/mol P_i/min ($r=0.99$) for α-tocopherol and −64 μmol/mol P_i/min ($r=0.98$) for ubiquinol.

In the present study a significant shortening in the depletion time of LDL ubiquinol and α-tocopherol during radical attack was observed during lovastatin therapy. This was associated with diminished resistance of LDL to oxidative stress produced by $CuSO_4$. Radical exposure rate produced by the thermal decomposition of an azo-compound was constant for each sample regardless of its LDL and antioxidant concentrations. Hence, the shortened depletion times of the ubiquinol and α-tocopherol in AMVN-induced oxidation can be expected. However, in Cu-induced oxidation LDL amount in oxidation cuvette was constant. The change in lag time describes decreased antioxidative defence during efficient lovastatin therapy. Both quantitative, and possibly qualitative change in LDL lipid composition may explain decrease in the maximal protein-normalized concentration of conjugated dienes.

Considering the smaller decrease of apoB than that of LDL antioxidants, the antioxidative defence in an individual LDL particle is weaker during effective lovastatin therapy. This may implicate loss of water-phase antioxidants. We did not measure the levels of ascorbic acid, protein-SH groups [27] or bilirubin [28,29]. Nor were the plasma hydroperoxides measured, which have been reported to be elevated in hypercholesterolemic patients [30]. Serum urate did not change significantly during lovastatin treatment (Table 1).

A recent study by the group of Esterbauer has shown that apoB as a lipid core protein participates in the first line LDL oxidation processes [31]. In the angiographic MARS study, the lovastatin treatment reduced LDL more than apoB, by 45 and 26%, respectively [32]. ApoB was reduced less than

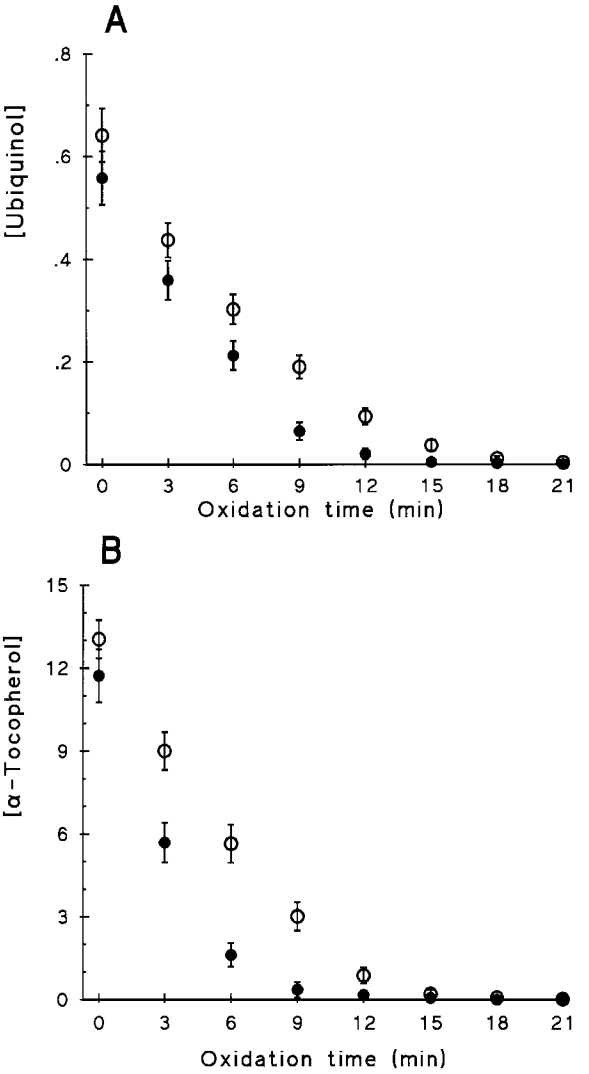

Fig. 2. Consumption of ubiquinol (A) and α-tocopherol (B) in LDL during oxidation with AMVN. Concentrations are corrected with LDL-phophorus: mmol/mol P_i. Mean ± S.E.M. ($n=27$). Symbols: open, placebo; closed, lovastatin.

LDL cholesterol on lovastatin (20 mg once daily) treatment in both high-fat and low-fat diet periods, 21 vs. 27% and 20 vs. 27%, respectively [33]. Our results agree with these previous studies.

When AMVN was used as a radical generator, LDL α-tocopherol was depleted faster than LDL ubiquinol, which is in contrast to earlier studies [5,27,34]. The reason for this phenomenon could be the high AMVN concentration we used, and thus faster generation of radicals. With copper-induced oxidation an increase in pro-oxidant concentration has

Table 4
Conjugated diene formation measured as absorbance at 247 nm during Cu-induced oxidation of LDL before the study and after 6-week treatment periods

Parmater	Before treatments and after wash-out	After placebo	After 60 mg lovastatin	Between treatment P
Lag time min	64.4 ± 1.6	63.5 ± 2.2	59.5 ± 1.5	0.0077
Maximal oxidation rate μmol/l/min	.484 ± .021	.468 ± .017	.455 ± .019	N.S.
Maximal protein-normalized conjugated dienes μmol/mg	516.4 ± 15.3	526.3 ± 13.5	482.6 ± 12.7	0.0001

Mean ± S.E.M. ($n=27$). ANOVA with equal replication and with the factors treatment and treatment order. N.S.: $P>0.05$.

caused a significant decrease in the lag time of conjugated diene formation [35]. We made all antioxidant measurements after the study. Therefore, it is possible that in spite of careful precautions, minimal oxidation had happened before the analysis. This possibility does not affect our results, because of the placebo-controlled, double-masked and cross-over design of the study. No period or carry-over effect was observed in the study. Even if LDL had already been minimally oxidized before analysis, the results indicate that lovastatin has made LDL, in vivo or ex vivo, more vulnerable.

One explanation to our findings could be the relatively short treatment period of 6 weeks used in this study. It may happen that during the first weeks of statin treatment a shift in the age distribution of the LDL particle pool to older particles occurs because of the depletion of younger ones by the inhibited cholesterol synthesis. Older LDL particles per se are less resistant to oxidation than younger ones [36]. In another study, an early increase in autoantibodies to oxidized LDL was associated with a decrease in LDL during statin treatment [37]. However, a later decrease of IgG autoantibodies by 12 months of treatment was also observed [37]. Another reason for a seeming discrepancy with the previous studies [23,38] might be the dose of the statin. The daily dose of 60 mg lovastatin was chosen to ensure the therapeutic efficacy on LDL cholesterol in the hypercholesterolemic CHD men.

In conclusion, lovastatin shortened significantly the depletion time of the LDL antioxidants ubiquinol and α-tocopherol during AMVN-induced LDL peroxidation. Parallelly, lag time in Cu-induced oxidation of LDL was also reduced. It needs to be clarified whether ubiquinone and/or vitamin E substitution could preserve the antioxidative capacity of LDL in the early phase of oxidative stress during statin therapy.

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References

[1] Goldstein, J.L. and Brown, M.S. (1990) Nature (London) 343, 425–430.
[2] Åberg, F., Appelkvist, E.-L., Dallner, G. and Ernster, L. (1992) Arch. Biochem. Biophys. 295, 230–234.
[3] Hanaki, Y., Sugiyama, S., Ozawa, T. and Ohno, M. (1993) Clin. Invest. 71, S112–S115.
[4] Mohr, D., Bowry, V.W. and Stocker, R. (1992) Biochim. Biophys. Acta 1126, 247–254.
[5] Maquire, J.J., Kagan, V., Ackrell, B.A.C., Serbinova, E. and Packer, L. (1992) Arch. Biochem. Biophys. 292, 47–53.
[6] Kontush, A., Hübner, C., Finckh, B., Kohlschütter, A. and Beisiegel, U. (1995) Biochim. Biophys. Acta 1258, 177–187.
[7] Tribble, D.L., Van den Berg, J.J.M., Motchnik, P.A., Ames, B.N., Lewis, D.M., Chait, A. and Krauss, R.M. (1994) Proc. Natl. Acad. Sci. USA 91, 1183–1187.
[8] EC Note for Guidance (1990) Good Clinical Practice for Trials on Medicinal Products in the European Community. III/3976/88-EN.
[9] Wieland, H. and Seidel, D. (1983) J. Lipid Res. 24, 904–909.
[10] Niki, E., Yamamoto, Y., Komuro, E. and Sato, K. (1991) Am. J. Clin. Nutr. 53, 201S–205S.
[11] Lang, J.K., Gohil, K. and Packer, L. (1986) Anal. Biochem. 157, 106–116.
[12] Peterson, G.L. (1983) Methods Enzymol. 91, 95–119.
[13] Esterbauer, H., Striegl, G., Puhl, H. and Rotheneder, M. (1989) Free Radic. Res. Commun. 6, 67–75.
[14] Fiske, C.H. and SubbaRow, Y. (1925) J. Biol. Chem. 66, 375–400.
[15] Itaya, K. and Ui, M. (1966) Clin. Chim. Acta 14, 361–366.
[16] Lehtimäki, T., Moilanen, T., Viikari, J., Åkerblom, H., Ehnholm, C., Rönnemaa, T., Marniemi, J., Dahlen, G. and Nikkari, T. (1990) J. Lipid Res. 31, 487–495.
[17] Superko, H.R. and Krauss, R.M. (1994) Circulation 90, 1056–1069.
[18] Scandinavian Simvastatin Survival Study Group (1994) Lancet 344, 1383–1389.
[19] Shepherd, J., Cobbe, S.M., Ford, I., Isles, C.G., Lorimer, A.R., Macfarlane, P.W., McKillop, J.H., Packard, C.J. and West of Scotland Coronary Prevention Study Group (1995) N. Engl. J. Med. 333, 1301–1307.
[20] Sacks, F.M., Pfeffer, M.A., Moye, L.A. and Rouleau, J.L. et al. (1996) N. Engl. J. Med. 335, 1001–1009.
[21] Tikkanen, M.J., Helve, E., Jäättelä, A., Kaarsalo, E., Lehtonen, A., Malbecq, W., Oksa, H., Pääkkönen, P., Salmi, J., Veharanta, T., Viikari, J., Äärynen, M. and Finnish Lovastatin Study Group (1988) Am. J. Cardiol. 62, 35–43.
[22] Bargossi, A.M., Battino, M., Gaddi, A., Fiorella, P.L., Grossi, G., Barozzi, G., Di Giulio, R., Descovich, G., Sassi, S., Genova, M.L. and Lenaz, G. (1994) Int. J. Clin. Lab. Res. 24, 171–176.
[23] Chirlanda, G., Oradei, A., Manto, A., Lippa, S., Uccioli, L., Caputo, S., Greco, A.V. and Littarru, G.P. (1993) J. Clin. Pharmacol. 33, 226–229.
[24] Watts, G.F., Castelluccio, C., Rice-Evans, C., Taub, N.A., Baum, H. and Quinn, P.J. (1993) J. Clin. Pathol. 46, 1055–1057.
[25] Laaksonen, R., Ojala, J.-P., Tikkanen, M.J. and Himberg, J.J. (1994) Eur. J. Clin. Pharmacol. 46, 313–317.
[26] Appelkvist, E.L., Edlund, C., Löw, P., Schedin, S., Kaln, A. and Dallner, G. (1993) Clin. Invest. 71, S97–S102.
[27] Halliwell, B., Hu, M.-L., Louie, S., Duvall, T.R., Tarkington, B.K., Motchnik, P. and Cross, C.E. (1992) FEBS Lett. 313, 62–66.
[28] Neuzil, J. and Stocker, R. (1994) J. Biol. Chem. 269, 16712–16719.
[29] Wu, T.-W., Fung, K.-P. and Yang, C.-C. (1994) Pharmacol. Lett. 54, 477–481.
[30] Chirico, S., Smith, C., Marchant, C., Mitchinson, M.J. and Halliwell, B. (1993) Free Radic. Res. Commun. 19, 51–57.
[31] Giessauf, A., Steiner, E. and Esterbauer, H. (1995) Biochim. Biophys. Acta 1256, 221–232.
[32] Blankenhorn, D.H., Azen, S.P., Kramsch, D.M., Mack, W.J., Cashin-Hemphill, L., Nodis, H.N., DeBoer, L.W.V., Mahrer, P.R., Masteller, M.J., Vailas, L.I., Alaupovic, P., Hirsch, L.J. and MARS Resarch Group (1993) Ann. Intern. Med. 119, 969–976.
[33] Hunninghake, D.B., Stein, E.A., Dujovne, C.A., Harris, W.S., Feldman, E.B., Miller, V.T., Tobert, J.A., Laskarzewski, P.M., Quiter, E., Held, J., Taylor, A.M., Hopper, S., Leonard, S.B. and Brewer, B.K. (1993) N. Engl. J. Med. 328, 1213–1219.
[34] Stocker, R., Bowry, V.W. and Frei, B. (1991) Proc. Natl. Acad. Sci. USA 88, 1646–1650.
[35] Gieseg, S.P. and Esterbauer, H. (1994) FEBS Lett. 343, 188–194.
[36] Walzem, R.L., Watkins, S., Frankel, E.N., Hansen, R.J. and German, J.B. (1995) Proc. Natl. Acad. Sci. USA 92, 7460–7464.
[37] Bui, M.N., Caulfield, M.T., Katz III, P.C., Richard, O. and Rackley, C.E. (1996) J. Am. Coll. Cardiol. 27, 413A.
[38] Laaksonen, R., Jokelainen, K., Laakso, J., Sahi, T., Härkönen, M., Tikkanen, M.J. and Himberg, J.J. (1996) Am. J. Cardiol. 77, 851–854.