

# Ubiquinone supplementation during lovastatin treatment: effect on LDL oxidation ex vivo<sup>1</sup>

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**Abstract** A randomized, double-masked, placebo-controlled cross-over trial was carried out to evaluate whether ubiquinone supplementation (180 mg daily) corrects impaired defence against initiation of oxidation of low density lipoprotein (LDL) related to effective (60 mg daily) lovastatin treatment. Nineteen men with coronary heart disease and hypercholesterolemia received lovastatin with or without ubiquinone during 6-week periods after wash-out. The depletion times for LDL ubiquinol and reduced  $\alpha$ -tocopherol were determined during oxidation induced by 2,2-azobis(2,4-dimethylvaleronitrile) (AMVN). Copper-mediated oxidation of LDL isolated by rapid density-gradient ultracentrifugation was used to measure the lag time to the propagation phase of conjugated diene formation. Compared to mere lovastatin therapy, ubiquinone supplementation lead to a 4.4-fold concentration of LDL ubiquinol ( $P < 0.0001$ ). In spite of the 49% lengthening in depletion time ( $P < 0.0001$ ) of LDL ubiquinol, the lag time in copper-mediated oxidation increased only by 5% ( $P = 0.02$ ). Ubiquinone loading had no statistically significant effect on LDL  $\alpha$ -tocopherol redox kinetics during high radical flux ex vivo. The faster depletion of LDL ubiquinol and shortened lag time in conjugated diene formation during high-dose lovastatin therapy may, at least partially, be restored with ubiquinone supplementation. However, the observed improvement in LDL antioxidative capacity was scarce, and the clinical relevance of ubiquinone supplementation during statin therapy remains open.—Palomäki, A., K. Malminiemi, T. Solakivi, and O. Malminiemi. Ubiquinone supplementation during lovastatin treatment: effect on LDL oxidation ex vivo. *J. Lipid Res.* 1998. 39: 1430–1437.

**Supplementary key words** AMVN •  $\alpha$ -tocopherol • clinical trial • coenzyme Q<sub>10</sub> • copper-induced oxidation • HMG-CoA reductase inhibitors • lipid oxidation • oxidation kinetics • statins • ubiquinol

The statins or 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase) inhibitors are the most effective drugs in lowering serum low density lipoprotein (LDL) concentration. They decrease cardiovascular morbidity and mortality of hypercholesterolemic patients, even in primary prevention (1). Large controlled prospective studies have shown that statins as a group improve the

prognosis of coronary heart disease (CHD) patients (2, 3). The HMG-CoA reductase inhibitors affect competitively the early key enzyme of the mevalonate pathway (Fig. 1), thus inhibiting the synthesis of cholesterol and other non-sterol end products (4, 5). One of them is coenzyme Q<sub>10</sub> (2,3-dimethoxy-5-methyl-6-decaprenyl benzoquinone), also known as ubiquinone (5–8). It may be estimated that on a normal diet, 60% of plasma ubiquinone is endogenous (9).

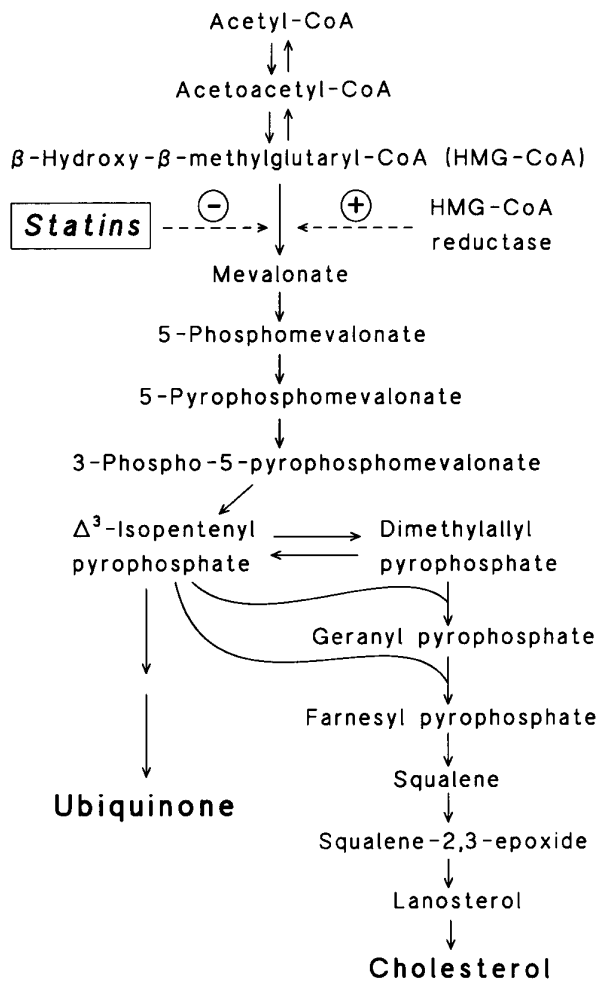
Except in the brain and lungs, coenzyme Q<sub>10</sub> (CoQ<sub>10</sub>) appears in human tissues mainly in its reduced form, ubiquinol (CoQ<sub>10</sub>OH), which acts as an antioxidant (10). LDL oxidizability by copper correlates negatively with its initial ubiquinol content, especially during the early stage of oxidation (11). In both animal and human studies, tissue or plasma levels of coenzyme Q<sub>10</sub> diminished during treatment with an HMG-CoA reductase inhibitor (5–8, 12, 13), while the combination of ubiquinone with statin preserved the pretreatment concentration of ubiquinol (12, 13). Effects of CoQ<sub>10</sub> treatment on the initial phase of oxidation without statin therapy have been studied thoroughly, suggesting that ubiquinol can act as an effective first line antioxidant when radical attack to LDL has occurred (14). Incorporation of very low amounts of ubiquinol into LDL in vitro makes LDL significantly more resistant to copper-mediated oxidation than native LDL (15). The significance of ubiquinol as a clinically important antioxidant remains uncertain, mainly because of its low content, i.e., there is no more than one ubiquinol molecule in 1 or 2 LDL particles (14, 16, 17).

We have observed that the LDL ubiquinol content ex

Abbreviations: AMVN, 2,2-azobis(2,4-dimethylvaleronitrile); CHD, coronary heart disease; CoQ<sub>10</sub>, coenzyme Q<sub>10</sub>; CoQ<sub>10</sub>OH, ubiquinol; HDL, high density lipoprotein; HMG-CoA reductase, 3-hydroxy-3-methylglutarylcoenzyme A reductase; LDL, low density lipoprotein; PBS, phosphate-buffered saline.

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**Fig. 1.** Endogenous synthesis of ubiquinone and cholesterol. Formation of mevalonate is the rate limiting step in synthesis.

pressed per phosphate is diminished during efficient lovastatin treatment (18). On lovastatin therapy, the depletion times of reduced LDL ubiquinol and  $\alpha$ -tocopherol were shortened significantly during peroxidative insult *ex vivo*. The finding was associated with a shortened lag time of conjugated diene formation, suggesting diminished resis-

tance of LDL particles to the early phase of oxidative stress (18).

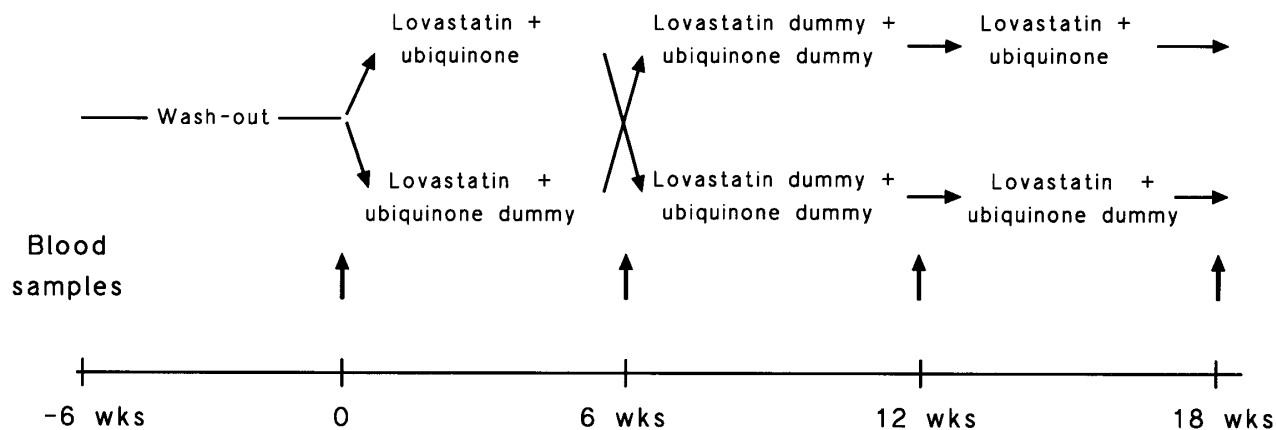
This is the first placebo-controlled, double-blinded clinical trial exploring the antioxidative effects of ubiquinone supplementation during lovastatin treatment. The questions, whether it prolongs the depletion time of LDL ubiquinol and  $\alpha$ -tocopherol during radical attack, or the lag time of copper-induced formation of conjugated dienes, were of special interest.

## MATERIALS AND METHODS

### Study design

In a randomized, double-blinded cross-over study the effects of ubiquinone supplementation were compared to those of placebo in patients receiving lovastatin (Fig. 2). The study was preceded by at least a 6-week wash-out period with no antioxidants or treatments affecting lipid metabolism. The two 6-week active treatment periods were separated by a 6-week placebo treatment period. Lovastatin was administered in both active treatment periods concomitantly with either ubiquinone or matched placebo capsules. The study was carried out in the cardiology outpatient clinic at Kanta-Häme Central Hospital, Hämeenlinna, Finland. It was approved by the Ethical Committee of the hospital and the National Agency for Medicines. Every patient gave written informed consent before entering the study. The recommendations of the Declaration of Helsinki were practiced and monitoring was carried out according to Good Clinical Trial Practice (19).

The inclusion criteria were checked on the prestudy visit. The following products were used in the trial: Kino Q10 (30 mg ubiquinone; Leiras, Finland) and Lovacol® (20 mg lovastatin; Orion, Finland under license from MSD). The patients took two ubiquinone or identical placebo capsules three times daily during the 1st and 3rd periods. One 20-mg tablet of lovastatin was taken daily during the first week, two tablets per day on the second week, and three tablets daily on the weeks 3 to 6 of the active treatment periods. Lovastatin placebos were taken after an identical protocol to the active treatments during the 2nd period. No concomitant lipid-lowering agents, antioxidants, or vitamins were allowed during the whole study, while all other medications were kept constant. Compliance was checked by tablet counting, and dietary habits were assessed on each visit. In addition to clinical examinations, safety of the therapy was evaluated by analyzing serum enzymes indicating the function of the muscles, liver, and kidneys.



**Fig. 2.** Schedule of the trial. Randomization of the treatment order, and start of the therapy occurred at the second visit (0).

## Subjects

Twenty well-informed men were included in the trial, but one subject was excluded in the early phase due to a protocol violation and was not included in the analyses. The rest of the study population, 19 co-operative outpatients ages  $55 \pm 7$  years (mean  $\pm$  SD) had coronary heart disease (CHD) and primary hyperlipidemia. The diagnosis of CHD was confirmed either by coronary angiography (13 patients) or by anamnesis of verified previous heart infarction (6 patients) at least 6 months before the study. The inclusion criteria for fasting serum lipids were LDL-cholesterol  $>4.0$  mm or total/HDL-cholesterol ratio  $>5.5$ . Exclusion criteria included concomitant steroid therapy, diabetes, alcoholism, or misuse of narcotics, overt hypertriglyceridemia ( $>5.0$  mm) and hepatic, renal, neoplastic, or endocrine disease. All the patients were on a cholesterol-lowering diet. Their body mass index was  $27.2 \pm 0.7$  kg/m<sup>2</sup> and sitting arterial blood pressure  $144 \pm 4$  mmHg/87  $\pm$  2 mmHg at start. Five of the men were current smokers. Fourteen were on beta-1-selective adrenoceptor antagonist therapy, and 3 used a non-selective beta blocker. Two patients used ACE-inhibitors and 8 used calcium channel blocking agents. Five of the patients had concomitant long-acting nitrate and 18 out of 19 took acetosalicylic acid.

## Blood samples

Venous blood samples were drawn between 8 and 9 am at the study visits (see Fig. 2) in a sitting position after a rest of at least 15 min. The patients were advised to fast and take no medication, coffee, or other beverages, and to refrain from smoking 12 h before the blood sampling. Alcohol was prohibited for 36 h before sampling. EDTA plasma was separated by centrifugation immediately after cooling (5 min) the sample in ice in the dark. The samples were frozen and stored at  $-80^{\circ}\text{C}$  until analyzed. 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 were measured by a nephelometric method using highly specific antisera (Behring, Marburg, Germany).

## Oxidation of LDL with CuSO<sub>4</sub>

To minimize the risk of oxidation during isolation, LDL was prepared using a rapid nonequilibrium density-gradient ultracentrifugation method (20). The density of plasma was adjusted to 1.21 g/ml by solid anhydrous KBr and 0.57 ml of the plasma was layered beneath 1.43 ml of saline containing 0.1% EDTA (d 1.006 g/ml) in a 2-ml Quick-Seal tube (Beckman, Palo Alto, CA). The tubes were centrifuged in a Beckman TL-100 bench-top ultracentrifuge using a Beckman TLV-100 vertical rotor at 100,000 rpm for 30 min at  $10^{\circ}\text{C}$ . The distinct LDL band was withdrawn through the side of the tube with a needle and a syringe. Immediately after isolation, 0.3 ml of the LDL fraction was applied to a gel filtration column (Econo-Pac 10 DG, Bio-Rad Laboratories, Hercules, CA) to remove the salt and EDTA. The column was washed with 2.5 ml of PBS (10 mM sodium phosphate buffer, pH 7.4, containing 0.15 M NaCl) and 2.8 ml of the eluate was discarded. Thereafter the LDL was collected in 0.7 ml of PBS. The protein concentration of the eluate was measured with a modification of the Lowry method (21) using bovine serum albumin as standard. Oxidation was determined as the production of hydroperoxides with conjugated double bonds (conjugated dienes) by continuously monitoring the change in absorbance at 234 nm as described by Esterbauer et al. (22). For the oxidation experiments, the LDL preparations were diluted with PBS to contain 0.05 g/l of protein ( $\sim 0.1$   $\mu\text{M}$  LDL). Oxidation was started by adding 10  $\mu\text{l}$  of freshly prepared 0.167 mM CuSO<sub>4</sub> to 1.0 ml of LDL solution in 1-cm quartz cuvettes. For these measurements we used a Perkin-Elmer Lambda Bio 10 spectrophotometer

equipped with a 9-position automatic cell changer fitted with a Peltier element to keep the temperature at  $37^{\circ}\text{C}$ . The absorbance was automatically recorded at 1-min intervals for 5 h. The spectrophotometer was connected to a computer for data collection and analysis. Several characteristic indices were obtained from the resulting absorbance versus time curves (23). Lag time (min) was determined from the intercept of lines drawn through the linear portions of the lag phase and propagation phase. The maximal rate of propagation (expressed as  $\mu\text{M}$  dienes formed per min) was obtained from the slope of the absorbance curve during the propagation phase using the molar absorptivity  $\epsilon_{234\text{nm}}$  of 29,500 l/mol per cm for conjugated dienes (22). Maximum concentration of dienes formed (expressed as  $\mu\text{mol/g}$  LDL protein) was calculated from the difference in absorbance at zero time and at diene peak. To minimize the effect of method variation on the results, all samples of an individual study subject were analyzed simultaneously. In addition, one control sample was always processed with the patient samples. The control sample was EDTA-plasma (containing 0.6% saccharose) drawn from one healthy subject and kept at  $-80^{\circ}\text{C}$  in small portions.

## Measurement of antioxidant consumption during oxidation of LDL with AMVN

LDL was precipitated from 2 ml EDTA-plasma with 75  $\mu\text{l}$  heparin (5,000 IU/ml, Noparin, Novo Nordisk) and 7.5 ml trisodium citrate (64 mM, pH 5.0) in acid-washed Kimax glass tubes (24). After careful removal of the supernatant, the LDL precipitate was dissolved in chloroform-methanol 1:1 to extract lipids. After shaking, aliquots were taken for the measurement of phosphate, lipid-soluble vitamin E, and ubiquinol, while the rest of the sample was used in the LDL oxidation experiment. Reduced  $\alpha$ -tocopherol and ubiquinol were determined with an HPLC method, using a redox-sensitive electrochemical detector (Antec, Leyden, Netherlands) (18). Because the recovery was slightly variable due to manual extraction of LDL, the results were standardized with LDL-phospholipids and expressed as millimoles of  $\alpha$ -tocopherol or CoQ<sub>10</sub>OH per mol of LDL-phosphate. Phosphate (Pi) was determined using a colorimetric method and inorganic ammonium molybdate reaction.

LDL in chloroform-methanol was oxidized by adding 2,2-azobis(2,4-dimethylvaleronitrile) (AMVN, Polysciences Inc., Warrington, PA) dissolved in benzene. The final concentration of AMVN was 2.1 mM. The reaction mixture was placed in a temperature-controlled incubator ( $37^{\circ}\text{C}$ ) and 100- $\mu\text{l}$  samples were taken every 3 min for the determination of  $\alpha$ -tocopherol and ubiquinol. Oxidation was stopped by deep-freezing the samples in liquid nitrogen where they were also stored until HPLC analyses. The rates of individual consumption of ubiquinol and reduced  $\alpha$ -tocopherol were calculated using linear regression analysis.

## Statistical analyses

The effects of the three different interventions were compared using the two-way analysis of variance with equal replications (RANOVA), and with the factors treatment and treatment order (25). When a statistical significance was observed in overall RANOVA, the comparison was continued using a parametric contrast analysis (*t*-test-based User contrast in BMDP SOLO) between the active phases (samples at 6 and 18 weeks). To evaluate a possible carry-over effect, RANOVA with active treatment phases only was performed (26). A carry-over effect was defined to be significant when the *P*-value for the treatment order was less than 0.1. Statistical analyses were carried out using the statistical program package BMDP SOLO v. 4.0 (27). A result with a two-sided *P*-value less than 0.05 is regarded as statistically significant in contrast analysis. Mean  $\pm$  standard error of the mean are presented if not otherwise cited.



## RESULTS

### Patient compliance and clinical variables

No severe adverse effects were reported or observed during the trial. Patient compliance was good as measured by protocol adherence and the counts of consumed tablets, which were 94.6% for lovastatin and 93.1% for ubiquinone (or identical placebo). This indicates good tolerance for the study medications. No clinically significant changes were observed in the weight, diet, living habits, serum enzymes (including creatine kinase), hematological parameters, or clinical status of the patients. The mean reported alcohol consumption was 139 g/week, and it did not change during the trial. There was no carry-over effect in any variable analyzed.

A small but statistically significant increase in alanine aminotransferase was observed earlier during lovastatin therapy (60 mg daily) (18). The increase was also seen in the present study, while the combination of ubiquinone and lovastatin resulted in the same ALAT level as that after the placebo period. The difference between the combination and lovastatin only was not statistically significant ( $P = 0.11$ ).

### Lipids and lipoproteins

Serum fasting lipid values are presented in **Table 1**. Compared to the placebo period, lovastatin treatment, with or without ubiquinone, decreased total cholesterol on average by 33%, LDL cholesterol by 46%, triglycerides by 20%, and the LDL/HDL cholesterol ratio by 55%. HDL cholesterol increased by 18%. Apolipoprotein B decreased on average by 34% and phospholipids in LDL-fraction by 39% during lovastatin therapy periods. All these changes were statistically significant. Ubiquinone did not affect the cholesterol-lowering effect of lovastatin.

### LDL antioxidant consumption

LDL ubiquinol content decreased on average by 17% during lovastatin therapy ( $P = 0.06$ ) (**Table 2**). Ubiquinone supplementation increased LDL ubiquinol content more than 4-fold compared to treatment with lovastatin alone. Starting at this higher concentration, the ubiquinol consumption curve was significantly steeper (+153%) during

AMVN exposure than that after lovastatin therapy without antioxidant supplementation (**Fig. 3**). The calculated total depletion time of LDL ubiquinol was shortened on average by 3 min (−25%) during the lovastatin treatment period, which was restored by ubiquinone supplementation. Despite the steeper consumption slope during AMVN-induced oxidation, the total depletion time of LDL ubiquinol was 49.6% longer after lovastatin treatment with ubiquinone supplementation than without it. After ubiquinone supplementation to lovastatin therapy, ubiquinol depletion time was comparable to that without lovastatin treatment (**Table 2**).

The changes in LDL ubiquinol level were also reflected in LDL tocopherol levels, but were not statistically significant. Tocopherol depletion was faster during lovastatin therapy (**Fig. 4**). The rate of consumption increased on average by 52% ( $P = 0.0015$ ) and total depletion time was shortened by 38% ( $P < 0.0001$ ). Some recovery, although statistically not significant, occurred in the depletion slope and in the total consumption time of LDL  $\alpha$ -tocopherol during ubiquinone supplementation (**Table 2**).

### Diene formation during oxidative stress

When LDL was oxidized with  $\text{CuSO}_4$ , the shortened lag time during lovastatin therapy was recovered after ubiquinone supplementation ( $P = 0.02$ ) to the level of double-dummy period (**Table 3** and **Fig. 5**). The maximal protein-normalized conjugated diene concentration decreased significantly ( $P = 0.005$ ), and on average by 6% during lovastatin treatment. Ubiquinone supplementation had no effect on the maximal rate of diene production or the maximal amount of dienes produced, expressed per gram of LDL protein.

Using multiple linear regression analysis the LDL ubiquinol level and the rate of ubiquinol consumption during AMVN-induced oxidation explained ( $r^2$ ) only 5% (NS) of the Cu-oxidation lag time after the wash-out period. However, after the lovastatin + placebo (LD) period these parameters explained about 35% ( $P = 0.034$ ), and after the lovastatin + ubiquinone (LU) therapy 49% ( $P = 0.004$ ) of the lag time. The changes in LDL ubiquinol concentration and consumption rate between LD and LU periods together explained 39% of the change in the lag time ( $P = 0.014$ ).

TABLE 1. Fasting serum lipid levels before the study and after 6-week treatment periods

Fasting Serum Lipid Parameter	Before Treatments and after Wash-out (WO)	After Placebos	Lovastatin + Placebo (LD)	Lovastatin + Ubiquinone (LU)	<i>P</i> between Treatments	
					LD vs. WO	LU vs. LD
Cholesterol, mm	7.29 ± 0.29	6.94 ± 0.31	4.59 ± 0.22	4.65 ± 0.22	<0.0001	NS
LDL-cholesterol, mm	5.12 ± 0.26	4.93 ± 0.27	2.63 ± 0.14	2.70 ± 0.15	<0.0001	NS
HDL-cholesterol, mm	0.91 ± 0.06	0.91 ± 0.05	1.07 ± 0.07	1.08 ± 0.07	0.0003	NS
LDL/HDL-ratio	6.19 ± 0.64	6.16 ± 0.91	2.76 ± 0.31	2.75 ± 0.26	<0.0001	NS
Triglycerides, mm	2.73 ± 0.34	2.41 ± 0.27	1.97 ± 0.23	1.91 ± 0.22	0.0004	NS
Apolipoprotein A-I, g/l	1.20 ± 0.05	1.24 ± 0.06	1.31 ± 0.06	1.32 ± 0.07	0.010	NS
Apolipoprotein B, g/l	1.43 ± 0.06	1.38 ± 0.07	0.91 ± 0.06	0.90 ± 0.05	<0.0001	NS
LDL-Chol/apoB, mmol/g	3.65 ± 0.18	3.62 ± 0.14	2.95 ± 0.11	3.07 ± 0.12	<0.0001	NS
Alanine amino transferase, U/l	26.8 ± 3.1	31.7 ± 4.0	39.2 ± 6.4	32.6 ± 3.0	0.037	NS
LDL-phosphorus <sup>a</sup> , mm	1.03 ± 0.06	1.02 ± 0.07	0.59 ± 0.05	0.64 ± 0.06	<0.0001	NS

Mean ± standard error of the mean ( $n = 19$ ) and the  $P$ -values from User contrast analyses (BMDP Solo) are presented. Contrast analysis was carried out after ANOVA with repeated measurements (RANOVA) and with the factors treatment and treatment order; NS:  $P > 0.05$ .

<sup>a</sup>EDTA plasma

TABLE 2. Kinetic parameters of LDL-antioxidants before the study and after 6-week treatment periods

Parameter	Before Treatments and after Wash-out (WO)	After Placebos	Lovastatin + Placebo (LD)	Lovastatin + Ubiquinone (LU)	<i>P</i> between Treatments	
					LD vs. WO	LU vs. LD
<b>LDL ubiquinol</b>						
Observed value before oxidation, mmol/mol Pi	0.734 ± 0.085	0.709 ± 0.065	0.585 ± 0.070	2.44 ± 0.266	0.031	<0.0001
Rate of consumption, mmol/mol Pi/min	62.9 ± 7.1	59.4 ± 6.1	64.5 ± 7.7	163.3 ± 13.4	NS	<0.0001
Calculated total depletion time, min	11.3 ± 0.78	11.8 ± 0.67	8.9 ± 0.82	13.3 ± 0.50	0.039	<0.0001
<b>LDL tocopherol</b>						
Observed value before oxidation, mmol/mol Pi	13.5 ± 0.8	13.4 ± 0.8	12.5 ± 1.1	12.9 ± 0.9	NS	NS
Rate of consumption, mmol/mol Pi/min	1.47 ± 0.20	1.46 ± 0.15	2.22 ± 0.24	1.92 ± 0.15	0.0015	NS
Calculated total depletion time, min	11.1 ± 1.1	10.5 ± 1.0	6.6 ± 0.8	7.2 ± 0.7	<0.0001	NS

Mean ± standard error of the mean (n = 19) and the *p*-values from User contrast analysis (after RANOVA) are presented; NS, *P* > 0.05.

Ubiquinone effect was associated with serum LDL-cholesterol level. In the low-LDL subgroup (n=10) with serum LDL-cholesterol less than 2.6 mm on statin therapy (LD period), the average lag time increased from 58.6 ± 2.7 min (LD period) to 63.9 ± 2.6 min (LU period) during ubiquinone supplementation (*P* = 0.009). In the high-LDL subgroup (n = 9, LD LDL > 2.6 mm), ubiquinone supplementation did not affect the lag time, which was 61.8 ± 1.9 min for the LD period and 61.7 ± 2.3 min for the LU period.

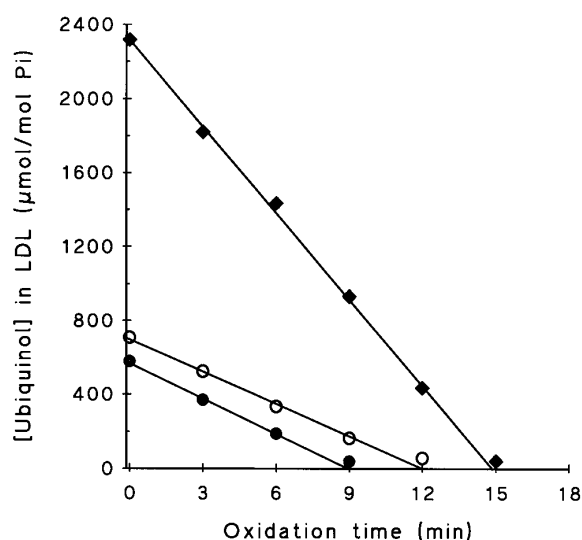
## DISCUSSION

Early oxidative changes in LDL seem to be related to antioxidant depletion, which occurs during the lag phase in oxidation of LDL *ex vivo* (14, 28). It has been questioned whether the lowered coenzyme Q<sub>10</sub> concentration

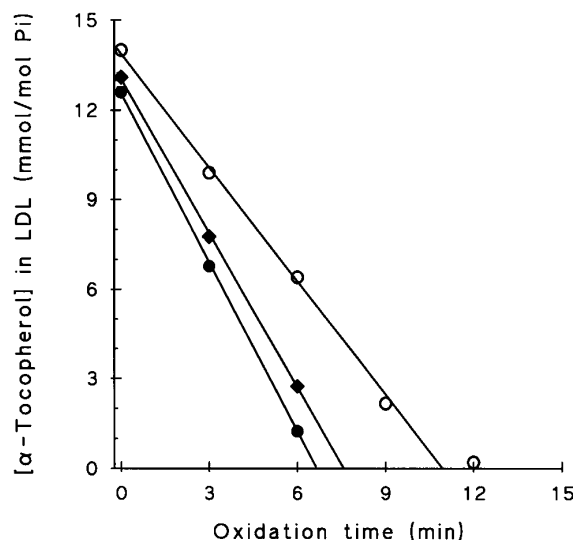
during statin treatment can provide sufficient antioxidant potential (5). According to the results of a randomized double-blind cross-over trial (18), effective lovastatin treatment was followed by the shortening of both ubiquinol and reduced α-tocopherol depletion times. This finding was partly due to relative reduction of these antioxidants in LDL. Lovastatin therapy was also related to a qualitatively unfavorable effect in the early phase of LDL oxidation, measured as the shortening of the lag time of diene formation (18). The present trial was designed to elucidate whether peroral ubiquinone supplementation has any effect on the impaired initial antioxidative defence in LDL related to effective statin treatment.

## Radical exposure

Of the two procedures used, the AMVN-method is fairly non-standardized, but the other, copper-induced oxidation, is a more commonly used, thoroughly characterized



**Fig. 3.** An example of the consumption of ubiquinol in LDL during oxidation with AMVN. LDL was precipitated from EDTA-plasma with heparin and trisodiumcitrate. Concentrations of LDL ubiquinol are corrected with LDL-phosphorus (Pi). Treatments: (○) wash-out; (●) lovastatin + placebo; (◆) lovastatin + ubiquinone. Consumption rates (and goodness of linear fit): after wash-out, 58 μmol/mol Pi per min (*r*<sup>2</sup> = 0.96); during lovastatin + placebo, 62 μmol/mol Pi per min (*r*<sup>2</sup> = 0.98), and during lovastatin + ubiquinone, 151 μmol/mol Pi per min (*r*<sup>2</sup> = 0.96).



**Fig. 4.** An example of the consumption of reduced α-tocopherol in LDL during oxidation with AMVN. LDL was precipitated from EDTA-plasma with heparin and trisodiumcitrate. Concentrations of LDL α-tocopherol are corrected with LDL-phosphorus (Pi). Treatments: (○) wash-out; (●) lovastatin + placebo; (◆) lovastatin + ubiquinone. Consumption rates (and goodness of linear fit): after wash-out, 1.4 mmol/mol Pi per min (*r*<sup>2</sup> = 0.95); during lovastatin + placebo, 2.0 mmol/mol Pi per min (*r*<sup>2</sup> = 0.99); and during lovastatin + ubiquinone, 1.8 mmol/mol Pi per min (*r*<sup>2</sup> = 0.97).

TABLE 3. Conjugated diene formation measured as change in absorbance at 234 nm during Cu-induced oxidation of LDL before the study and after 6-week treatment periods

Parameter	Before Treatments and after Wash-out (WO)	After Placebos	Lovastatin + Placebo (LD)	Lovastatin + Ubiquinone (LU)	<i>P</i> between Treatments	
					LD vs. WO	LU vs. LD
Lag time, min	64.8 ± 1.9	62.3 ± 2.0	60.1 ± 1.7	62.9 ± 1.7	0.0002	0.0214
Maximal oxidation rate, mm/min	0.495 ± 0.025	0.476 ± 0.018	0.457 ± 0.020	0.449 ± 0.020	0.0053	NS
Maximal protein-normalized conjugated dienes, mmol/g	520.6 ± 20.2	520.9 ± 15.5	489.0 ± 15.4	492.0 ± 16.5	0.0056	NS

Mean ± standard error of the mean (n = 19) and the *P*-values from User contrast analysis (after RANOVA) are presented; NS, *P* > 0.05.

kinetic method (22). Previous studies have shown that besides the intrinsic properties of LDL, the concentrations of LDL and copper and temperature during the incubation have profound effects on the indices of oxidation (29). We used 1.67 μM Cu<sup>2+</sup>, and a Cu<sup>2+</sup>/LDL molar ratio of 16 (30), which in our hands also produces a constant lag time. The copper concentration 1.67 μM also approximates the mean concentration of Cu<sup>2+</sup> used in copper-induced oxidation systems in literature (31). Although being about one-tenth of the reference value for the total copper concentration in serum, the concentration has been suggested as physiologically irrelevant considering the very initial stages of lipid peroxidation chain reaction (32).

#### Ubiquinone supplementation

In this study, CoQ<sub>10</sub> supplementation statistically significantly increased LDL ubiquinol content and prolonged the depletion time of LDL ubiquinol during oxidation *ex vivo*. It tended to maintain the prestudy lag time of diene formation, partially preventing the shortening caused by lovastatin. This is parallel to earlier studies, where ubiquinol

was shown to be an effective antioxidant in LDL (15, 28, 33–35). Although statistically significant, the prolongation of the lag time after ubiquinone supplementation was only 5% and not very impressive.

Apparently opposite results have been obtained in a cross-over study, where 4-week exogenous ubiquinone with a daily dose of 180 mg did not prolong the mean lag time despite a 3-fold increase in LDL ubiquinone concentration (36). However, the study subjects were quite different from those in our trial, consisting of 12 healthy young men. In another trial, effects of 2-month oral ubiquinone supplementation were studied in 60 smoking men divided in three parallel groups. In this single-blinded placebo-controlled trial the two different CoQ<sub>10</sub> preparations at 90 mg/day did not affect the susceptibility of the VLDL/LDL fraction to oxidation (37). In our study where the ubiquinone dose was 180 mg daily, the exclusion of the five smoking men did not affect the results statistically significantly.

#### Lag time in copper-induced oxidation

In the above-mentioned studies it was questioned whether ubiquinol can affect the lag phase in copper-mediated oxidation. According to the regression and subgroup analyses, the effect of ubiquinol in our trial seemed to be related to serum LDL-cholesterol level, and hence also to lovastatin therapy. However, although ubiquinone supplementation increased ubiquinol depletion time by 49%, the change in lag time was only 5%. The physiological significance of the lag time in copper-induced oxidation of LDL is not yet fully established.

The changes in the lag time reported to be caused by α-tocopherol therapy, although without concomitant statin treatment, are on a totally different scale from those observed here (38). However, the effect of α-tocopherol is contradictory. It seems not only to have an antioxidant but also a pro-oxidant effect, which may be prevented by coenzyme Q<sub>10</sub> (39).

Antioxidants such as probucol and α-tocopherol may prevent atherosclerosis by novel mechanisms independent of making LDL resistant to oxidation (40). They can inhibit the release of interleukin-1, increase the expression of cholesteryl ester transfer protein, modify intracellular oxidative metabolism, and prevent endothelial dysfunction (41, 42). The relevance of the lag time of conjugated diene formation to the biological effects of antioxidants is unclear (43).

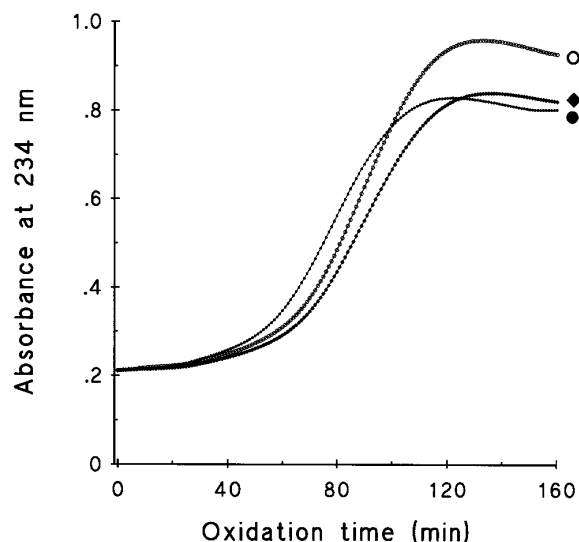


Fig. 5. A representative example of the formation of conjugated dienes during copper-induced oxidation of LDL. LDL was isolated using rapid density-gradient ultracentrifugation and desalted with a gel filtration column. The final concentration of CuSO<sub>4</sub> was 1.67 μM. Formation of conjugated dienes was followed by measuring absorbance change at 234 nm. Treatments: (○) wash-out, (●) lovastatin + placebo, (◆) lovastatin + ubiquinone.

## Antioxidant consumption

An interesting and unexpected finding was that higher LDL ubiquinol concentrations were associated with faster consumption rates of the antioxidant during AMVN-induced radical attack. The phenomenon was very consistent both within- and between-subjects, and every patient had a steeper ubiquinol consumption rate after oral CoQ<sub>10</sub> loading. However, the reason is highly speculative.  $\alpha$ -Tocopherol in LDL has a recycling mechanism that reduces the oxidized form back to reduced  $\alpha$ -tocopherol (44). Ubiquinol may also have such a system in LDL, as it has in mitochondria (45). If this regenerating system is down-regulated during ubiquinone supplementation, the net result is faster consumption of ubiquinol during oxidative stress. Another reason might be that ubiquinol is, whenever available, preferably consumed as the first line antioxidant. However, concentration-dependent consumption of an antioxidant would produce a non-linear depletion curve, whereas it was very linear in our study. It is possible that lovastatin itself acts as a pro-oxidant or changes the LDL ubiquinol/ubiquinone balance in favor of oxidized CoQ<sub>10</sub>. In a recent study (46) the ubiquinol/ubiquinone redox status in dense LDL appeared to be a marker for oxidative changes in LDL. Using only redox-sensitive electrodes in the HPLC system, we did not analyze the initial levels of oxidized ubiquinone in LDL.

It has been suggested that ubiquinol may protect  $\alpha$ -tocopherol from consumption by reducing the tocopheroxyl radical (15, 47). The present study does not give additional information to such a sparing effect, at least during AMVN-induced high radical flux, as the slight elevation in LDL  $\alpha$ -tocopherol content and the prolongation of the  $\alpha$ -tocopherol depletion time during ubiquinone supplementation were not statistically significant.

## Conclusion

There is no prognostic indication for routine ubiquinone supplementation for patients with statin treatment. Our present and earlier findings (18) may have clinical value, if the use of lipid-lowering drugs (48) increases in order to achieve the recommended (49) LDL cholesterol level of 2.6 mm in CHD patients. The lag time of conjugated diene formation reflects a series of chemical events occurring before accelerated peroxidation of LDL, whereas LDL ubiquinol is recognized as participating in the initial antioxidative defense (28). Due to this, even a substantial increase in LDL ubiquinol concentration has only a minor effect on the lag time. ■

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