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Comparison of LDL Trap Assay to Other Tests of Antioxidant Capacity; Effect of Vitamin E and Lovastatin Treatment

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Oxidized low density lipoprotein (LDL) has a major impact in the development of atherosclerosis. Risk for oxidative modification of LDL is usually determined indirectly by measuring the capability of LDL to resist radical insult. We compared three different methods quantifying the antioxidative capacity of LDL *ex vivo* in dyslipidemic patients with coronary heart disease. Plasma samples were obtained from two double-blinded cross-over trials. The duration of all interventions (placebo, lovastatin 60 mg/day, RRR-α-tocopherol 300 mg/day and lovastatin + RRR-α-tocopherol combined) was 6 weeks. The total radical capturing capacity of LDL (TRAP) in plasma was determined using 2,2-azo-bis(2,4-dimethyl-valeronitrile) (AMVN) -induced oxidation, and measuring the extinction time of chemiluminescence. TRAP was compared to the variables characterizing formation of conjugated dienes in copper-induced oxidation. Also the initial concentrations and consumption times of reduced α-tocopherol (α-TOH) and ubiquinol in AMVN-induced oxidation were determined.

Repeatability of TRAP was comparable to that of the lag time in conjugated diene formation. Coefficient of variation within TRAP assay was 4.4% and between TRAP assays 5.9%. Tocopherol supplementation produced statistically significant changes in all antioxidant variables except those related to LDL ubiquinol. TRAP increased by 57%, the lag time in conjugated diene formation by 34% and consumption time of α-TOH by 88%. When data of all interventions were included in the analyses, TRAP correlated with the lag time ($r = 0.75$, $p < 10^{-6}$), with LDL α -TOH ($r = 0.50$, $p < 0.001$) and with the consumption time of α-TOH ($r = 0.58$, $p < 0.0001$). In the baseline data, the associations between different antioxidant variables were weaker. TRAP correlated with the lag time ($r = 0.55$, $p < 0.001$) and α-TOH consumption time ($r = 0.48$, $p < 0.05$), and inversely with apolipoprotein Al ($r = -0.51$, $p < 0.05$). Lag time at the baseline did not correlate with ubiquinol or tocopherol parameters, or with any plasma lipid or lipoprotein levels analyzed. Lovastatin treatment did not significantly affect the antioxidant capacity of LDL. In conclusion, TRAP reflects slightly different properties of LDL compared to the lag time. Thus, LDL TRAP assay may complement the other methods used to quantify the antioxidant capacity of LDL. However, TRAP and the lag time react similarly to vitamin E supplementation.

Keywords: assay performance; antioxidant capacity; coronary heart disease; LDL-cholesterol; vitamin E supplementation

INTRODUCTION

Oxidation of LDL (low density lipoprotein fraction in plasma) is among the initial events in the

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pathogenesis of atherosclerosis[1]. Minimally modified LDL has high affinity to the scavenger receptor of a macrophage. This results in unregulated uptake of the lipoprotein in the arterial wall and formation of foam cells. Oxidatively modified LDL triggers an immunological response with synthesis of specific antibodies and recruitment of monocytes and macrophages[2]. Susceptibility of LDL to oxidation and antioxidant defence capacity in plasma are postulated to reflect the situation in the vascular wall as well. Elevated levels of oxidized lipids in serum suggest that lipid peroxidation may be accelerated also in the intima. Inhibitors of hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase are widely used to suppress endogenous cholesterol synthesis[3]. Intensive lovastatin treatment may affect oxidative defence mechanisms in LDL by decreasing ubiquinol synthesis[4,5]. Tocopherol supplementation has been reported to increase the level of reduced tocopherol in LDL[6], protecting its lipids from oxidative modification *in vitro*[7] and *ex vivo*[8]. Numerous methods have been developed for the determination of the total antioxidant capacity in plasma (or serum). The reduced forms of known antioxidants can be directly quantified, but most of the methods are based on the initiation or rate of peroxidation of fatty acids in LDL. These include eg. measurement of reactive oxidants[9], production of lipid peroxides[10], thiobarbituric acid reactive substances[11,12] or other aldehydes than malonaldehyde[13], conjugated dienes[14] and fluorescent proteins or lipids[15]. Other methods include quantitation of the disappearance of LDL antioxidants and polyunsaturated fatty acids, fragmentation of apolipoprotein B[16], and increase in the relative electrophoretic mobility of LDL[17]. Oxidatively modified LDL is taken up by macrophages *in vivo*. The macrophage uptake experiments are thus biologically highly relevant, but may be too laborious and insensitive for the measurement of the oxidizability of LDL.

The measurement of the total antioxidant capacity (or total peroxyl radical trapping activity, TRAP) in plasma *ex vivo* was first introduced by Burton *et al.* in 1982[18]. Our assay to measure antioxidants in LDL fraction is based on continuous monitoring of chemiluminescence, and it was developed by Dr. Metsä-Ketelä. It was modified from TRAP assay performed in water phase[19,20,21]. In most of the previous studies[22,23], a water-soluble azo-compound, 2,2-azo-bis (2-amidinopropane) HCI (AAPH), has been used in the LDL TRAP assay to induce oxidation. The method with the more lipid-soluble 2,2-azo-bis(2,4-dimethylvaleronitrile) (AMVN) has not been previously published in detail. We compared the repeatability and sensitivity of this assay to reflect changes in the antioxidant capacity of LDL to those of two other methods where oxidation of LDL is induced by copper or an azo-compound. Plasma samples of dyslipidemic patients with verified atherosclerotic coronary heart disease (CHD) were used. Samples taken during lovastatin therapy and tocopherol supplementation were considered to represent conditions where the antioxidative capacity of LDL is altered.

MATERIAL AND METHODS

Plasma samples were obtained from 10 male volunteers with coronary heart disease and hyperlipidemia. All the subjects had occlusions/obliterations determined angiographically at least in two major coronary vessels. Their average age was 56 ± 8 years (mean ± SD, range 40 to 69) and body mass index 25.4 ± 1.4 kg/m2. At the baseline, their fasting LDL cholesterol (mean ± SD) was 4.8 ± 0.9 mM, total / HDL -ratio 6.9 ± 1.2, and triglyceride level 2.5 ± 1.4 mM. Nine of the patients were on beta-blocking agent therapy, 2 used ACE-inhibitors, 4 long-acting nitrates and 2 calcium channel blocking agents. None of them were current smokers. The medication and dosage were not changed during the trial.

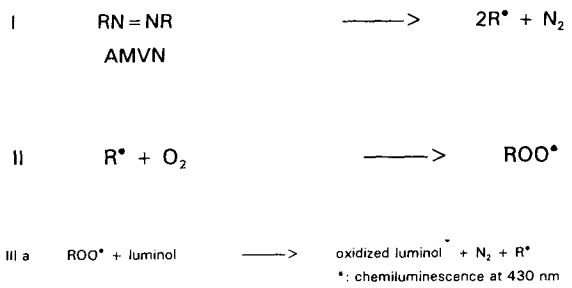
The patients participated in two cross-over trials which had double-blinded, placebo-masked and randomized cross-over designs. The mean of the measured variable after the two 2-month wash-out and one placebo period is considered here as an individual baseline. The active randomized intervention periods consisted of lovastatin, RRR-α-tocopherol and lovastatin + RRR-α-tocopherol phases. All intervention periods had a duration of 6 weeks. The dosage of lovastatin (Lovacol, Orion, Finland) was 60 mg daily and that of RRR-α-tocopherol (Esol, Leiras, Finland) was 300 mg (450 IU of vitamin E) daily. This investigation conforms with the principles outlined in the Declaration of Helsinki.

Fasting venous samples were drawn totally six times at 8 a.m. at the beginning and end of the three active study periods. The patients were advised to fast and not to take any medication, coffee or other beverages for 12 h before the blood samples. Alcohol was prohibited for 36 h before sampling. Blood samples were drawn in sitting position after a rest of at least 15 minutes. EDTA plasma was separated by centrifugation immediately after cooling (5 min) the sample in ice in the dark. Separated samples were kept frozen at –80°C until analyzed. Determination of lipids, apolipoproteins and inorganic phosphorus in LDL have been described earlier[4].

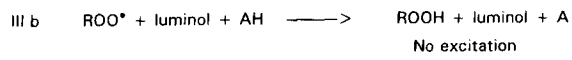
Determination of Total Radical Antiperoxiding Capacity (TRAP)

The principle of total radical antiperoxiding capacity (TRAP) determination for water-soluble compounds (plasma, buffer solutions) has been previously described in detail[19,20,21]. Here we describe a modification of that method for lipid-soluble samples. LDL was precipitated from 2 ml EDTA-plasma by 75 µl heparin and 7.4 ml Na-citrate (64 mM, pH 5.04) in 10 ml acid-washed Kimax-tubes[24]. The precipitated LDL fraction was extracted with 2 ml chloroform-methanol (1:1). After shaking, two aliquots of 100 µl each were deep-frozen for the measurement of inorganic phosphate, and 1 ml for the determination of lipid-soluble α-tocopherol and ubiquinol, while the rest of the sample was used without delay in the TRAP experiment.

In the determination of LDL TRAP, peroxyl radicals are produced at a constant rate by thermal decomposition of 2,2-azobis(2,4-dimethylvaleronitrile) (AMVN; Polysciences Inc., Warrington, PA, USA): see reaction I below. Peroxyl radicals in reaction II oxidize luminol which emits light as chemiluminescence (III a). Antioxidants in the sample added temporarily extinguish chemiluminescence (III b).



In the presence of antioxidant AH:



Luminescence reappears when some antioxidants in LDL have run out (Figure 1). The duration to the time point with half maximal generation of chemiluminescence is the most reproducible parameter. This is directly and linearly proportional to the peroxyl radical trapping capacity in the sample.

The reaction was initiated by mixing 600 µl chloroform-methanol (6:4) with 50 µl AMVN (0.4 M in benzene) and 50 µl luminol (5-amino-2,3,-dihydro-1,4-phthalazinedione; Bio-Orbit, Turku, Finland) mixture, where luminol 10 mM was dissolved in 20 mM H_2BO_4-$Na_2B_4O_2$ • 10 H_2O and 200 mM KHCO-MeOH (1:1:2). The cuvette was placed in the temperature-controlled (32°C) sample carou-

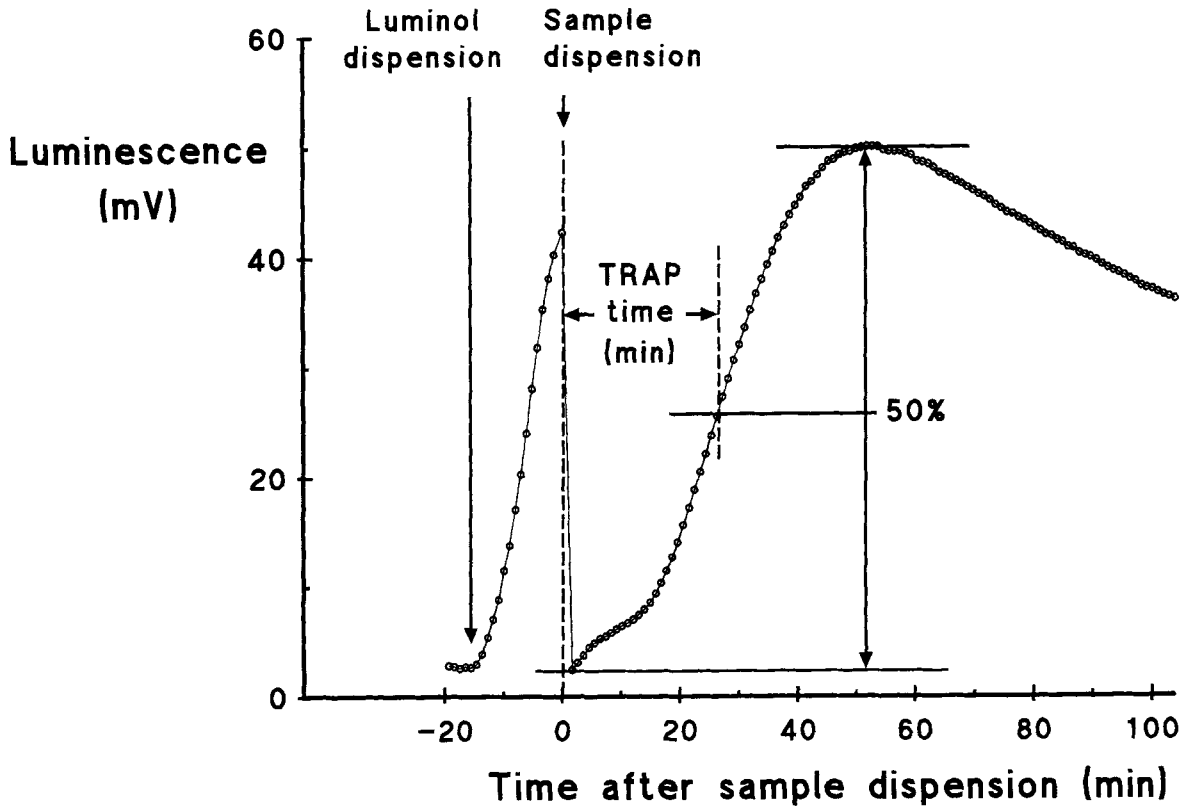


FIGURE 1 Measurement of total radical antiperoxidizing capacity (TRAP) in LDL. TRAP is calculated from TRAP time using a calibration coefficient, and is expressed as tocopherol equivalents (see Figure 2). Luminol is dispensed 15 min before the sample into the cuvette containing AMVN solution at 32°C

sel of the luminometer (1251 Luminometer, LKB Wallac, Turku, Finland). The processed LDL sample (room temperature) of 100 µl was injected directly into the cuvette after 15 min at time point 00. The computer automatically measured the chemiluminescence of cuvettes at 50 s intervals during the oxidation experiment. Tocopherol is known to trap two radicals per molecule. AMVN-oxidation experiments are carried out completely in lipid-phase, and the stoichiometric factor of α-TOH is assumed to be 2.0. The system is calibrated using four concentrations (5, 10, 20 and 40 µM) of a tocopherol solution (Trolox C, Aldrich, Deisenhofen, Germany). The linear correlation coefficient between TRAP time (50% recovery of luminescence) and tocopherol concentration usually is not less than 0.999 (Figure 2). The LDL TRAP results are expressed as micromole per millimole of in-organic phosphorus in LDL.

Measurement of Antioxidant Consumption During Oxidation of LDL with AMVN

The kinetic oxidation method with isolated LDL has been described earlier[4]. Shortly, LDL in chloroform-methanol mixture was oxidized by 2.1 mM AMVN in a temperature-controlled incubator (37°C). A sample was taken every 3 minutes, and reduced α-tocopherol (α-TOH) and ubiquinol were determined with HPLC[25]. The individual depletion times of α-TOH and ubiq-

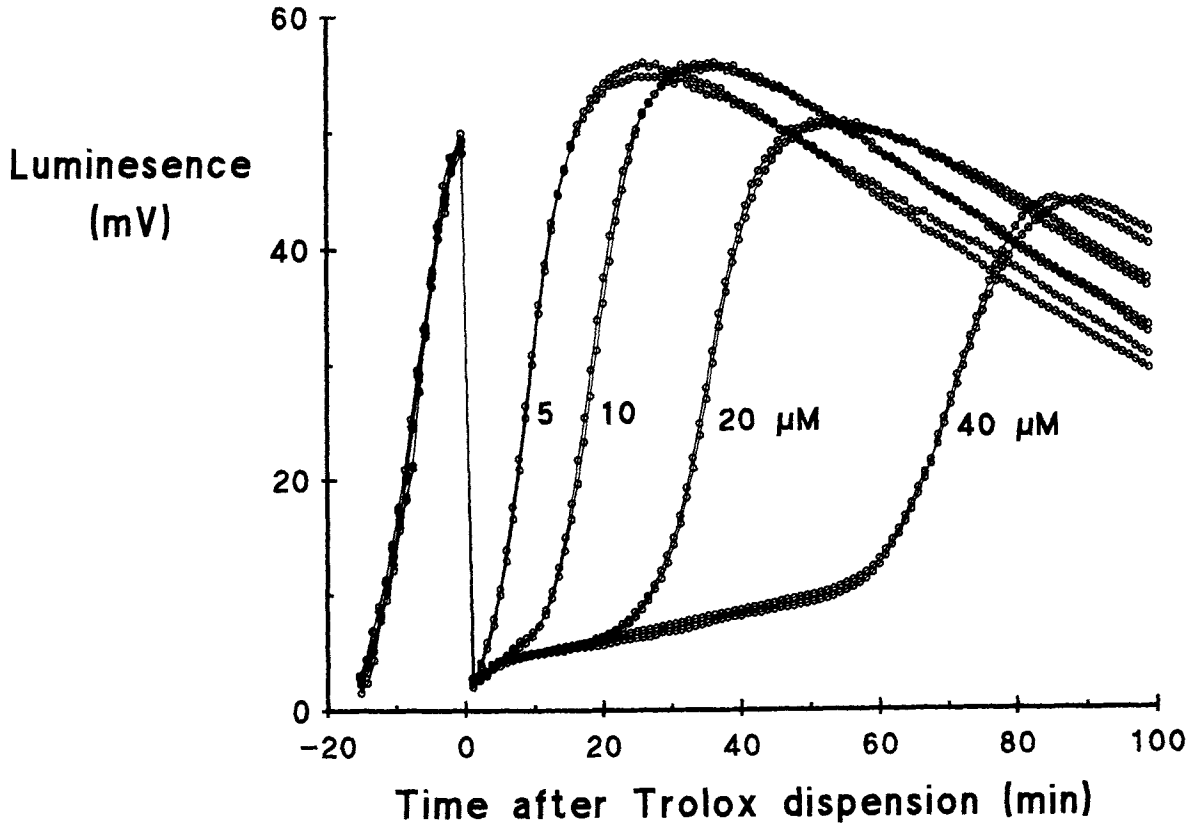


FIGURE 2 Determination of the calibration coefficient. Trolox (synthetic tocopherol) at concentrations of 5, 10, 20 and 40 µM was used in determination of the coefficient between TRAP time and tocopherol concentration. Linear correlation coefficient was 0.999, and coefficient of variation between the duplicates was on average 1.1%

uinol were calculated using linear regression analysis. Because recovery is variable due to manual extraction of LDL, the results were divided with LDL phosphorus (inorganic). In this report, exhaustion of the antioxidants (consumption time) is expressed by dividing the depletion time with LDL phosphorus.

Oxidation of LDL with CuSO4

The formation of hydroperoxides with conjugated double bonds (conjugated dienes) during copper-induced oxidation of LDL was detected by measuring UV-absorbance at 234 nm. The final concentration of $CuSO_4$ in the mixture was 1.67 µM. The method has been described earlier[14,5]. The lag time to the start of the propagation phase of diene formation, the maximal rate of formation and the maximal concentration of conjugated dienes formed (expressed as µmoles/g LDL protein) were determined. All samples of a participant were analyzed simultaneously. One control sample was always processed with the patient samples. It was EDTA plasma (containing 0.6% saccharose) drawn from a healthy subject and kept at –80°C divided in small aliquots.

Calculation of Repeatability of the Tests

In AMVN-induced oxidation experiments, the within-assay coefficient of variation (CV) was

calculated using duplicates of 76 samples which consisted of both healthy subjects and dyslipidemic CHD patients (I). Each TRAP experiment day included a common control sample. A plasma sample pool of healthy subjects kept deep-frozen was analyzed totally sixteen times to analyze between-day (between-assay) repeatability of TRAP and kinetic variables of lipid-soluble antioxidants. The within-subject CV of dyslipidemic CHD patients was calculated using the three baseline measurements of the ten participants: two from the wash-out periods and one from the placebo period. The between-subject variation is the standard deviation of the average baseline values divided by the mean of these three baseline measurements. In copper-induced oxidation experiments, the within-assay CV was calculated using duplicates of totally 192 samples. The control sample sizes for estimation of between-assay variation varied from 10 to 25 measurements each. The weighted mean of between-assay CV of ten different control specimens are presented in I.

Statistical analyses were carried out using the statistical program package BMDP SOLO v. 4.0[26]. The effects of the three different interventions were compared using the analysis of variance with equal replications (RANOVA), and with the factors treatment and treatment order. If a statistical significance was observed in overall RANOVA, the comparison was continued using a parametric contrast analysis, Tukey test, between the active phases and baseline values. A result with a two-sided p-value less than 0.05 was regarded as statistically significant. The complete data set with 6 repeated measurements was used in evaluating possible associations between antioxidant and lipid variables. Pearson's linear correlation coefficients, and mean ± standard deviation are presented if not otherwise cited.

TABLE I Repeatability and biological variation (coefficient of variation, %) of different tests for measuring antioxidative capacity in LDL

	AMVN – induced oxidation					*Cu – induced oxidation*		
	TRAP	*LDL*	*LDL*	*Consumption time*		*Lag time*	*Rate*	*Max*
		α-TOH	*CoQ*	*α-TOH*	*CoQ*			
Healthy control subjects								
Within-assay CV%	4.4	–	–	–	–	2.8	3.5	2.0
Between-assay CV%	5.9	7.5	11.3	12.8	14.2	5.4	6.4	4.9
Dyslipidemic CHD patients								
Within-subject CV%	12.4	17.2	25.2	22.3	20.9	7.0	10.4	6.3
Between-subject CV%	18.1	34.7	30.1	38.2	23.7	18.4	24.0	11.6

AMVN	2,2-azobis(2,4-dimethylvaleronitrile)
Cu	copper 1.67 μM
TRAP	Total radical antiperoxidizing capacity in plasma LDL
Lag time	Initial delay in formation of conjugated dienes
Rate	Maximal formation rate of conjugated dienes
Max	Maximal concentration of protein-normalized conjugated dienes
α -TOH	reduced α-tocopherol concentration divided by inorganic phosphorus (Pi)
CoQ	ubiquinol concentration divided by Pi
CV%	coefficient of variation in percentage
CHD	coronary heart disease

TABLE II Variables (mean ± SD) describing antioxidant capacity of LDL in 10 hypercholesterolemic CHD patients. The relative changes (%) were measured after 6-week treatments with lovastatin 60 mg and RRR-α-tocopherol 300 mg daily. Analysis of variance with repeated measurements and Tukey test was used to test the significance of the change compared to the baseline value

Fasting serum or plasma LDL		*Baseline*	*Lovastatin*		*Vitamin E*		*Lovastatin + Vitamin E*	
			%	*p*	%	*p*	%	*p*
AMVN – induced oxidation of LDL								
TRAP	μmol/mmol Pi	26.5 ± 4.8	−2.9		+57.8[a]		+56.2[a]	
Reduced LDL α-tocopherol								
Observed value before oxidation	μmol/mmol Pi	14.1 ± 4.9	+3.6		+118.3[a]		+129.0[a]	
Consumption time	min/mmol Pi	11.6 ± 2.7	+9.6		+87.8[a]		+67.4[a]	
LDL ubiquinol								
Observed value before oxidation	μmol/mmol Pi	0.70 ± 0.21	−19.1		+14.1		−12.1	
Consumption time	min/mmol Pi	12.9 ± 4.1	+26.0		−18.4		+39.5[b]	
Cu – induced oxidation of LDL								
Lag time	min	64.6 ± 11.9	−7.4		+34.4[a]		+27.9[a]	
Maximal oxidation rate	μM/min	.454 ± 0.11	−2.2		−11.8[b]		−17.8[c]	
Maximal protein-normalized conjugated dienes	μmol/g	516 ± 57	−12.7 [b]		−8.9		−16.9[c]	

TRAP = total radical antiperoxidizing capacity in plasma LDL
Pi = inorganic phosphorus content in LDL

a. $p < 0.0001$.
b. $p < 0.05$.
c. $p < 0.01$.

TABLE III Linear correlation coefficients between different variables in LDL. All data are pooled together (n = 60). Significancies:

	AMVN – induced oxidation				
	TRAP	*LDLα-TOH*	*LDL CoQ*	*Consumption time*	
				α-TOH	*CoQ*
TRAP	–	0.50[a]	0.01	0.58[b]	−0.06
LDL α-tocopherol	0.50[a]	–	0.35[c]	0.61[b]	0.15
LDL ubiquinol	0.01	0.35[c]	–	0.33[c]	0.23
Consumption time of α-tocopherol	0.58[b]	0.61[b]	0.33[c]	–	0.29
Consumption time of ubiquinol	−0.06	0.15	0.23	0.29	–
Copper – induced oxidation					
Lag time	0.75[b]	0.55[b]	0.25	0.61[b]	0.02
Rate	−0.20	−0.41[c]	−0.07	−0.27	−0.13
Max	−0.25	−0.50[a]	−0.05	−0.25	0.01

TRAP	Total radical antiperoxidizing capacity in plasma LDL
α-TOH	Reduced α-tocopherol concentration divided by Pi
CoQ	Ubiquinol concentration divided by inorganic phosphorus (Pi)
Lag time	Initial delay in formation of conjugated dienes
Rate	Maximal formation rate of conjugated dienes
Max	Maximal concentration of protein-normalized conjugated dienes

a. $p < 0.001$.
b. $p < 0.0001$.
c. $p < 0.01$.

RESULTS

The within- and between-assay repeatabilities of TRAP assay were poorer than those of the lag time or maximal concentration of conjugated dienes during Cu^{2+}-induced oxidation (Max (I). Coefficients of variation (CV% = sd./mean) of the duplicates of the four standard samples describing within-assay repeatability were between 1.0 and 1.3% (Figure 2). The between-assay CV% measured using six determinations of the standard line was 4.5 % for low standard (5 μM) and 3.1% for high standard (40 μM). The repeatability of the consumption times of α-TOH and ubiquinol during non-metal-ion-induced oxidation was clearly poorer; the intra-individual variability in three baseline samples was over 20%. This may be partly due to a long interval (3 min) between the measurements during AMVN-induced oxidation. Also the reduced α-TOH and ubiquinol concentrations in the LDL of fasting plasma samples after wash-out and placebo periods (within-subject CV) were highly variable. This may be due to seasonal variation in nutrition. The between-subject CV% of reduced α-TOH was 32.5% if the antioxidant concentrations were corrected with apoB levels instead of LDL phosphorus.

Vitamin E supplementation increased antioxidant capacity in LDL (II). The average relative change was greater in TRAP than in the lag time. Concentration of α-TOH in LDL, increased and consumption time of α-TOH was prolonged significantly. However, this did not affect LDL ubiquinol concentration or consumption rate during AMVN-induced oxidation. The decrease in TRAP or the lag time during lovastatin treatment were not significant. Highly significant changes ($p < 0.0001$) occured in serum lipids and lipoproteins during lovastatin treatment, as expected. Total cholesterol, LDL, apolipoprotein B (apoB), and LDL phosphorus decreased by 31%, 43%, 28% and 34%, respectively. LDL / apoB -ratio decreased by 21 % ($p < 0.01$). Interestingly, fasting serum LDL cholesterol tended to decrease (-4.4%) and HDL increase (+6.4%) also during supplementation of vitamin E. These lead to a statistically significant decrease in LDL/HDL -ratio (-11%, $p < 0.05$). The weight or living habits of the patients did not change during the whole follow-up period. Association between TRAP and the lag time was highly significant in all data ($r = 0.75$, III) and during tocopherol period ($r = 0.896$, $p < 10^{-6}$, Figure 3a). At the baseline, correlation between TRAP and lag time ($r = 0.55$, $p < 0.001$) was not impressive. The small changes in TRAP and the lag time induced by lovastatin did not correlate (Figure 3b). Reduced α -TOH in LDL and its consumption time correlated significantly with TRAP and the lag time (III) when vitamin E supplementation period was included. In the baseline (open circles in Figure 4), LDL α-TOH did not predict antioxidative capacity in LDL measured with techniques where lipid peroxidation is initiated either with a metal-ion or a non-metal-ion.

The associations between the changes in ubiquinol-related variables in LDL and TRAP or the lag time were not significant. However, ubiquinol consumption time was associated with all lipid and lipoprotein variables except Apo Al, whereas tocopherol consumption time did not associate with any of the lipid variables. Also TRAP and lag time did not correlate with lipid variables, which was somewhat unexpected. In the baseline data TRAP was inversely associated only with apolipoprotein Al ($r = -0.51$, $p < 0.01$).

DISCUSSION

In this six-phase cross-over data set lovastatin treatment represents a period when antioxidative defence mechanisms in LDL are possibly affected[4]. RRR-α-tocopherol supplementation represents a period when the reducing capacity of LDL is fortified[27,28,8]. The different standardization of LDL sample in the copper- and AMVN-induced oxidation assays may partly

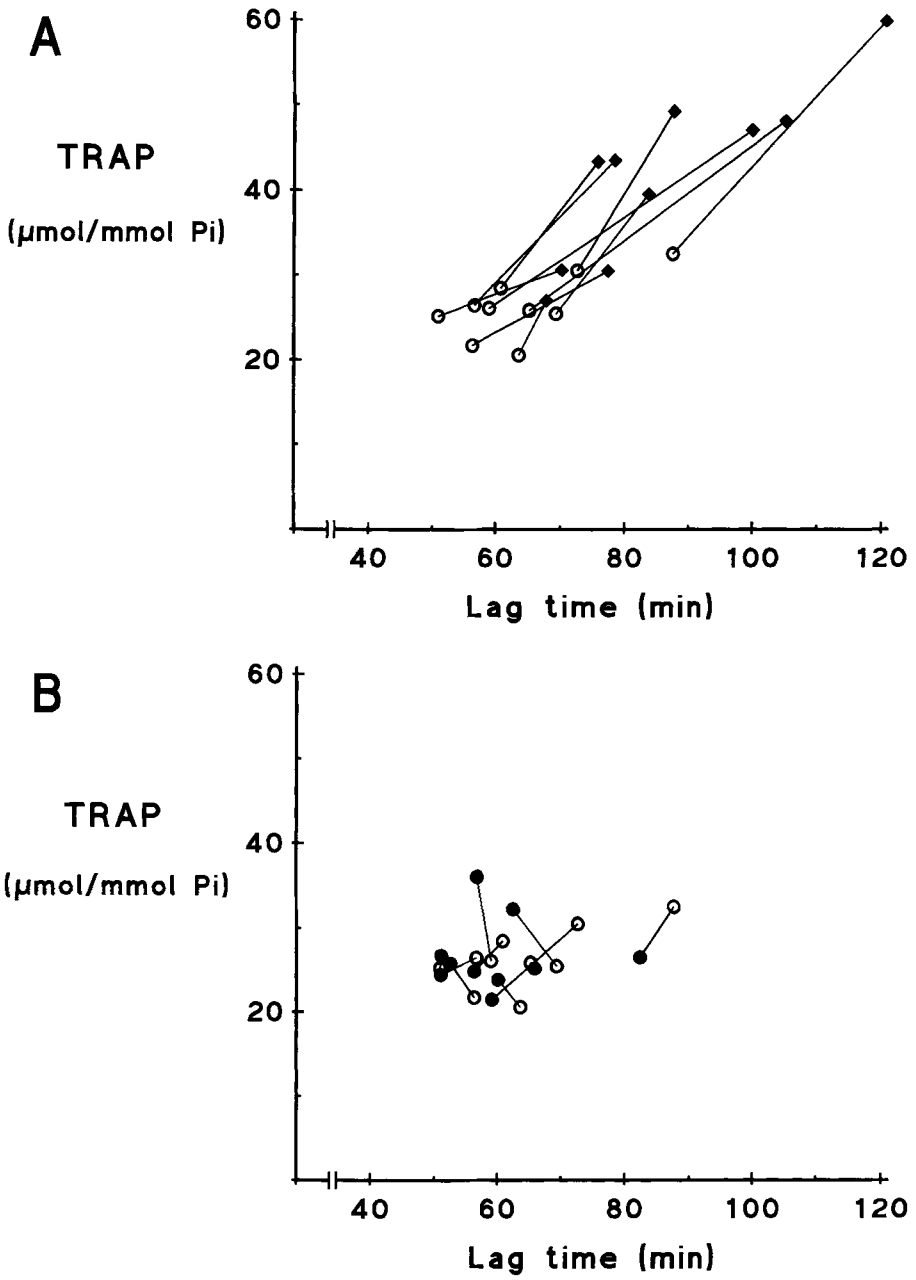


FIGURE 3 Individual changes of TRAP and lag time in Cu^{2+}-induced oxidation. A) ◦ = baseline, ♦ = RRR-α-tocopherol 300 mg daily. B) ◦ = baseline, • = lovastatin 60 mg daily

explain the different behaviour of TRAP and lag time during lovastatin therapy. In metal ion -induced oxidation of LDL copper vs. lipoprotein concentration was constant. In the other experiments the amount of AMVN per sample was always constant independent of the LDL

level in serum, and the results were normalized using the inorganic phosphorus concentration in LDL (Pi). Also the consumption times were divided with Pi, and can not be directly compared with those published previously[4,5,8].

The daily dose of tocopherol in this trial was 300 mg of RRR-α-tocopherol, which is about equivalent with 450 mg (450 IU) of *all-racemic* α-tocopheryl acetate (*d,l*-α-tocopheryl acetate) when a bioactivity ratio of 1.36 is used between these two compounds. This ratio has been suggested to be considerably higher[29,30].

ApoB contains thiols which may participate in the initial radical-scavenging events in LDL[31]. Also the particle size may affect oxidation[32]. The correlation analyses were also carried out with TRAP data where Trap time was divided with apoB level, that is, with the number of LDL particles. However, this did not significantly change the correlation coefficients between TRAP and the variables of Cu^{2+}-induced oxidation.

This investigation supports previous findings concerning the role of α-TOH in *ex vivo* experiments on antioxidative capacity in LDL. In a study with 16 healthy volunteers, the lag time and LDL α-TOH (per phosphate) did not correlate in copper-mediated oxidation, but the association was inverse ($r = -0.42$) in the AAPH-induced oxidation[33]. In another study with 20 healthy volunteers no significant association was found between the lag time and α-TOH, ubiquinol, or polyunsaturated fatty acids in LDL[34], whereas in primates exposed to different diets the correlation between the lag time in AAPH-mediated oxidation assay and LDL α-TOH was significant[35]. However, the lag time in copper-mediated oxidation did not correlate with LDL α-TOH during a diet rich in monounsaturated or saturated fats. In a placebo-controlled trial with 40 healthy smoking men and supplementation of α-TOH 200 mg/day for 2 months, LDL TRAP increased by 58% and the lag time by 34%[36], which is exactly the same we found with older men with CHD. The relative changes of α -tocopherol level in LDL or LDL + VLDL levels corresponded well in these studies: 118% increase with 300 mg/day and 90% increase with 200 mg/day. The results differ only in that Porkkala-Sarataho et al. found stronger correlation between the changes in LDL-tocopherol and in LDL TRAP. In another trial with healthy athletic men, a 4-week supplementation with 294 mg tocopherol daily raised LDL TRAP by 40% and serum tocopherol concentration by 59%[37].

Belcher et al.[38] compared the lag times of conjugated diene formation induced by copper (10 μM $CuSO_4$) and by hemin + hydrogen peroxide. Healthy volunteers took vitamin E (800 IU/day) or vitamin C (1000 mg/day) for 2 weeks. The linear correlation of the lag times calculated from both supplemented and non-supplemented samples was 0.929 between the two different methods. In 21 NIDDM patients the lag time was lengthened about 1.5-fold during vitamin E supplementation (1600 IU/day). As in our material, no correlation was noted between LDL α-TOH and the lag time at baseline, whereas the correlation was significant ($r = 0.69$) after the supplementation[39].

Our results are also in concordance with a study, where α-TOH/cholesterol ratio correlated significantly with total antioxidant activity of LDL during vitamin E supplementation (300 mg daily for 9 weeks) but not in the non-supplemented group[40]. In that study oxidation of LDL was induced with ABTS (2,2-azinobis(3-ethylbenzothiazoline-6-sulphonic acid)) radical cation.

In conclusion, duration of extinction of AMVN-induced chemiluminescence, TRAP, associates with the lag time in copper-induced formation of conjugated dienes in LDL. This correlation is stronger during vitamin E supplementation, and weaker during lovastatin treatment when LDL particles contain less cholesterol. The repeatability of LDL TRAP assay is acceptable but slightly inferior to that of the lag time. In all

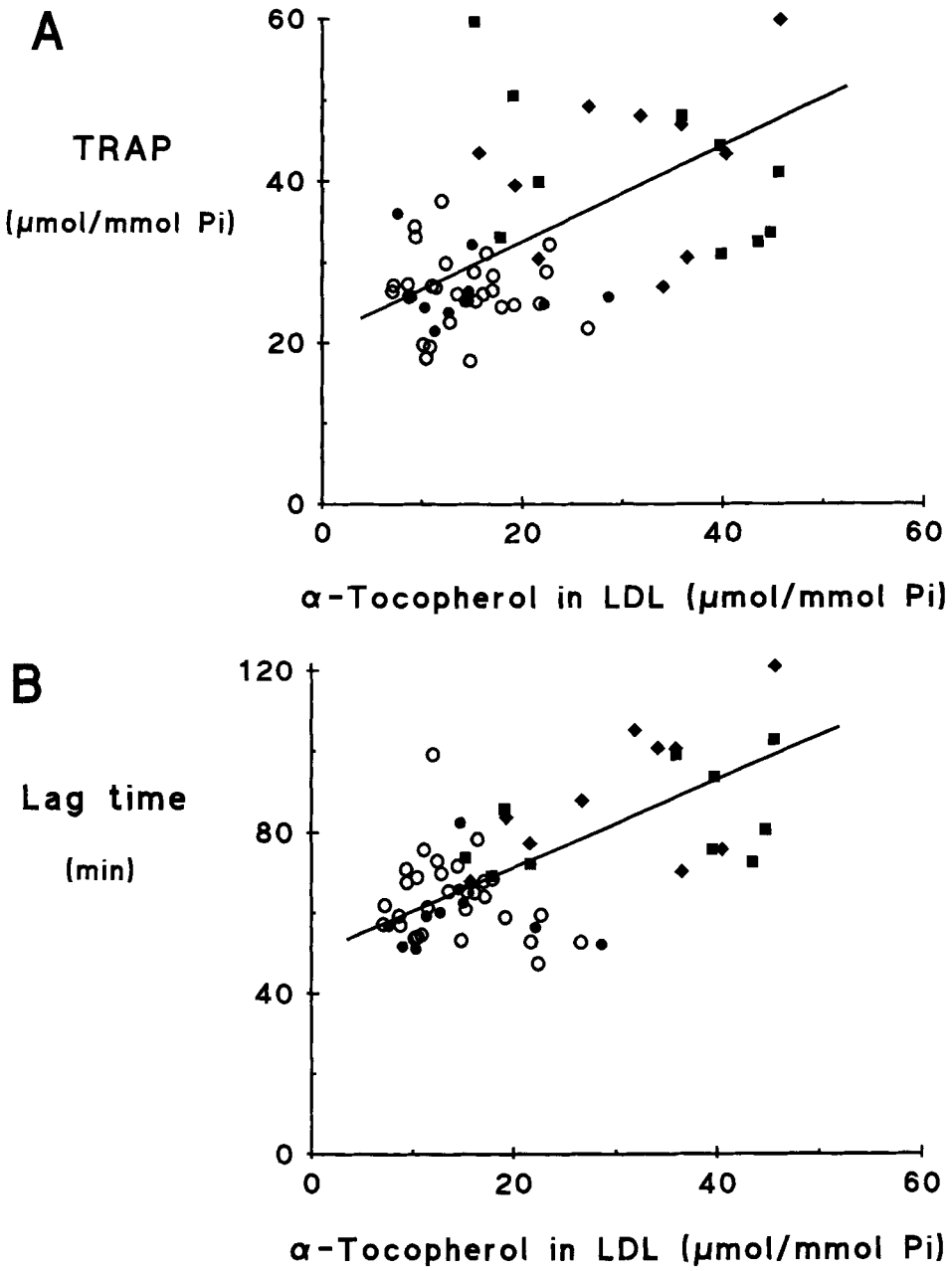


FIGURE 4 Association of reduced α-tocopherol in LDL and LDL TRAP (A) and lag time of conjugated diene formation in $CuSO_4$-induced oxidation (B). ◦ = baseline, • = lovastatin 60 mg daily, ♦ = RRR-α-tocopherol 300 mg daily, ■ = lovastatin 60 mg daily + RRR-α-tocopherol 300 mg daily

three methods used in this investigation water-phase antioxidants were excluded. Regardless of the method, α-tocopherol appears to play a significant role as an antioxidant only in tocopherol-supplemented LDL. LDL TRAP may complement other indicators of antioxida-

tive capacity in LDL, but further trials are needed to elucidate if it has any role in clinical practice.

Acknowledgements

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