MECHANISMS OF ANTIOXIDANT PROTECTION OF LOW-DENSITY LIPOPROTEIN PARTICLES AGAINST FREE RADICAL OXIDATION

© 2025 V. Z. Lankin[a]*, K. B. Shumaev[a,b], V. A. Medvedeva[a], A. K. Tikhaze[a], and G. G. Konovalova[a]

[a] *National Medical Research Center of Cardiology Named after Academician E. I. Chazov, Ministry of Health of the Russian Federation, Moscow, Russia;*

[b] *Bach Institute of Biochemistry, Research Center of Biotechnology, Russian Academy of Sciences, Moscow, Russia*

**e-mail: lankin0309@mail.ru*

Received September 24, 2024

Revised December 05, 2024

Accepted December 10, 2024

Abstract. It was found that when patients with atherosclerosis are orally administered ubiquinon Q_{10} (CoQ_{10}), the oxidation (lipohydroperoxide content) of low-density lipoprotein (LDL) particles sharply decreases, which confirms the important role of this natural antioxidant in protecting LDL particles from free radical oxidation *in vivo*. The influence of lipophilic natural antioxidants ubiquinol Q_{10} ($CoQ_{10}H_2$) and α-tocopherol (α-TOH) on the kinetic parameters of Cu^{2+}-initiated free radical oxidation of LDL particles was investigated. In this model system, the possible synergism of the antioxidant action of $CoQ_{10}H_2$ and α-TOH is shown. The probable mechanisms of bioregeneration of the lipophilic antioxidants in LDL particles, including regeneration of α-TOH from the tocopheroxyl radical (α-TO$^{\bullet}$) with the participation of $CoQ_{10}H_2$ and/or ascorbate, are discussed.

Keywords: *low-density lipoproteins, free radical oxidation, antioxidants, ubiquinol Q_{10}, α-tocopherol, antioxidant bioregeneration*

DOI: 10.31857/S03209725250108e5

INTRODUCTION

Low-density lipoprotein (LDL) nanoparticles of the lipid-transport system circulating in the bloodstream, unlike various cells and subcellular structures (including blood cells), do not contain antioxidant enzymes (superoxide and hydroperoxide oxidoreductases) that protect lipid-protein supramolecular complexes from the damaging effects of free radical oxidation (FRO) [1]. Nevertheless, LDL need effective protection against SRO due to the presence of a large amount of oxidation substrate, polyunsaturated fatty acid acyls (PUFAs) in the particulate phospholipids [2]. The outer phospholipid monolayer of LDL, as well as bilayer phospholipid membranes, is readily oxidized under aerobic conditions *in vitro* [3] and *in vivo* [4] both spontaneously and when catalyzed by initiators of diverse nature [5]. The presence of high partial pressure of oxygen (pO_2) in blood plasma, as well as inducers of SRO (such as metal ions of variable valence, hemoproteins, Cu-containing proteins, lipohydroperoxides (LOOH)), various generators of H_2O_2, superoxide anion radical ($O_2^{\bullet -}$) and other reactive oxygen species ROS) [5] create an increased risk of damage to LDL particles due to their SRO in the process of circulation in the bloodstream. Complete absence of any antioxidant enzymes in these structural formations repeatedly increases their susceptibility to SRO. Obviously, based on the pragmatism of evolution, the presence of antioxidant proteins in LDL particles, whose specific function is the transport of lipids synthesized in the liver to peripheral tissues, is inappropriate. At the same time, all cells without exception would have to have enzymatic systems for utilization of these proteins after completion of lipid transport, which would be associated with additional useless energy consumption. Nature has found a clever way out of this situation, which consists in the creation of an effective system of non-enzymatic control of SRO in LDL, represented, first of all, by fat-soluble phenolic antioxidants - the reduced form of coenzyme Q_{10} (ubiquinol Q_{10}, $CoQ_{(10)}H_{(2)}$) [6] and vitamin E ($CoQ_{(10)}H_2$). [6] and vitamin E (α-tocopherol, α-TON) [7]. Importantly, unlike the true vitamin α-TON (not synthesized in the body), a significant portion of $CoQ_{10}H_2$ (about 60%) does not enter tissues from food sources but is formed by *de novo* biosynthesis [8]. Fat-soluble vitamins (including α-TON) are transported by LDL particles and localized in the hydrophobic core of nanoparticles [9]. The literature suggests that $CoQ_{10}H_2$ is the main (most effective) antioxidant protecting LDL particles from oxidation [10-12]. Nevertheless, according to calculations there is no more than 1 molecule of $CoQ_{10}H_2$ per 1 LDL particle [13], which makes it impossible for this antioxidant to fulfill its effective protective function without the existence of a mechanism of its bioregeneration (reduction) in LDL.

It is believed thatα -TON and $CoQ_{10}H_2$ can interact with peroxyl radicals ($LOO^{\bullet}$) of lipids in the following reactions [14-16]:

α-TOH + LOO $^{\bullet}\longrightarrow$ α-TOH$^{\bullet}$ + LOOH, (1)

$CoQ_{10}H_{(2)\,LOO}$+ $^{\bullet}\longrightarrow$ $CoQ_{10}H^{\bullet}$ + LOOH, (2)

CoQ(10)H$^{\bullet}$ + LOO$^{\bullet}$ $\longrightarrow$ CoQ_{10}+ LOOH. (3)

The tocopheroxyl radical (α-TO$^{\bullet}$) produced during reaction (1) can react again with LOO$^{\bullet}$ or another α-TO$^{\bullet}$ molecule to form non-radical products [14]. At the same time, $CoQ_{10}H_2$ is sequentially oxidized by LOO$^{\bullet}$ to ubisemiquinone ($CoQ_{10}H^{\bullet}$) and further to the fully oxidized form of CoQ_{10} (reactions (2) and (3)) [15]. At the same time, $CoQ_{10}H_2$ reduces not only lipid free radicals but also α-TO$^{\bullet}$ in reaction (4) [16]:

$CoQ_{10}H_2$ + α-TO $^{\bullet}\longrightarrow$ $CoQ_{10}H^{\bullet}$ + α-TON. (4)

This reaction may underlie the synergism of antioxidant action of $CoQ_{10}H_2$ andα -THO, thus participating in the bioregeneration of α-TOH, and it is possible that this mechanism may play an important role in preventing SRO of LDL particles. Thus, in the absence of $CoQ_{10}H_2$, the accumulation of tocopheroxyl radicals (α-TO$^{\bullet}$) may cause a prooxidant effect in LDL [17, 18], since α-TO$^{\bullet}$ under these conditions may act as an initiator of chain reactions:

α-TO LH$^{\bullet}$ + $\rightarrow$ α-TON L + $^{\bullet}$, (5)

L $^{\bullet}$+ $O_2\rightarrow$ LOO$^{\bullet}$, (6)

where LH– acyls of PUFAs; L $^{\bullet}$–alkyl radicals of lipids.

Nevertheless, the effects observed in the interaction of $CoQ_{10}H_2$ and α-TON in model systems cannot fully explain the bioregeneration of these lipophilic antioxidants under *in vivo* conditions, because the presumed localization of $CoQ_{10}H_2$ and α-TON in LDL particles [19] and the above described interaction reactions of these fat-soluble antioxidants under *in vivo* conditions are not obvious. In the present work, we have attempted to experimentally substantiate possible mechanisms of bioregeneration of $CoQ_{10}H_2$ andα -TON, which provide protection of LDL particles by these antioxidants from SRO during their circulation in the bloodstream.

MATERIALS AND METHODS

Reagents. For clinical studies, the preparation ubiquinone Q_{10} ("Bioquinone"; Phrama Nord, Denmark) in soybean oil capsules was used in this work. SkQ1 (10-(6′-plastoquinonyl)decyltriphenylphosphonium) was kindly provided by Academician V.P. Skulachev (Belozersky Research Institute of Physical and Chemical Biology, Lomonosov Moscow State University). The remaining reagents were obtained from Sigma-Aldrich (USA).

Clinical study of the effect of long-term *per os* administration of ubiquinone Q_{10} (CoQ_{10}) preparation in CHD patients on plasma LDL lipohydroperoxide content. The clinical study included 10 men (49± 2.5 years) with chronic ischemic heart disease CHD) and hyperlipidemia, who were treated in NMIC of cardiology of the Ministry of Health of Russia. Patients did not receive lipotropic drugs and took *per os* preparation ubiquinone Q_{10} at a daily dose of 60 mg for 6 months. To monitor LOOH levels in LDL from patients, fasting venous blood was drawn in the presence of 1 mg/mL EDTA as an anticoagulant and antioxidant, after which LDL was isolated by preparative ultracentrifugation as described below. Data on changes in LDL LOOH content in IBS patients upon ubiquinone Q_{10} administration were presented as % of baseline (100%) LDL LOOH level.

LDL isolation by preparative ultracentrifugation. LDL from blood plasma of practically healthy donors was obtained by centrifugation in NaBr density gradient on a Beckman Optima XPN-80 ultracentrifuge (Beckman, USA) [20]. Lipoproteins were isolated from three blood plasma samples obtained from three different healthy donors. Donor plasma containing 1 mM EDTA was added to a centrifuge tube, gently layered with NaBr solution with a density of 1.006 g/mL and centrifuged (105,000 *g* for 18 h at 4 ^{o}C) in a Ti-60 rotor. After removing the top layer containing very low density lipoproteins, a calculated amount of NaBr was added to the contents of the tube under stirring and the salt was dissolved to create a density of 1.065 g/mL, after which the tube was supplemented with NaBr solution of the same density. After centrifugation (105,000 *g* for 18 h at 4 ^{o}C), the top layer containing the flotated LDL was collected. The resulting LDL fraction was dialyzed for 18 h at 4 ^{o}C against 2000 volumes of 145 mM NaCl in 50 mM K,Na-phosphate buffer (pH 7.4).

Determination of lipohydroperoxide content in LDL using Fe^{2+}-xyleneolorange reagent. The content of lipid hydroperoxides (LOOH) in LDL was determined using oxidation of Fe^{2+} ions by reaction with LOOH:

$$LOOH + Fe^{2+} \longrightarrow LO^{\bullet} + OH^{-} + Fe^{3+}, \quad (7)$$

where $LO^{\bullet}$ is the alkoxyl radical of lipids.

Fe^{3+} ions (equimolar concentrations of LOOH) form a colored complex with xylenol

orange (o-cresolsulfophthalein-3,3'-bismethyliminodiacetic acid tetrasodium salt), the concentration of which was measured on a UV-2600 Shimadzu spectrophotometer (ε_{560}= 1.5× 10^4 M^{-1} - $cm^{(-)(1))}$) [21]. For the assays, FOX reagent containing 1 mM xylenol orange and 2.5 mM Mora's salt $Fe(NH_4)_{(2)}(SO_{(4)})_{(2)}$in 25 mM H_2SO_4 was pre-prepared with the addition of 9 volumes of 4.4 mM butylhydroxytoluene (BHT) in 90% methanol (purity grade for HPLC). In control samples, LOOH was quantitatively reduced by triphenylphosphine to hydroperoxide and the absorbance of these samples was subtracted from the readings of the experimental samples [21]. Tert-butyl hydroperoxide was used as a standard.

Study of the kinetics of free-radical Cu^{2+}-initiated oxidation of LDL. After dialysis, the protein content of LDL samples was determined by the Lowry method, and then samples were diluted to 50 μg protein/mL with a solution containing 154 mM NaCl in 50 mM K,Na-phosphate buffer (pH 7.4), and oxidation of LDL particles at 37 °C was induced by introducing 30 μM $CuSO_4$ into the incubation medium, after which the accumulation of conjugated dienes was measured at fixed time intervals on a UV-2600 Shimadzu spectrophotometer [22 - 24]. Conjugated double bonds absorbing at 233 nm are formed during the formation of PUFA hydroperoxides (LOOH). The LOOH content (ΔD_{233}) in LDL particles can be calculated based on a molar extinction coefficient of 22,000 $M^{-(1}$)-$cm^{(-)(1)}$. The duration of the induction period (τ) on the kinetic curves of lipid SRO in LDL was determined as described previously [25]. The kinetics of Cu^{2+}-dependent SRO of LDL particles *in vitro* was studied using LDL isolated from blood plasma of three donors (LDL isolated from blood plasma of each donor was used in independent experiments). Using the kinetic model of Cu^{2+}-initiated SRO of LDL particles, we studied the inhibitory effect of natural phenolic lipophilic antioxidants (α-tocopherol, ubiquinol $Q_{10)}$ and synthetic plastoquinone (skQ1), the structural formulas of which are shown in Fig. 1.

Fig. 1. Structural formulas of the investigated lipophilic phenolic antioxidants: ubiquinol Q_{10} ($CoQ_{10}H_{2)}$,α -tocopherol, 10-(6′-plastoquinonyl)decyltriphenylphosphonium (skQ1). The tested antioxidants were added to the incubation mixture containing LDL in ethanol solutions (final alcohol concentration not exceeding 2%), followed by shaking for 1 min in a shaker and incubation for 4 min at 37 °C

Preparation of ubiquinol Q_{10} and free radicalsα -tocopherol and probucol. $CoQ_{10}H_2$ was prepared by reducing CoQ_{10} with sodium borohydride ($NaBH_{4)}$, for which equimolar solutions of these compounds in ethanol were mixed [15]. The $NaBH_4$ solution was prepared immediately before its use. The tocopheroxyl radical (α-$TO^{\bullet}$) was prepared by oxidizing a 400 mM solution

of α-TOH in isopropanol with manganese dioxide (MnO_2) powder. For this purpose, 1 mL ofα - tocopherol solution was mixed with 100 mg of manganese dioxide and shaken on a shaker for 20 min, after which the mixture was centrifuged and the supernatant was filtered to completely remove MnO_2 particles. The phenoxyl radical of probucol (4,4'-[(1-methylethylidene)bis(thio)]bis[2,6-bis(1,1-dimethylethyl)phenol]) was prepared similarly by oxidizing a 0.2 M solution of probucol with manganese dioxide.

Electron paramagnetic resonance (ESR) spectroscopy. ESR spectra were measured on an automated ESR 70-03 XD/2 spectrometer ESR 70-03 XD/2 UP "KBST" BSU (Belarus). Free radical solutions of α-tocopherol and probucol in isopropanol were mixed with ethanol and 160 mM Na-phosphate buffer (pH 7.4) in the ratio 8/1/1. This reaction mixture (40 μL) was injected into a gas-permeable PTFE Sub-Lite-Wall capillary tube (inner diameter, 0.635 mm; wall thickness, 0.051 mm; Zeus Industrial Products, Inc., USA). The capillary was folded twice and inserted into a quartz tube with a diameter of ~4 mm, which was placed in the resonator of the EPR spectrometer. The α-$TO^{\bullet}$ spectra were recorded at 25° C and the following conditions: microwave attenuation - 1 dB; RF modulation amplitude - 0.05 mT; CB frequency - ~9.32 GHz; gain - 1000; sweep range - 5 mT. Spectra recording was started 2 min after mixing the components of the reaction mixture.

Statistical Analysis. In the clinical study, SPSS 14 Windows Version 7.0 programs were used for statistical analysis. The Kolmogorov-Smirnov criterion was used to assess the normality of the distribution of variables. Student's t-criterion was used to assess the significance of the differences of experimental data in relation to the control. The results obtained *in in vitro* experiments were analyzed using Origin Pro 8 software (Origin Lab Corp., USA). One-factor analysis of variance was used to compare mean values, followed by Student's t-criterion and Dunnett's criterion to assess the reliability of differences between groups. Data of all conducted studies are presented as mean values (M)± standard deviation (σ). Differences were considered statistically significant at $p< 0.05$.

RESEARCH RESULTS

Effect of ubiquinone Q_{10} on the content of lipohydroperoxides in plasma LDL of IBS patients. During the first month of CoQ_{10} therapy the LOOH content in LDL of IBS patients sharply decreases (by 61% of the initial level), and by the second month of observation it decreases to 19% and remains at the similar level during the next 4 months of therapy (Fig. 2).

Fig. 2. Effect of oral administration of ubiquinone Q_{10} in CHD patients on LOOH content in plasma LDL. Differences with baseline level of LOOH in LDL were statistically significant; * $p< 0.05$

It should be noted that the observed pronounced antioxidant effect (in terms of reduction of LOOH content in LDL) observed already 1 month after administration of CoQ_{10} to patients may indicate the existence of its effective bioregeneration (reduction) with the formation of $CoQ_{10}H_2$ under *in vivo* conditions, study of the mechanism of which was the subject of further research.

Effect of exogenous ubiquinol Q_{10} and α-tocopherol on free-radical oxidation of LDL particles *in vitro*. The effect of exogenous hydrophobic antioxidants $CoQ_{10}H_2$,α -TON and synthetic antioxidant skQ1 (plastoquinone analog) on the SRO kinetics of LDL particles in the concentration range of 5-80 μM was investigated in in *vitro* experiments. The kinetic curves of LOOH accumulation formed in LDL during Cu^{2+}-initiated ER in the presence of the studied phenolic antioxidants at a concentration of 80 μM are shown in Fig. 3. As can be seen from these data, the studied antioxidants affect the initial rate of LOOH accumulation and the duration of the induction period (τ) on the kinetic curves of the SRO of LDL particles (Fig. 3).

Fig. 3. Effect of different lipophilic antioxidants on the kinetics of LOOH (diene conjugates) accumulation during Cu^{2+}-initiated SRO of LDL particles. *1* - oxidation of native LDL without addition of antioxidants (control); 2 - LDL+ 80 μM ubiquinol Q_{10}; *3* - LDL+ 80 μMα -tocopherol; *4* - LDL+ 80 μM ubiquinol Q_{10}+ 80 μMα -tocopherol; *5* LDL+ 80 μM skQ1. Differences of groups *4* and *5* with respect to group *1* (control) are statistically significant; * $p< 0.05$

Obviously, the investigated kinetic parameters can be correctly used to evaluate the efficiency of antioxidant action in the model system. It is known that the duration of the SRO induction period (τ) is directly proportional to the concentration of SRO inhibitor in the system - [InH] and inversely proportional to the rate of SRO initiation - ω, i.e. $\tau = [InH]/\omega$ [26]. In the oxidation system we used when studying LDL particles with unchanged lipid composition, ω is a constant (constanta) value, based on which the duration of the SRO induction period (τ) should be proportional to the antioxidant content (or its antioxidant activity). From Fig. 3 (curve *2*)it can be seen that $CoQ_{10}H_2$ at a concentration of 80 μM causes a significant increase in the duration τ during the SRO of LDL particles, as well as a decrease in the initial rate of their oxidation. When using the same concentration ofα -TON (Fig. 3, curve *3*)or joint action of $CoQ_{10}H_2$ andα -TON at a

concentration of 80 μM (Fig. 3, curve *4*), almost complete suppression of LDL oxidation was observed. Plastoquinone skQ1 at a concentration of 80 μM had a more pronounced antioxidant effect (Fig. 3, curve *5*) compared to the action of $CoQ_{10}H_2$ (Fig. 3, curve *2*).

The data on the effect of different concentrations of the investigated antioxidants on durationat the SRO of LDL particles are presented in Fig. 4. From these results it can be seen (Fig. 4, curve *1*)that the introduction of 5 μM $CoQ_{10}H_2$ into the oxidation medium results in a more than 3-fold increase in the τ duration (from 8 min (control) to 20 min), but a much larger increase in the concentration of $CoQ_{10}H_{(2)}$does not lead to a marked increase in antioxidant action (τ= 14 min at 80 μM $CoQ_{10}H_{2)}$. At the same time, increasing the concentration ofα -TON at the TPO of LDL particles from 5 to 80 μM causes a very dramatic increase in τ duration from 36 to 160 min, respectively (Fig. 4, curve *2*). Notably, addition of $CoQ_{10}H_2$ to LDL particles at concentrations of 40 and 80 μM together with 10 μMα -TON causes a marked increase in τ duration to 65 and 80 min, respectively. However, when 10 μMα -TON was combined with $CoQ_{10}H_2$ at low concentrations (5 and 10 μM), the duration ofwas almost the same as in the presence of $CoQ_{10}H_2$ alone (Figure 4, curve *3*). Plastoquinone skQ1, which, like $CoQ_{10}H_2$, is a reduced quinone, affected τ duration to a lesser extent than other antioxidants, including $CoQ_{10}H_2$ (Fig. 4, curve *4*).

Fig. 4. Dependence of the duration of the period of induction (τ) of LDL particles SRO (by accumulation of diene conjugates) on the concentration of added phenolic lipophilic antioxidants: ubiquinol Q_{10} (*1*), α-tocopherol (*2*), combination of 10 μM α-tocopherol with different concentrations (5-80 μM) of ubiquinol Q_{10} (*3*) and skQ1 (*4*). * $p< 0.05$ - statistically significant differences of groups *1*, *3* and *4* in relation to group *2*; # $p< 0.05$ - statistically significant differences of groups *1* and *4* in relation to group *3* at concentrations of ubiquinol Q_{10} and skQ1 equal to 40 and 80 μM

At a concentration of 5 μM $CoQ_{10}H_2$ did not markedly affect the magnitude of the initial rate of LOOH accumulation during the SRO of LDL particles, whereas when the concentration of $CoQ_{(10)}H_2$ was increased to 10-80 μM, the initial rate of LDL oxidation decreased 2-2.5-fold (Fig. 5).

Fig. 5. Effect of different concentrations of exogenous α-tocopherol, ubiquinol Q_{10}, and skQ1 on the initial rate of Cu^{2+}-initiated lipid ER in LDL particles; *1* - native LDL without addition of antioxidants (control); *2* - LDL+ ubiquinol Q_{10}; *3* - LDL+ α-TON; *4* - LDL+ α-TON +ubiquinol Q_{10} (antioxidants were added at equimolar concentrations); *5* - LDL+ skQ1. * $p< 0.05$ - statistically

significant differences of experimental groups with respect to control (initial rate of LOOH accumulation in the absence of antioxidants)

In contrast, when 5 μM α-TON was introduced into the incubation medium, the initial rate of LDL oxidation decreased dramatically (more than), and at a concentration of α-TON equal to 10 μM, the initial rate of LOOH accumulation dropped almost 6-fold and decreased progressively with increasing antioxidant content in the SRO medium (Fig. 5). At high concentrations (40-80 μM), α-TOH inhibited the initial rate of SRO of LDL particles much more effectively than $CoQ_{10}H_2$ and skQ1 (Figure 5). At the same time, when equal concentrations of $CoQ_{10}H_2$ and α-TON were added simultaneously to the incubation medium, their additive effects were not observed over the entire range of antioxidant concentrations used (Fig. 5). The initial rate of LOOH accumulation when these antioxidants were introduced into the oxidation medium of LDL particles at a concentration of 10 μM was only slightly lower than in the presence of $CoQ_{10}H_2$ alone (Fig. 5). When other concentrations were used, the combined action of these antioxidants was almost identical to that of α-tocopherol alone (Fig. 5).

Nevertheless, the addition of $CoQ_{10}H_2$ at concentrations of 40 or 80 μM together with 10 μM α-TON to the LDL oxidation medium enhanced the antioxidant effect of the latter by almost 2 and 9 times, respectively (Fig. 6, columns *4* and *5*). This fact is in agreement with the data on the increase in the duration τ on the kinetic curves of SRO in LDL under similar conditions (Fig. 4, curve *3*).

Fig. 6. Changes in the initial rate of Cu^{2+}-initiated SRO in LDL particles upon addition of 10 μM α-tocopherol and different concentrations (10-80 μM) of ubiquinol Q_{10}; *1* - oxidation of LDL without addition of antioxidants (control); *2* - LDL+ 10 μM α-TOH *; 3* - LDL+ 10 μM ubiquinol Q_{10}+ 10 μM α-TOH *; 4* - LDL+ 40 μM ubiquinol Q_{10}+ 10 μM α-TOH; *5* - LDL+ 80 μM ubiquinol Q_{10}+ 10 μM α-TOH. * $p< 0.05$ - statistically significant differences of groups *2-5* with respect to group *1* (control); # $p< 0.05$ - statistically significant differences of groups *3-5* with respect to group *2* (LDL+ 10 μM α-TOH)

As mentioned above, the combined introduction of $CoQ_{10}H_2$ and α-TOH at a concentration of 10 μM, on the contrary, has a prooxidant effect (Figs. 5 and 6). Thus, the analysis of the kinetic characteristics of SRO of LDL particles showed that the introduction of high concentrations of $CoQ_{10}H_2$ into the oxidation medium apparently leads to the reduction of α-TON radicals, as a result

of which low concentrations of the latter more effectively inhibit SRO processes in lipid-protein complexes.

Reduction of tocopheroxyl radical under the action of ubiquinol Q_{10} and ascorbate. The interaction of $CoQ_{10}H_2$ with α -TO$^{\bullet}$ was investigated by EPR spectroscopy (Fig. 7). As can be seen from the data presented in Fig. 7, $CoQ_{10}H_2$ is quantitatively reduced α -TO$^{\bullet}$ in aqueous-alcoholic medium (Fig. 7, *a*; spectra *1-4*). The tocopheroxyl radical is quite stable, which allowed us to estimate its concentration (Fig. 7, *b*). It should also be noted that α -TO$^{\bullet}$ is reduced by sodium ascorbate (Fig. 7, *a*; spectra *5-7*). At high concentrations of this antioxidant, a small doublet EPR signal characteristic of the free radical of ascorbic acid (semidehydroascorbate) is recorded (Fig. 7, *a*; spectrum *7*). At the same time, $CoQ_{10}H_2$ was found to reduce the phenoxyl radical of the synthetic hydrophobic antioxidant probucol (Fig. 7; spectra *8* and *9*). The action of this phenolic antioxidant is often compared with α -TON, including the inhibition of SRO processes in LDL [22, 27, 28].

The regeneration of the tocopheroxyl radical under the action of $CoQ_{10}H_2$ should occur according to reaction (4), the products of which, along with α -TON, are $CoQ_{10}H^{\bullet}$, and it cannot be excluded that this reaction is reversible. At the same time, $CoQ_{10}H^{\bullet}$ can participate in the regeneration of α -THOH according to the reaction:

$$CoQ_{10}H^{\bullet} + \alpha\text{-TO}^{\bullet} \longrightarrow CoQ_{10} + \alpha\text{-TOH}. \quad (8)$$

However, we failed to record $CoQ_{10}H^{\bullet}$ during the reduction of α -TO$^{\bullet}$. On the other hand, we have shown the formation of $CoQ_{10}H^{\bullet}$ during incomplete reduction of CoQ_{10} by sodium borohydride. In this case, $CoQ_{10}H^{\bullet}$, appears to be the product of a coproportionation reaction between CoQ_{10} and $CoQ_{10}H_2$ (Fig. 7, *c*):

$$CoQ_{10} + CoQ_{10}H_2 \longrightarrow 2\ CoQ_{10}H^{\bullet}\ . \quad (9)$$

The decrease in the level of $CoQ_{10}H^{\bullet}$ in the reaction medium (Fig. 7, *c*) can be attributed to its one-electron oxidation by molecular oxygen during the reaction:

$$CoQ_{10}H^{\bullet} + O_2 \longrightarrow CoQ_{10} + O_2^{\bullet-} + H^+\ , \quad (10)$$

resulting in the formation of superoxide anion radical ($O_2^{\bullet-}$) [19, 29, 30].

Fig. 7. Free radical reduction ofα -tocopherol (α -TO$^{\bullet}$) and probucol. Free radical EPR spectra were recorded in a reaction mixture containing 320 mM α-TOH or 160 mM probucol, which were pre-oxidized with MnO_2. *a* - Medium withα -TO$^{\bullet}$ without additives (*1*); medium withα -TO$^{\bullet}$ without additives after addition of $CoQ_{10}H_{(2}$) at concentrations of 0.25 (*2*), 0.5 (*3*) and 1 (*4*) mM; medium withα -TO$^{\bullet}$ without additives after addition of 1 (*5*), 2 (*6*) and 5 (*7*) mM Na ascorbate; medium containing probucol phenoxyl radical (*8*); medium containing probucol phenoxyl radical after addition of 0.5 mM $CoQ_{10}H_2$ (*9*). *b* - Concentration dependence of the reduction ofα -TO$^{\bullet}$ ubiquinol by Q_{10}. *c* - Formation of $CoQ_{10}H^{\bullet}$ in the reaction mixture upon incomplete reduction of CoQ_{10} by sodium borohydride. The reaction medium contained 100 μM CoQ_{10} and 50 μM $NaBH_4$. EPR spectra were recorded after 2 min (*1*), 10 min (*2*) and 30 min (*3*) incubation of the mixture

Thus, the absence of $CoQ_{10}H^{\bullet}$ at a concentration sufficient to register this radical in a model system containingα -TO$^{\bullet}$ and $CoQ_{(10)}H_2$ is probably due to the occurrence of reactions (8) and (10).

Nevertheless, it should be kept in mind that the system we used is homogeneous, unlike LDL, which are structural entities consisting of a phospholipid monolayer and a hydrophobic core containing cholesterol esters and triglycerides.

DISCUSSION OF RESULTS

In the early 90s the last century, the ideas that oxidized LDL play an important role in the etiology and pathogenesis of atherosclerosis were formulated [31-35]. Based on these works, numerous clinical studies were undertaken in which attempts were made to restrain the progression of atherosclerosis by reducing LDL oxidizability in IBS patients using high-dose vitamin E (α-TOH) therapy [36-39]. No beneficial effect was achieved in these clinical trails. This caused great disappointment to researchers and led some authors completely deny the possibility of the influence of oxidized LDL on atherogenesis [36-39]. Nevertheless, we found that the key factor of atherogenesis is not oxidized (containing LOOH) LDL, but LDL particles chemically modified by secondary products of ARD - low-molecular-weight dicarbonyls (malonic dialdehyde, etc.) [39-41]. In addition, it was suggested that protection of LDL particles from damage during their SRO is carried out not by α-TON but by $CoQ_{10}H_2$ [42]. This opinion contradicts the notion that α-TON is a more effective antioxidant than $CoQ_{10}H_2$ [19]. There is evidence that at low concentrations, $CoQ_{10}H_2$ inhibits Cu^{2+}-initiated SRO of LDL particles *in vitro* [43]. Orally administered CoQ_{10} is

reduced to $CoQ_{10}H_2$ after absorption and transported to the liver, where it is incorporated into LDL particles that deliver lipids to peripheral tissues [44]. After oral administration of CoQ_{10}, the maximum plasma concentration of $CoQ_{10}H_2$ is observed in 6-8 h, with a half-life of more than 30 h [44]. It has been found that administration of CoQ_{10} *per os* contributes to the increase of resistance of LDL particles to SRO [10-12, 45], and its content in LDL particles can increase almost 3-fold during long-term supplementation [45]. The above data are in good agreement with our results, which are presented in Fig. 2, where we can see that already 30 days after the beginning of oral therapy with inclusion of CoQ_{10} the initial content of LOOH in LDL decreases by 4 times and reaches the minimum level (8 times lower than the initial one) in 2 months after the beginning of pharmacotherapy, after which during the next 4 months the concentration of LOOH in LDL of CHD patients practically does not change (reaches "saturation") (Fig. 2). Thus, our results strongly suggest the possibility of efficient reduction of CoQ_{10} to $CoQ_{10}H_2$ *in vivo*, although this reduction may not be a consequence of bioregeneration of CoQ_{10} directly within LDL particles, but reflects the possibility of biotransformation of this antioxidant absorption and/or assembly of LDL particles in the liver [44]. Biosynthesis of $CoQ_{10}H_2$ provides the body's major requirement for this nutrient, as only about 40% of $CoQ_{10}H_2$ is supplied by food [8]. Accordingly, the use of cholesterol-lowering pharmacotherapy with the inclusion of HMG-CoA reductase inhibitors (statins), which simultaneously with the inhibition of cholesterol synthesis inhibit the biosynthesis of $CoQ_{10}H_2$, leads to the development of $CoQ_{10}H_2$ deficiency [46-48]. Thus, therapy with statins contributes to increased LDL oxidation in patients [49]. This is all the more undesirable, since deficiency of CoQ_{10} has been noted in cardiovascular diseases [50, 51], with atherosclerosis predominantly reducing the level of its reduced form [51]. It should be noted that dosing IBS patients with high oral doses of vitamin E (α- tocopherylsuccinate) practically does not affect LDL oxidizability [22]. This fact may indicate that α-tocopherol esters are not completely hydrolyzed in the organism of IBS patients with the formation of the active antioxidant form (free α-TON), although the corresponding aryl hydrolase is present in the intestinal wall cells. On the other hand, it can be assumed that *in vivo* the effect of α-TON on LDL oxidizability depends on its localization in these particles [22].

To study the inhibitory efficiency of various lipophilic phenolic antioxidants, we used the standard model of Cu^{2+}-initiated SRO of LDL particles [22-24]. This model is based on the formation of peroxyl ($LOO^{\bullet}$) and alkoxyl ($LO^{\bullet}$) lipid radicals in the following reactions:

$$LOOH + Cu^{2+} \rightarrow LOO^{\bullet} + H^{+} + Cu^{+}, \quad (11)$$

$$LOOH + Cu^{+} \rightarrow LO^{\bullet} + OH^{-} + Cu^{2+}, \quad (12)$$

as well as superoxide anion radical ($O_2^{\bullet -}$), hydrogen peroxide ($H_2O_{(2)}$), and hydroxyl radical (HO). •These AFCs are produced in copper ion catalyzed reactions:

$O_2 + Cu^+ \rightarrow O_2^{\bullet -} + Cu^{2+}$, (13)

$O_2^{\bullet -} + O_2^{\bullet -} + 4\ H^+ \rightarrow 2\ H_2O_2$, (14)

$H_2O_2 + Cu^+ \rightarrow HO^{\bullet} + OH^- + Cu^{2+}$. (15)

In turn, $NO^{\bullet}$, $LOO^{\bullet}$ and $LO^{\bullet}$ interact with the PUFA acyls (LH) of phospholipids of LDL particles and thereby participate in other ER chain reactions.

Consequently, the oxidation system we used actually simulates the process of LDL particle SRO occurring under oxidative stress under the action of AFCs in *vivo* and is widely used in studies of the mechanisms of oxidative processes in LDL *in vitro* [25, 52-54]. The results obtained using the model of Cu^{2+}-initiated SRO of LDL particles are shown in Figs. 3-6. Analysis of the kinetic curves of the SRO of LDL particles indicates that the most effective of the lipophilic antioxidants studied isα -tocopherol. A mixture of equimolar concentrations of $CoQ_{10}H_2$ and α-TON inhibits SRO in essentially the same way as α-TON alone , although some pro-oxidant effect is observed in the presence of 10 μM of these antioxidants compared with the same concentration of α-TON (Figures 5 and 6). This effect of $CoQ_{10}H_2$ can be explained by the participation of $CoQ_{(}10)H^{\bullet}$ in the production of $O_2^{\bullet -}$ (reaction (10)) and consequently other AFCs (reactions (14) and (15)). At high concentrations (40 and 80 μm) α-TON and its combination with $CoQ_{10}H_2$ causes almost complete inhibition of peroxidation reactions in LDL (Figures 3 and 5). It should be noted that, in contrast to α-TOH, the increase in the level of $CoQ_{10}H_2$ has almost no effect on the duration of the induction period (τ) on the kinetic curves of SRO (Fig. 4). Such an effect of $CoQ_{10}H_2$ is consistent with the data of Nohl et al. [29], where it was shown thatτ azoinitiator-induced ER of liposomes increases with increasing concentration of $CoQ_{10}H_2$ markedly less than in the presence of.

At the same time, addition of $CoQ_{10}H_2$ at a concentration of 80 μM together with 10 μM α-TON to LDL particles significantly enhanced the antioxidant effect of the latter (Figures 4 and 6). These results indicate the existence of synergism in the mechanism of the combined antioxidant action of $CoQ_{10}H_2$ and α-TON . The explanation for this synergism may be the reduction in the presence of $CoQ_{10}H_2$ (reaction (4)) of tocopheroxyl radicals capable of initiating further chain reactions of SRO in LDL particles (reaction (5)) [19]. Indeed, in a homogeneous model system (water-alcohol mixture), we have shown that when the molar ratio of α-$TO^{\bullet}$ and $CoQ_{10}H_2$ is 1/31, approximately 45% of α-$TO^{\bullet}$ is reduced (Fig. 7, *b*). Importantly, this mechanism of α-TOH bioregeneration may explain the effective antioxidant effect of CoQ_{10} when administered to patients with atherosclerosis (Fig. 2). Because the *in vivo* content of α-TON in LDL particles is

much higher than that of $CoQ_{10}H_2$, it is possible that reaction (4) is reversible. Thus, we can assume the participation of α-TON in the bioregeneration (reduction) of ubisemiquinone Q_{10} in LDL particles during the reaction:

$CoQ_{10}\,H^{\bullet} + \alpha\text{-TOH} \rightarrow CoQ_{10}H_2 + \alpha\text{-TO}^{\bullet}$. (16)

Indeed, it was previously shown that the addition of $CoQ_{10}H_2$ stimulates the formation of the probucol phenoxyl radical in LDL particles during their intensive oxidation [28]. It is believed that probucol, like possibly, is oxidized by $CoQ_{10}H$ formed under these conditions$^{\bullet}$. However, in the homogeneous model system we used, the phenoxyl radical of probucol, as well as α-TO$^{\bullet}$, was reduced by $CoQ_{10}H_2$ (Fig. 7, *a*).

At the same time, it is known that free radicals of probucol and α-tocopherol, but not $CoQ_{10}H^{\bullet}$, are regenerated by ascorbic acid (Asc-OH) [27, 28, 55-57]. This mechanism of α-TOH regeneration is consistent with our results (Fig. 7, *a*; spectra *5-7*) and involves the following reactions:

$Asc\text{–}OH + \alpha\text{-TO}^{\bullet} \rightarrow Asc\text{–}O^{\bullet -} + \alpha\text{-TOH}$, (17)

$Asc\text{–}O^{\bullet -} + \alpha\text{-TO}^{\bullet} \rightarrow Asc{=}O + \alpha\text{-TOH}$, (18)

where Asc-$O^{\bullet -}$ is semidehydroascorbate (ascorbate free radical); Asc= O is dehydroascorbic acid.

Nevertheless, the role of ascorbic acid in the process of protection of LDL particles from SRD is not fully elucidated. It is obvious that high concentration of ascorbate in blood plasma and the presence of semidehydroascorbate reductase (Asc-$O^{\bullet -}$) in erythrocyte membrane [55] are necessary, first of all, to suppress the interaction of AFCs formed during oxidative stress (such as $O_2^{\bullet -}$ and NO$^{\bullet}$) with LDL particles. As noted, Asc-OH does not regenerate $CoQ_{10}H_2$ from $CoQ_{(10)}H^{\bullet}$, however, evidence in the literature suggests that short-chain analogs of CoQ_{10} are reduced to the corresponding ubisemiquinones in reactions with ascorbate and semihydroascorbate [30, 58]. Based on the above, it can be assumed that ascorbate reduces α-TO$^{\bullet}$ to α-TON and CoQ_{10} to $CoQ_{10}H^{\bullet}$. The possible participation of various natural antioxidants in the conjugated processes of protection of LDL particles from SRB chain processes is shown in the following scheme (Fig. 8).

Fig. 8. Hypothetical scheme illustrating possible bioremediation mechanisms of $CoQ_{10}H_2$ and α-TON in the process of LDL particle SRO. Notations adopted: $CoQ_{10}H_2$, $CoQ_{(10)}H^{\bullet}$ and CoQ_{10} are ubiquinol $Q_{(10)}$(reduced form of coenzyme $Q_{(10)}$), ubisemiquinol $Q_{(10)}$and ubiquinone $Q_{(10)}$(oxidized

form of coenzyme Q_{10}), respectively; α-TON and α-TO$^{\bullet}$ are α-tocopherol and tocopheroxyl radical; Asc-OH, Asc-O$^{\bullet}$ and Asc= O - ascorbic acid, semidehydroascorbate and dehydroascorbate, respectively; LOO$^{\bullet}$ and LO$^{\bullet}$ - peroxyl and alkoxyl radicals of PUFAs; LOOH and LOH - lipid hydroperoxide and hydroxide

Apparently, the system of conjugated reactions of lipophilic and hydrophilic antioxidants functions as a kind of redox-buffer providing the most effective antioxidant protection of LDL particles under oxidative stress.

It is important to note that the nature of action of $CoQ_{10}H_2$ and the synthetic antioxidant skQ1 on Cu^{2+}-induced SRO in LDL particles is similar (Fig. 3-5), despite the significant difference of the nonbenzoquinone part of their molecules (Fig. 1). The mechanisms of bioregeneration of coenzyme Q_{10} and α-tocopherol proposed by us are also discussed by other authors [16, 19]. The possibility of such reactions is indicated by the existing ideas about the proximity of $CoQ_{10}H_2$ and α-TON in LDL particles, in which the benzoquinone or chromanone parts of the molecules of these antioxidants are adjacent to hydrophilic phosphate groupings of phospholipids, and the hydrophobic "tails" are located near the acyls of PUFA phospholipids [19].

It should be taken into account that in each LDL particle there are up to 12 molecules of α-TON per 1 molecule of $CoQ_{10}H_2$ [13, 17], and the amount of the substrate of SRO, acyl PUFAs, in LDL phospholipids in the molar ratio is about 20-30 per 1 mole of α-TON [59]. If we assume rounded molar ratios between $CoQ_{10}H_2$/α-TON/ PUFA= 1/10/300, it becomes clear that, taking into account the assumed mechanisms of bioregeneration of lipophilic antioxidants, the inhibition of SRO in LDL particles should be very effective. In fact, this efficiency may be even higher, since one molecule of reduced coenzyme Q_{10} is able to scavenge two free radicals, acting first as $CoQ_{(10)}H_2$ and then as $CoQ_{10}H^{\bullet}$ (reactions (2) and (3)). Nevertheless, the specific mechanisms of antioxidant defense of LDL particles and the participation of various natural antioxidants in the regulation of SRD processes in LDL particles need additional experimental substantiation.

AUTHORS' CONTRIBUTION

Lankin V.Z. - study concept, writing the version of the article and discussion of the results; Shumayev K.B. - conducting EPR-spectrometric studies, calculation of the results, making figures, writing the version of the article and discussion of the results; Medvedeva V.A. - conducting kinetic studies; Tihaze A.K. - preparation of the literature list, participation in writing the discussion of the article; Konovalova G.G. - conducting a clinical study.

FUNDING

This work was financially supported by the Russian Science Foundation (grant No. 22-15-00013).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICS DECLARATION

The clinical part of the study was conducted in accordance with the ethical standards of the National Committee on Research ethics and the 1964 Declaration of Helsinki and its subsequent revisions or comparable ethical standards. Informed voluntary consent was obtained from each of the participants included in the study. Experimental protocols were approved by the Ethical Committee of the Institute of Clinical Cardiology of the NMICC Institute of Clinical Cardiology of the Russian Ministry of Health January 29, 2018 (project identification code 233).

REFERENCES

1. Sharapov, M. G., Gudkov, S. V., and Lankin, V. Z. (2021) Hydroperoxide reducing enzymes in the regulation of free radical processes, *Biochemistry (Moscow)*, **86**, 1256-1274, doi: 10.1134/S0006297921100084.
2. Shen, B. W., Scanu, A. M., and Kezdy, F. J. (1977) Structure of human serum lipoproteins inferred from compositional analysis, *Proc. Natl. Acad. Sci. USA*, **74**, 837-841, doi: 10.1073/pnas.74.3.837.
3. Lankin, V. Z., Tikhaze, A. K., and Kosach, V. Ya. (2022) Comparative susceptibility to oxidation of different classes of blood plasma lipoproteins*, Biochemistry (Moscow)*, **87**, 1335-1341, doi: 10.1134/S0006297922110128.
4. Sharapov, M. G., Gudkov, S. V., and Lankin, V. Z. (2021) Role of glutathione peroxidases and peroxiredoxins in free radical induced pathologies*, Biochemistry (Moscow)*, **86**, 1418-1433, doi: 10.1134/S0006297921110067.
5. Lankin, V. Z., and Tikhaze, A. K. (2017) Role of oxidative stress in the genesis of atherosclerosis and diabetes mellitus: a personal look back on 50 years of research, *Curr. Aging Sci*., **10**, 18-25, doi: 10.2174/1874609809666160926142640.
6. Mellors, A., and Tappel, A. L. (1966) The inhibition of mitochondrial peroxidation by ubiquinone and ubiquinol, *J. Biol. Chem*., **241**, 4353-4356.
7. Witting, L. A. (1980) Vitamin E and lipid antioxidants in free-radical-initiated reactions, *Free Radic. Biol*., **4**, 295-319.
8. Bliznakov, E. G., and Wilkins, D. J. (1998) Biochemical and clinical consequences of

inhibiting coenzyme Q10 biosynthesis by lipid-lowering HMG-CoA reductase inhibitors (statins): a critical overview, *Adv. Ther.*, **15**, 218-228.

9. Hevonoja, T., Pentikäinen, M. O., Hyvönen, M. T., Kovanen, P. T., and Ala-Korpela, M. (2000) Structure of low density lipoprotein (LDL) particles: basis for understanding molecular changes in modified LDL, *Biochim. Biophys. Acta*., **15**, 189-210, doi: 10.1016/s1388-1981(00)00123-2.
10. Thomas, S. R., Neuzil, J., and Stocker, R. (1997) Inhibition of LDL oxidation by ubiquinol-10. A protective mechanism for coenzyme Q in atherogenesis? *Mol. Aspects Med*., **18**, 85-103, doi: 10.1016/s0098-2997(97)00031-9.
11. Thomas, S. R., Witting, P. K., and Stocker, R. (1999) A role for reduced coenzyme Q in atherosclerosis? *Biofactors*, **9**, 207-224, doi: 10.1002/biof.5520090216.
12. Littarru, G. P., Battino, M., Tomasetti, M., Mordente, A., Santini, S., Oradei, A., Manto, A., and Ghirlanda, G. (1994) Metabolic implications of coenzyme Q_{10} in red blood cells and plasma lipoproteins, *Mol. Aspects Med*., **15**, 67-72, doi: 10.1016/0098-2997(94)90014-0.
13. Stocker, R. (1993) Natural antioxidants and atherosclerosis, *Asia Pac. J. Clin. Nutr*., **2**, 15-20.
14. Niki, E. (2014) Role of vitamin E as a lipid-soluble peroxyl radical scavenger: *in vitro* and *in vivo* evidence, *Free Radic. Biol. Med*., **66**, 3-12, doi: 10.1016/j.freeradbiomed.2013.03.022.
15. Frei, B., Kim, M. C., and Ames, B. N. (1990) Ubiquinol-10 is an effective lipid-soluble antioxidant at physiological concentrations*, Proc. Natl. Acad. Sci. USA*, **87**, 4879-4883, doi: 10.1073/pnas.87.12.4879.
16. Kagan, V. E., Fabisiak, J. E., and Quinn, E. J. (2000) Coenzyme Q and vitamin E need each other as antioxidants, *Protoplasma*, **214**, 11-18, doi: 10.1007/BF02524257.
17. Bowry, V. W., Stocker, R. (1993) Tocopherol-mediated peroxidation. The prooxidant effect of vitamin E on the radical-initiated oxidation of human low-density lipoprotein, *J. Am. Chem. Soc.*, **115**, 6029-6044, https://doi.org/10.1021/ja00067a019.
18. Witting, P. K., Upston, J. M., and Stocker, R. (1997) Role of alpha-tocopheroxyl radical in the initiation of lipid peroxidation in human low-density lipoprotein exposed to horse radish peroxidase, *Biochemistry*, **36**, 1251-1258, doi: 10.1021/bi962493j.
19. Fong, C. W. (2023) Coenzyme Q 10 and Vitamin E synergy, electron transfer, antioxidation in cell membranes, and interaction with cholesterol, hal-03976270.
20. Lindgren, F. T. (1975) Preparative ultracentrifugal laboratory procedures and suggestions for lipoprotein analysis, in *Analysis of Lipids and Lipoproteins* (Perkins, E. G., ed) *Champaign: Amer. Oil. Chemists Soc*., pp. 204-224.

21. Nourooz-Zadeh, J., Tajaddini-Sarmadi, J., and Wolf, S. P. (1994) Measurement of plasma hydroperoxide concentrations by the ferrous oxidation-xylenol orange assay in conjunction with triphenylphosphine*, Anal. Biochem*., **220**, 403-409.
22. Lankin, V. Z., Konovalova, G. G., Tikhaze, A. K., Shumaev, K. B., Kumskova, E. M., and Viigimaa, M. (2014) The initiation of the free radical peroxidation of low-density lipoproteins by glucose and its metabolite methylglyoxal: a common molecular mechanism of vascular wall injuries in atherosclerosis and diabetes, *Mol. Cell. Biochem*., **395**, 241-252, doi: 10.1007/s11010-014-2131-2.
23. Mark, J., and Burkitt, A. (2001) Critical overview of the chemistry of copper-dependent low density lipoprotein oxidation: roles of lipid hydroperoxides, a-tocopherol, thiols, and ceruloplasmin, *Arch. Biochem. Biophys*., **394**, 117-135, doi: 10.1006/abbi.2001.2509.
24. Patel, R. P., and Darley-Usmar, V. (1999) Molecular mechanisms of the copper dependent oxidation of low-density lipoprotein, *Free Radic. Res.*, **30**, 1-9, doi: 10.1080/10715769900300011.
25. Kontush, A., Hubner, C., Finckh, B., Kohlschutter, A., and Beisiegel, U. (1994) Low density lipoprotein oxidizability by copper correlates with its initial ubiquinol-10 and polyunsaturated fatty acid content, *FEBS Lett*., **341**, 69-73, doi: 10.1016/0014-5793(94)80242-4.
26. Emanuel N. M., Denisov E. T., Maizus Z. K. (1965) Chain reactions of hydrocarbon oxidation in liquid phase, Moscow, Nauka, 375 p.
27. Kagan, V. E., Freisleben, H. J., Tsuchiya, M., Forte, T., and Packer, L. (1991) Generation of probucol radicals and their reduction by ascorbate and dihydrolipoic acid in human low density lipoproteins, *Free Radic. Res. Commun*., **15**, 265-276, https://doi.org/10.3109/10715769109105222.
28. Shumaev, K. B., Ruuge, E. K., Dmitrovsky, A. A., Bykhovsky, V. Ya., and Kukharchuk, V. V. (1997) Effect of lipid peroxidation products and antioxidants on the formation of probucol radicals in low density lipoproteins, *Biochemistry***, 62**, 657-660.
29. Nohl, H., Gille, L., and Kozlov, A. V. (1998) Antioxidant-derived prooxidant formation from ubiquinol, *Free Radic. Biol. Med.*, **25**, 666-675, doi.org/10.1016/S0891-5849(98)00105-1.
30. Roginsky, V. A., Mohr, D., and Stocker, R. (1996) Reduction of ubiquinone-1 by ascorbic acid is a catalytic and reversible process controlled by the concentration of molecular oxygen, *Redox Rep*., **2**, 55-62, doi: 10.1080/13510002.1996.11747027.
31. Steinberg, D., Parthasarathy, S., Carew, T. E., Khoo, J. C., and Witztum, J. L. (1989) Beyond cholesterol. Modifications of low-density lipoprotein that increase its

atherogenicity, *New Eng. J. Med.*, **320**, 915-924.

32. Kita, T., Ishii, K., Yokode, M., Kume, N., Nagano, Y., Arai, H., Arai, H., and Kawai, C. (1990) The role of oxidized low density lipoprotein in the pathogenesis of atherosclerosis, *Eur. Heart J.*, **11**, 122-127, doi: 10.1093/eurheartj/11.suppl_e.122.
33. Witztum, J. L. (1994) The oxidation hypothesis of atherosclerosis, *Lancet*, **344**, 793-795.
34. Yla-Herttuala, S. (1994) Role of lipid and lipoprotein oxidation in the pathogenesis of atherosclerosis, *Drugs Today*, **30**, 507-514.
35. Steinberg, D. (1995) Role of oxidized LDL and antioxidants in atherosclerosis, *Adv. Exp. Med. Biol.*, **369**, 39-48.
36. Lankin, V. Z., and Tikhaze, A. K. (2017) Role of oxidative stress in the genesis of atherosclerosis and diabetes mellitus: a personal look back on 50 years of research, *Curr. Aging Sci.*, **10**, 18-25.
37. Lankin, V. Z., Tikhaze, A. K., and Melkumyants, A. M. (2022) Dicarbonyl-dependent modification of LDL as a key factor in endothelial dysfunction and atherosclerotic vascular wall damage, *Antioxidants***, 11**, 1565.
38. Lankin, V. Z., Tikhaze, A. K., and Melkumyants, A. M. (2022) Malondialdehyde as an important key factor in molecular mechanisms of vascular wall damage under heart disease development, *Int. J. Mol. Sci.*, **24**, 128.
39. Lankin, V. Z., Tikhaze, A. K., Sharapov, M. G., and Konovalova, G. G. (2024) The role of natural low molecular weight dicarbonyls in atherogenesis and diabetogenesis*, Rev. Cardiovasc. Med.*, **25**, 295, doi: 10.31083/j.rcm2508295.
40. Lankin, V. Z., Tikhaze, A. K., and Konovalova, G. G. (2023) Differences in structural changes and pathophysiological effects of low-density lipoprotein particles upon accumulation of acylhydroperoxy derivatives in their outer phospholipid monolayer or upon modification of apoprotein B-100 by natural dicarbonyls, *Biochemistry (Moscow)*, **88**, 1910-1919.
41. Lankin, V. Z., Sharapov, M. G., Tikhaze, A. K., Goncharov, R. G., Antonova, O. A., Konovalova, G. G., and Novoselov, V. I. (2023) Dicarbonyl-modified low-density lipoproteins are key inducers of LOX-1 and NOX1 gene expression in the cultured human umbilical vein endotheliocytes, *Biochemistry (Moscow)*, **88**, 2125-2136.
42. Stocker, R., Bowry, V. W., and Frei, B. (1991) Ubiquinol-10 protects human low density lipoprotein more efficiently against lipid peroxidation than does α-tocopherol*, Proc. Natl. Acad. Sci. USA*, **88**, 1646-1650.
43. Ahmadvand, H., Mabuchi, H., Nohara, A., Kobayahi, J., and Kawashiri, M. A. (2013) Effects of coenzyme Q(10) on LDL oxidation *in vitro, Acta Med. Iran*, **51**, 12-18.

44. Raizner, A. E. (2019) Coenzyme Q_{10}, *Methodist Debakey Cardiovasc. J.*, **15**,185-191, doi: 10.14797/mdcj-15-3-185.
45. Mohr, D., Bowry, V. W., and Stocker, R. (1992) Dietary supplementation with coenzyme Q_{10} results in increased levels of ubiquinol-10 within circulating lipoproteins and increased resistance of human low-density lipoprotein to the initiation of lipid peroxidation, *Biochim. Biophys. Acta*, **1126**, 247-254.
46. Bargossi, A. M., Grossi, G., Fiorella, P. L., Gaddi, A., Di Giulio, R., and Battino, M. (1994) Exogenous CoQ_{10} supplementation prevents plasma ubiquinone reduction induced by HMG-CoA reductase inhibitors, *Mol. Aspects Med.*, **15**, 187-193, doi: 10.1016/0098-2997(94)90028-0.
47. Garrido-Maraver, J., Cordero, M. D., Oropesa-Avila, M., Vega, A. F., de la Mata, M., Pavon A. D., Alcocer-Gomez, E., Calero, C. P., Paz, M. V., Alanis, M., de Lavera, I., Cotan, D., and Sanchez-Alcazar, J. A. (2014) Clinical applications of coenzyme Q_{10}, *Front. Biosci.*, **19**, 619-633, doi: 10.2741/4231.
48. Gutierrez-Mariscal, F. M., de la Cruz-Ares, S., Torres-Peña, J. D., Alcalá-Diaz, J. F., Yubero-Serrano, E. M., and López-Miranda, J. (2021) Coenzyme Q_{10} and Cardiovascular diseases,, Antioxidants, **10**, 906, doi: 10.3390/antiox10060906.
49. Lankin, V. Z., and Tikhaze, A. K. (2003) Atherosclerosis as a free radical pathology and antioxidative therapy of this disease, *Free Radic.*, **344**, 218-231.
50. Lankin, V. Z., Tikhaze, A. K., Kapel'ko, V. I., Shepel'kova, G. S., Shumaev, K. B., Panasenko, O. M., Konovalova, G. G., and Belenkov, Y. N. (2007) Mechanisms of oxidative modification of low density lipoproteins under conditions of oxidative and carbonyl stress, *Biochemistry (Moscow)*, **72**, 1081-1090, doi: 10.1134/s0006297907100069.
51. Kontush, A., Reich, A., Baum, K., Spranger, T., Finckh, B., Kohlschùtter, A., and Beisiegel, U. (1997) Plasma ubiquinol-10 is decreased in patients with hyperlipidaemia, *Atherosclerosis*, **129**, 119-126, doi: 10.1016/s0021-9150(96)06021-2.
52. Miura, S., Watanabe, J., Tomita, T., Sano, M., and Tomita, I. (1994) The inhibitory effects of tea polyphenols (flavan-3-ol derivatives) on Cu^{2+}-mediated oxidative modification of low density lipoprotein, *Biol. Pharm. Bull.*, **17**, 1567-1572, doi: 10.1248/bpb.17.1567.
53. Yeomans, V. C., Linseisen, J., and Wolfram, G. (2005) Interactive effects of polyphenols, tocopherol and ascorbic acid on the Cu^{2+}-mediated oxidative modification of human low density lipoproteins, *Eur. J. Nutr.*, **44**, 422-428, doi: 10.1007/s00394-005-0546-y.
54. Jayaraman, S., Gantz, D. L., Gursky, O. (2007) Effects of oxidation on the structure and

stability of human low-density lipoprotein, *Biochemistry*, **46**, 5790-5797, doi: 10.1021/bi700225a.

55. Beyer, R. E. (1994) The role of ascorbate in antioxidant protection of biomembranes: interaction with vitamin E and coenzyme Q, *J. Bioenerg. Biomembr.*, **26**, 349-358, doi: 10.1007/BF00762775.
56. Neuzil, J., Thomas, S. R., and Stocker, R. (1997) Requirement for, promotion of, or inhibition by alpha-tocopherol of radical-induced initiation of plasma lipoprotein lipid peroxidation*, Free Radic Biol. Med.*, **22**, 57-71, doi: 10.1016/s0891-5849(96)00224-9.
57. Carr, A. C., Zhu, B. Z., and Frei, B. (2000) Potential antiatherogenic mechanisms of ascorbate (vitamin C) and alpha-tocopherol (vitamin E), *Circ. Res.*, **87**, 349-354, doi: 10.1161/01.res.87.5.354, doi: 10.1161/01.res.87.5.349.
58. Guo, Q., and Packer, L. (1999) ESR studies of ascorbic acid-dependent recycling of the vitamin E homologue Trolox by coenzyme Q_0 in murine skin homogenates, *Redox Rep.*, **4**, 105-111, doi: 10.1179/135100099101534783.
59. Chancharme, L., Thérond, P., Nigon, F., Zarev, S., Mallet, A., Bruckert, E., and Chapman, M. J. (2002) LDL particle subclasses in hypercholesterolemia. Molecular determinants of reduced lipid hydroperoxide stability, *J. Lipid Res.*, **43**, 453-462.